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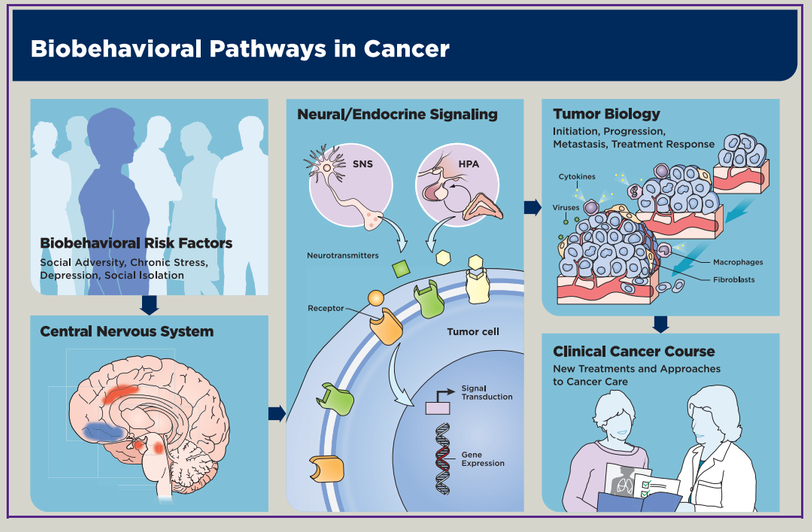
The Official Journal of the Psychoneuroimmunology Research Society



Special Issue on Advances in Cancer and Brain, Behavior, and Immunity: A Decade of Progress
Edited by Paige McDonald and Susan Lutgendorf

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Cover photo. This supplement issue summarizes scientific contributions to cancer research by scientists in brain, behavior, and immunity, beginning with biobehavioral risk factors and ending with new clinical approaches for cancer treatments. See article by McDonald, O'Connell and Lutgendorf on pages S1 to S9.

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Volume 30, 15 March 2013

Special Issue on Advances in Cancer and Brain, Behavior, and Immunity: A Decade of Progress
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CONTENTS

Continued

Brain, Behavior, and Immunity 30 (2013) S1–S9

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Brief Commentary

Psychoneuroimmunology and cancer: A decade of discovery, paradigm shifts, and methodological innovations

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ABSTRACT

This article introduces the supplement Advances in *Cancer and Brain, Behavior, and Immunity* and outlines important discoveries, paradigm shifts, and methodological innovations that have emerged in the past decade to advance mechanistic and translational understanding of biobehavioral influences on tumor biology, cancer treatment-related sequelae, and cancer outcomes. We offer a heuristic framework for research on biobehavioral pathways in cancer. The shifting survivorship landscape is highlighted, and we propose that the changing demographics suggest prudent adoption of a life course perspective of cancer and cancer survivorship. We note opportunities for psychoneuroimmunology (PNI) research to ameliorate the long-term, unintended consequences of aggressive curative intent and call attention to the critical role of reciprocal translational pathways between animal and human studies. Lastly, we briefly summarize the articles included in this compilation and offer our perspectives on future research directions.

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1. Introduction

In 2002, the National Cancer Institute (NCI), in collaboration with other Institutes and Centers of the National Institutes of Health, convened a meeting of scientific experts to discuss seminal research on behavioral, neural, endocrine, and immune system interactions in health and disease. To inform the development of a biobehavioral research agenda in cancer control, knowledge was extracted from contemporary studies of neuroimmune mechanisms of subjective experiences (e.g., stress, loneliness, and pain), biological processes (e.g., circadian rhythmicity, sleep, wound healing, sickness behavior, and apoptosis), and disease outcomes (e.g., human immunodeficiency virus, depression, and post-traumatic stress disorder). *Brain, Behavior, and Immunity* published the *Biological Mechanisms of Psychosocial Effects on Disease* supplement in February 2003. This seminal volume captured state-of-the-science reviews and commentaries by leading experts in psychoneuroimmunology (PNI) and served as a catalyst for biobehavioral[1] research conducted in a cancer context. In the decade prior to the NCI commissioned supplement, *Brain, Behavior, and Immunity* published only 12 cancer-relevant articles. Since the 2003 supplement, the journal has featured 128 cancer-relevant papers that have generated 3361 citations (data from SCOPUS, retrieved November 1, 2012), relative to 55 papers on PNI and cancer, published in other peer review journals during the same time period. These bibliometric data highlight *Brain, Behavior, and Immunity* as a leading scholarly outlet for research on the biology of psychological and social experiences and the integrated mechanisms associated with cancer as a complex disease process. The current volume celebrates the 10-year anniversary of the 2003 supplement. This collection of invited reviews and research articles captures important discoveries, paradigm shifts, and methodological innovations that have emerged in the past decade to advance mechanistic and translational understanding of biobehavioral influences on tumor biology, cancer treatment-related sequelae, and cancer outcomes.

2. Transition from cellular immunity in peripheral blood to tumor-relevant measurements

Early clinical investigations focused almost exclusively on psychosocial modulations of the humoral and cellular immune response and, to some extent, on DNA repair (Andersen et al.,

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[1] For the purpose of this introduction, we use the terms "biobehavioral" and "psychosocial" interchangeably.

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1994; Antoni, 2003; Kiecolt-Glaser and Glaser, 1999; Kiecolt-Glaser et al., 2002). Women at an increased genetic risk for cancer exhibited specific immune impairments and abnormalities in their endocrine response to stress (Bovbjerg and Valdimarsdottir, 1993; Dettenborn et al., 2005; Gold et al., 2003). Clinical studies documented associations between depression, social support, and natural killer cell activity in breast cancer patients (Levy et al., 1987; Levy et al., 1985; Levy et al., 1990). Other research groups observed distress/stress and social isolation-associated impairments in immune function among breast, cervical and ovarian patients (Andersen et al., 1998; Antoni et al., 2009; Lutgendorf et al., 2005; Nelson et al., 2008; Sephton et al., 2009; Thornton et al., 2007); however, the prognostic relevance of these associations remained uncertain (Cohen and Rabin, 1998). Building on the clinical significance of immune cells in ascites (Lotzova et al., 1986; Lotzova et al., 1984) and tumor-infiltrating lymphocytes (Lai et al., 1996) in ovarian cancer, Lutgendorf and colleagues observed significant associations between psychosocial factors and the cellular immune response at the tumor level in a clinical sample (Lutgendorf et al., 2005). This study, among others, signaled an important contextual transition for PNI studies of cancer, a transition aligned closely to advances in cancer cell biology and emerging appreciation for target tissues and the context in which tumors thrive (Marx, 2008).

3. Emergence of the tumor microenvironment

DeVita and Rosenberg (2012) recently chronicled significant discoveries and major events in cancer research since the founding of the *New England Journal of Medicine* nearly 200 years ago (DeVita and Rosenberg, 2012). Basic understanding of cancer biology has matured substantially beyond Virchow's observation of the cellular origin of cancer and the view of tumors as ''insular masses of proliferating cancer cells'' (p. 646, Hanahan and Weinberg, 2011). Progress has been led by milestones[2] like 'observations from a ploughman' (Dell, 2006; Hart and Fidler, 1980; Paget, 1889), 'bloodlines' (Farrell, 2006; Folkman, 1971), 'environmental awareness' (Schuldt, 2006), and the 'hallmarks of cancer' (Hanahan and Weinberg, 2000, 2011). Cancers have come to be seen as inherently complex collections of heterogeneous pathologies that vary by tissue of origin and constellation of genomic, proteomic, and metabolic alterations (Fidler, 2003; Hanahan and Weinberg, 2000, 2011; Vogelstein and Kinzler, 2004). Incipient mutated cells must acquire several biological capabilities to reach full malignancy, and several environments – i.e., the primary, invasive and metastatic tumor microenvironments – are created during tumorigenesis (Hanahan and Weinberg, 2011). In the case of solid tumors, commonly derived from epithelial cells, these microenvironments provide a safe haven for bidirectional communication between cancer cells and the tumor-associated stroma. Cells that construct the microenvironments include pericytes, cancer-associated fibroblasts, endothelial cells, local and bone marrow-derived stromal stem and progenitor cells, cancer stem cells, invasive cancer cells, and immune inflammatory cells (Albini and Sporn, 2007; Joyce, 2005; Joyce and Pollard, 2009; Langley and Fidler, 2007; McAllister and Weinberg, 2010; McCawley and Matrisian, 2001). Inflammation, a seminal biological process in the onset and progression of many diseases (Haroon et al., 2012; Nathan, 2002), has emerged as an essential enabling process for tumor growth and metastasis (Hanahan and Weinberg, 2011; Mantovani, 2009). Cytokines, chemokines, macrophages, and leukocyte infiltrates contribute to tumor progression by promoting invasion, migration, and angiogenesis (Gonda et al., 2009; Mantovani et al., 2008; Medrek et al., 2012; Pitroda et al., 2012; Solinas et al., 2009). Truly, it takes a village of distinct cell types and signaling systems to support the tumor ecosystem.

4. The central nervous system as a master regulator: implications for cancer

Renewed appreciation of the landscapes that enable tumor growth and metastatic dissemination inspire broader consideration of the macro-physiological milieus that potentially shape individual variability in the natural course of cancer and responsiveness to therapies (Castano et al., 2011; Schuller and Al-Wadei, 2010). We offer the following perspective (Fig. 1). The brain, as an adaptive and dynamic synthesizer of experiential and perceptual processes (Ganzel et al., 2010), can participate in the complex regulation of signaling systems used by the diverse array of cells and structures to enable tumorigenesis. Experimental and clinical studies suggest that downstream activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis exerts selective physiologic pressures that initiate molecular signaling pathways involved in DNA repair, angiogenesis, cell survival, inflammation, invasion, metastasis, and resistance to therapy (Antoni et al., 2006; Cole and Sood, 2012; Hara et al., 2011; Lutgendorf and Sood, 2011; Wu et al., 2004). Catecholamines (epinephrine, norepinephrine, dopamine) bind to α-adrenergic receptors (α-ARs) and β-adrenergic receptors (β-ARs), and acetylcholine binds to families of nicotinic (nAChRs) and muscarinic (mAChRs) receptors found on tumor cells and stromal compartments within the microenvironment (Schuller, 2008). Neuroendocrine receptor-mediated signaling has the documented ability to regulate leukocyte gene expression, molecular processes, and functional characteristics of cells within microenvironments (Badino et al., 1996; Cole and Sood, 2012; Lutgendorf et al., 2003; Lutgendorf et al., 2009; Schuller and Al-Wadei, 2010). Examples of observed effects include promotion of tumor cell growth, migration and invasive capacity, and stimulation of angiogenesis by inducing production of pro-angiogenic cytokines. Neuroendocrine hormones activate oncogenic viruses and alter several aspects of immune function, including antibody production, cell trafficking, and the production and release of proinflammatory cytokines (Glaser and Kiecolt-Glaser, 2005; Webster Marketon and Glaser, 2008). Although not explicitly reflected in our conceptual schema, peripherally generated inflammatory and other innate immune mediators can signal back into the central nervous system, stimulate afferent nerves that produce local cytokines, change neuronal function, and cause sickness behaviors as an adaptive response to systemic pressures (Dantzer and Kelley, 2007; Dantzer et al., 2012; Dantzer et al., 2008; Irwin and Cole, 2011; Kelley et al., 2003; Miller et al., 2008). Immune-to-brain communication cascades are thought to undergird cancer and treatment-related symptoms such as fatigue, depression, cognitive dysfunction, and sleep disturbance (Bower et al., 2011; Dantzer et al., 2012; Lutgendorf and Sood, 2011; Miller et al., 2008). Contemporary PNI remains poised to elucidate the prevalence, impact, and etiologies of cancer-related physical and affective sequelae at different phases of cancer survival (Bower, 2012; Dantzer et al., 2012; Haroon et al., 2012).

5. Shifting sands of survivorship

Advances in prevention, detection, and treatment (DeVita and Rosenberg, 2012) continue to yield significant declines in the incidence of most cancers and death rates for all cancers combined (Eheman et al., 2012; Siegel et al., 2012b). These trends, combined with overall increases in life expectancy, have created a ''booming [aging] cancer survivor population'' (p. 1996, Parry et al., 2011). Siegel et al. estimated 13.7 million American cancer survivors were alive in January 2012[3] (Siegel et al., 2012b). The majority

[2] See http://www.nature.com/milestones/milecancer/pdf/milecancer_all.pdf.

[3] Number of survivors projected to increase to nearly 18 million by 2022.

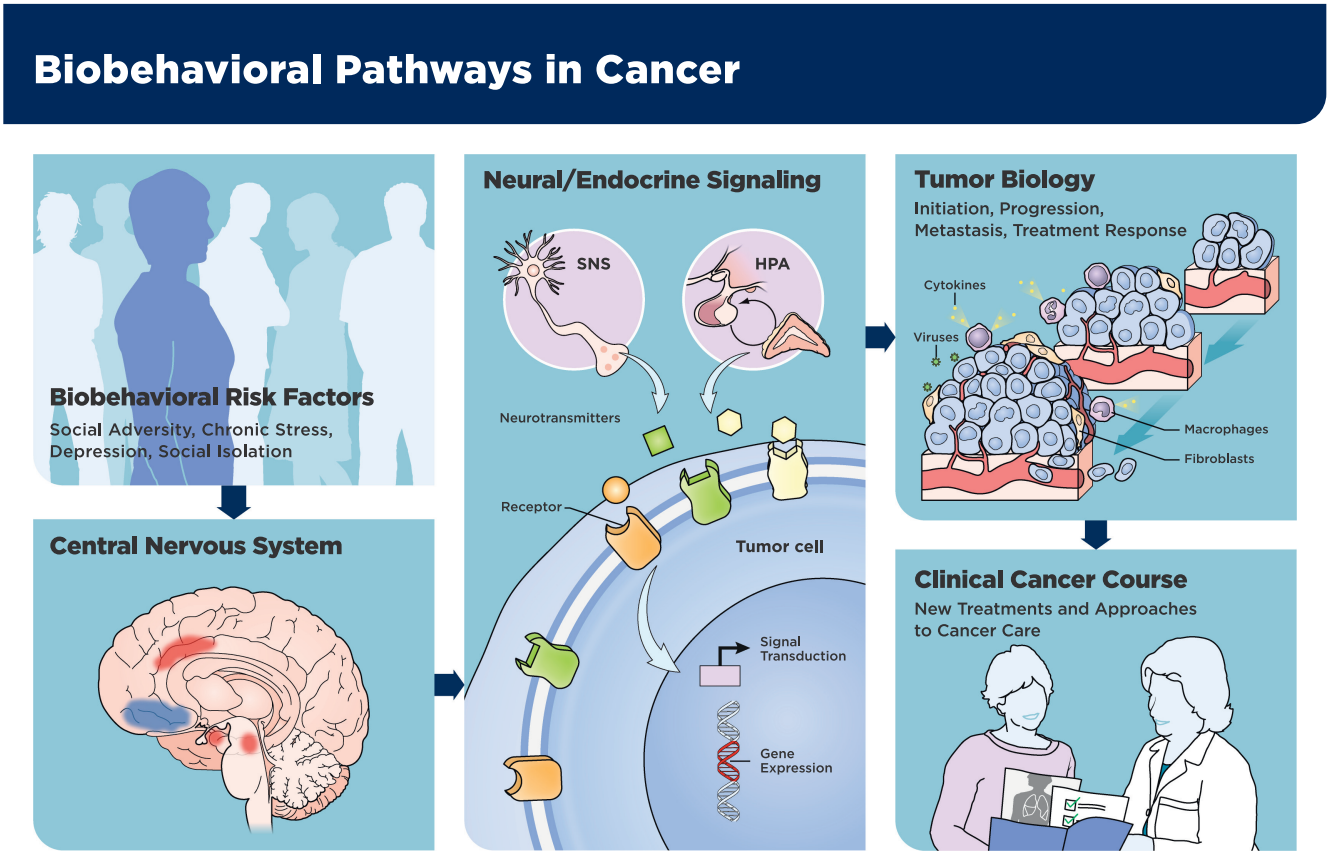

Fig. 1. Heuristic framework for research on biobehavioral risk factor influences on clinical cancer course.

of this emergent demographic had far exceeded the 5-year survival benchmark. Adolescent and young adult (AYA) survivors, diagnosed at ages 15 to 29 years, have an 82% probability of survival 30 years from diagnosis (Mertens et al., 2008). While this statistic is impressive, seminal research by Oeffinger, Lipshultz and others document profound adverse long-term health-related outcomes following exposure to highly aggressive curative intent therapies (Lipshultz et al., 2012; Oeffinger et al., 2006). Most notably relevant to PNI, childhood cancer treatments are associated with late effects on the cardiovascular, central nervous, endocrine, and immune systems. Further, survivors of adult, AYA, and pediatric cancers are at risk for recurrence and subsequent malignancies. Relative to the US population, survivors experience excess morbidity and mortality due to cardiac and vascular abnormalities and pulmonary complications (Choi et al., 2011; Mariotto et al., 2007; Oeffinger and Tonorezos, 2011; Siegel et al., 2012a; Valdivieso et al., 2012). This landscape highlights an opportunity to use PNI paradigms to understand cancer from a competing risk perspective in which multiple factors concurrently affect risks for morbidity and mortality (Mell et al., 2010; Schairer et al., 2004). Although not consistently observed (Zucca et al., 2012), age at diagnosis, general life expectancy trends, and long-term physiological sequelae of treatment exposure have converged to increase the prevalence of comorbidity or multimorbidity[4] in a cancer context (Braithwaite et al., 2012; Land et al., 2012; Patnaik et al., 2011; Ritchie et al., 2011; Yood et al., 2012).

[4] Multimorbidity is defined by the simultaneous existence of more than one pathophysiologic condition or clinical entity (p. 371; Ritchie et al., 2011).

6. Biobehavioral risk factors in the context of cancers as chronic diseases

Early prevention, detection, and treatment advances have shifted our conceptualization and management of most cancers from acute to chronic disease models, which are often modulated by psychosocial factors (Karelina and DeVries, 2011; Sullivan et al., 2012; Williams, 2008; Wyman et al., 2012). This paradigm shift further fuels our interest in psychosocial contributions to intra-individual variability in cancer outcomes. Meta-analytic reviews suggest stressful life experiences and depression are associated with poorer survival and higher mortality across a diverse array of cancer types (e.g., breast, lung, head and neck, hepatobiliary, lymphoid, and hematopoietic cancers) (Chida et al., 2008; Pinquart and Duberstein, 2010; Satin et al., 2009). Prospective endorsement of depressive symptoms, and cortisol slope were associated with decreased survival in patients with metastatic renal cell carcinoma (Cohen et al., 2012). Conversely, among women with metastatic breast cancer, a decline in depressive symptoms conferred survival benefit (Giese-Davis et al., 2011). A recent meta-analysis found the influence of social relationships on mortality comparable to risk conferred by tobacco and alcohol use. Further, the social relationship risk for mortality exceeded risks associated with physical activity (or lack thereof) and obesity (Holt-Lunstad et al., 2010). Inflammation often mediates associations between close relationships, depression, and chronic stress, and health (Kiecolt-Glaser et al., 2010). Extending prior cross-sectional findings of social support, depression and inflammatory gene expression associations, ovarian cancer patients with a greater sense of social attachment had a lower likelihood of death (Lutgendorf et al., 2012). Lastly, perceived social isolation or loneliness

predicts morbidity and mortality risk across different age groups (Perissinotto et al., 2012; Udell et al., 2012).

7. Bioecological perspective of cancer and cancer survivorship

These data highlight the potential utility of life course/life span or 'bioecological' perspectives of cancer and cancer survivorship. Most models of mortality and survival rely on tumor characteristics and treatment exposure as prognostic indicators (Merletti et al., 2011; Ward et al., 2004; Wei et al., 2010). Tumors develop within microenvironments, yet cancers develop within a person nested within several environmental contexts. Colditz and Wei (2012) assert that traditional projections of cancer mortality fail to account adequately for multilevel interactions and reciprocity among biologic pathways, physical/built environment, and social/behavioral factors (Colditz and Wei, 2012). Models that dynamically capture exposure to multiple risk and protective factors, certain to have pleiotropic effects across and within time and levels of analysis (Evans and Kim 2010), promise to reveal greater understanding of individual- and population-level differences in cancer risk and outcome (Gehlert et al., 2008; Hiatt and Breen, 2008; Warnecke et al., 2008). Inequalities in cancer incidence, mortality, and survival by race/ethnicity and socioeconomic status prevail[5] (Chang et al., 2012; Merletti et al., 2011; Ward et al., 2004). A growing literature defines the biology of [social] disadvantage and early adversity and offers tenable hypotheses and mechanistic pathways as explanations for disparities in health and disease outcomes across the lifespan (Adler and Stewart, 2010; Boyce et al., 2012; Kelly-Irving et al., 2012). We use this platform to encourage deliberate investment in research on biopsychosocial mechanisms associated with persistent disparities in cancer outcome (Parente et al., 2012).

8. Animal models of human disease: powerful tools for elucidation of mechanisms

Use of correlation studies to support 'weight of the evidence' has been a prevalent criticism levied against PNI studies of cancer. However, within the last decade, growing availability of transgenic and knockout mouse models of human cancer provides opportunities to understand how PNI-type interactions may modulate the molecular biology of cancer. Orthotopic and human tumor xenograft models more accurately recapitulate the dynamics of human cancer *in vivo* (Talmadge et al., 2007). Biologically sophisticated animal models of human cancer provide a context for experimental manipulation of psychosocial factors, such as environmental enrichment (Cao et al., 2010), isolation (Hermes and McClintock, 2008), stress (Sheridan et al., 2004; Thaker et al., 2006), and depression (Lamkin et al., 2011). In addition, animal models advance the discovery of the consequent changes in neuronal structure and function, neuroendocrine and immune activity, and peripheral biology that influence tumor cells and their microenvironment. In this conceptualization, psychosocial factors set the stage for a "macroenvironment" that can shape tumor microenvironments to be more or less favorable to tumor growth. This systems-approach highlights the interactions of networks of pro-tumor and anti-tumor mechanisms, and underscores the multiple processes involved in both biobehavioral contributions to tumor growth, as well as in resistance to tumor growth. Such a broad, integrative approach will be necessary for the next steps in research that target both mechanisms and interventions.

[5] Social ecological theory predominately guides the exploration of the fundamental causes of disparities in health. A thoughtful and balanced reflection of the relative contributions of local, State, and Federal level policies, social and physical environments, and access to and quality of health services is beyond the scope of this commentary and research contained in this volume. For recent reviews and perspectives see; Colditz et al., 2012 and Esnaola and Ford, 2012.

9. Overview of invited reviews and empirical reports in Advances in *Cancer* and *Brain, Behavior, and Immunity*

Scholars in PNI and related disciplines and in cancer research were invited to author the papers contained in this volume. Reflective of the decade that bore witness to the sequencing of the human genome, the Cole review highlights several conceptual and methodological innovations that are transforming our knowledge of neural and endocrine regulation of the cancer genome (Cole, 2013). Sood and colleagues review studies that have converged to refine our understanding of sympathetic nervous system regulation of pathways relevant to cancer growth and progression (Armaiz-Pena et al., 2012). The nuanced and selective influences of neuroendocrine hormones on tumor cells, stromal cells and the metastatic cascade have come into focus and are beginning to reveal new therapeutic opportunities. Volden and Conzen present a complementary review of the influence of glucocorticoid signaling on tumor progression through cell context-specific transcriptional networks (Volden and Conzen, 2012 1045). In the clinical context, disruption of HPA rhythms, as indicated by diurnal cortisol slopes, predicted early metastatic breast cancer mortality (Sephton et al., 2000). Sephton and colleagues, as reported in this volume, replicate those findings in a small sample of lung cancer patients followed for a median of 4 years from date of diagnosis (Sephton et al., 2012). Volden and Conzen foreshadow emerging interest in stress regulation of epithelial cancer biology through metabolic pathways and energy regulators such as insulin, leptin, ghrelin, and adiponectin (Cao and During, 2012; Williams et al., 2009).

Convergence of animal models and human correlative studies led Neeman and Ben-Eliyahu to identify catecholamine and prostaglandin-mediated immunosuppression as a perioperative risk factor for cancer recurrence and metastasis (Neeman and Ben-Eliyahu, 2012). The authors advance a theoretical model that captures the cumulative risk and review mechanistic support for the use of pharmacological blockade of key mediators during the perioperative period. Sheridan and colleagues review the utility of a mouse model of repeated social defeat to elucidate neural-immune mechanisms in cancer (Powell et al., 2012). This review highlights the role of myeloid-derived cells in stress-primed inflammation, in tissue remodeling in non-immune and immune organs, and in support of behavioral states experienced as cancer-associated sickness behaviors (see reviews in this volume by Bower and Lamkin, 2012; Costanzo et al., 2012; Irwin et al., 2012). The empirical paper by Madden et al. examines the impact of social isolation on breast cancer pathogenesis in adult severe combined immunodeficiency mice using a human breast cancer cell line known to express β-ARs (Madden et al., 2012). The results raise implications of mild vs. chronic stress exposure, timing of exposure during the life span of experimental animals, and the need to capture transient shifts in target cell populations. Further, the study supports the importance of myeloid-derived suppressor cells and stress-associated leukocyte recruitment as indicated by changes in macrophage populations in tumor and spleen, similar to that observed with social disruption (SDR) stress paradigms (Engler et al., 2004; Powell et al., 2012).

Bower and Lamkin identify two questions that direct contemporary research on cancer-related fatigue, i.e., what are the neural underpinnings of fatigue that are distinct from depression, and what are the factors that contribute to inflammation and fatigue at different treatment phases (Bower and Lamkin, 2012)? The review calls for established animal models of cancer-related fatigue, attention to signal transduction pathways and downstream mark-

ers of inflammatory activation, prospective longitudinal studies of fatigue to map time course and recovery, and exploration of mechanisms that might explain observations of symptom clusters. The authors suggest early life stress as a plausible risk factor for inflammation that undergirds cancer-related fatigue. The empirical paper by Witek-Jansek et al. in this volume explores whether childhood adversity is associated with vulnerability for intense sustained behavioral symptoms, including fatigue and depressive symptoms, and quality of life and immune dysregulation (Witek Janusek et al., 2012).

Irwin and colleagues describe the common presentation of sleep disturbance and depression in cancer survivors (Irwin et al., 2012). The authors outline a model in which sleep disturbance drives alterations in inflammatory biology, which result in of depressive symptoms and in clinical depression for some. The model acknowledges depression history and other psychosocial, biobehavioral, and medical factors that might act as moderators. The Lutgendorf laboratory contributes an analysis of associations between cortisol, interleukin-6, depression, fatigue, and disability in ovarian cancer patients followed prospectively from pre-surgical baseline to one-year post surgery, and illustrates how chemotherapy acts to normalize these biological markers (Schrepf et al., 2012).

Although challenges exist, the review by Costanzo et al. identifies opportunities to explore clinically significant PNI relationships in a hematopoietic stem cell transplantation context (HSCT) (Costanzo et al., 2012). Improved understanding of the factors that moderate timely immune recovery and optimal immune regulation might confer improved short- and long-term outcomes for HSCT recipients. Noted as challenges for PNI researchers working in a HSCT context are the pace of change and evolution in HSCT medicine and associated technical innovations. The secondary data analysis by McGregor et al. investigating the effect of pre-transplantation distress on white blood cell count among autologous hematopoietic cell transplantation patients, highlights these challenges (McGregor et al., 2012).

Within the last decade, exercise has been established as an effective adjuvant therapy to control adverse consequences associated with cancer treatment. Jones et al. comprehensively reviews extant evidence linking exercise behavior, functional capacity/exercise capacity, disease recurrence, and cancer-specific and all-cause mortality (Betof et al., 2012). Further, the authors outline host and tumor-related mechanisms underlying the exercise/fitness and prognosis relationship and review evidence from pre-clinical animal models of cancer. This exciting work highlights exercise as one critical component of energy balance influences on cancer etiology, progression, and outcome (Hursting et al., 2012).

This volume would not be complete without a balanced synthesis of extant literature on psychological and physiological adaptation and psychosocial interventions following a diagnosis of cancer (Antoni, 2012). Antoni notes the opportunity to consider outcomes beyond survival and disease recurrence, the importance of determining optimal timing of interventions, acknowledgment of cancers as different diseases, and the need to identify individuals at high risk for poor outcomes. He discusses application of microarray and bioinformatic analyses (Cole, 2010; Cole et al., 2005) to demonstrate that an intervention can causally influence inflammatory and metastasis-regulated gene expression in circulating leukocytes from early-stage breast cancer patients (Antoni et al., 2012).

Three empirical papers in this volume focus on cognitive dysfunction due to cancer treatment exposure in breast cancer samples (Ganz et al., 2012; Kesler et al., 2012; McDonald et al., 2012). Ganz et al. conducted an interim cross-sectional analysis of a prospective, longitudinal, observational cohort study to explore associations between proinflammatory cytokines, cerebral functioning, and chemotherapy exposure (Ganz et al., 2012). Similarly, Kesler and colleagues investigated the correlations between hippocampal volume and peripheral cytokine levels in a sample of breast cancer survivors nearly five years post-chemotherapy exposure (Kesler et al., 2012). The Kesler et al. and Ganz et al. papers report associations between tumor necrosis factor-alpha (TNF-α) and memory impairments. McDonald and colleagues replicate and extend prior work by their group and others (Kesler et al., 2011; McDonald et al., 2010). Their current study reports chemotherapy-associated structural brain changes in frontal regions that correspond to concurrent perceptions of compromised executive function (McDonald et al., 2012). We recognize that these studies have limitations such as small samples sizes, discordance between objective cognitive performance and subjective complaints, and, in some cases, lack of pre/post-treatment and/or non-cancer control comparisons. These limitations beg for prospective longitudinal designs that facilitate pooling of data from different research groups, harmonization of measures, and the use of advanced statistical methods and modeling (Nelson and Suls, in press). Nevertheless, research presented by Ganz et al., Kesler et al., and McDonald et al. nicely illustrates the nexus of brain, behavior, and inflammation.

10. Through the looking glass

This supplement synthesizes contemporary understanding of PNI in a cancer context and suggests opportunities for further discovery of mechanisms and development of interventions to improve clinical cancer care. Multiple signaling pathways by which the ''macroenvironment'' can influence the tumor microenvironment are identified, but many unanswered questions remain. For example, numerous effects of catecholaminergic and glucocorticoid signaling on tumor growth and progression have begun to be mapped, but it is likely that there are multiple downstream effects on tumor growth processes, many of which have not been identified (for example, see Zappala et al., 2012). Hanahan and Weinberg (2011), in an update to their classic paper, highlighted 10 hallmarks of cancer that are necessary for tumor growth and progression. These include sustaining proliferative signaling; evading growth suppressors; avoiding immune destruction; enabling replicative immortality; tumor-promoting inflammation; activating invasion and metastasis, inducing angiogenesis; genome instability and mutation; resisting cell death; and deregulating cellular energetics. The work highlighted in this issue describes how the stress response can influence the macroenvironment to support these hallmarks.

In addition to effects on the tumor and microenvironment, there are likely multiple upstream biobehaviorally modulated pathways that may affect tumor growth, which will make productive targets for future investigation. These include the role of the parasympathetic nervous system, of biobehaviorally sensitive neuropeptides and hormones such as oxytocin, prolactin, growth hormone, and prostaglandins, as well as a variety of metabolic mediators (e.g. insulin growth factor-1, leptin, and ghrelin) that are sensitive to biobehavioral pathways. Biobehavioral mediators seldom work alone, and yet mechanistic research has focused on investigation of discrete pathways for the sake of defining mechanisms. However, to understand the relevant mechanisms, it will be important to understand downstream effects of interconnected pathways – e.g., the synergistic effects on tumor dynamics of NE and cortisol in chronic stress. We envision a complex web of systemic pathways that influence tumor growth and development at multiple levels. This critical information will guide understanding of whether therapies can be successful by blocking only adrenergic signaling (as in use of beta-blockers), or whether adrenergic signaling and prostaglandins must be jointly blocked (see Neeman and Ben-Eliyahu, 2012), or whether adrenergic and glucocorticoid

pathways must both be targeted. Understanding whether narrow or broad targeting of therapies is clinically indicated is critical in developing successful pharmacologic approaches. Behavioral interventions tend to be 'broad spectrum"- targeting many overlapping biobehavioral pathways; future research on behavioral interventions may benefit from analysis of which molecular pathways are active.

Future research will also benefit from parsing out effects of different biobehavioral states – e.g. stress, depression, social isolation – to determine if there is one final common pathway, or to what extent there are discrete biological signatures of these different psychological constructs. Molecular signatures of positive constructs also need further investigation. For example, does resilience just mean less sympathetic activation or less hormonal and inflammatory responsivity to stress, or does it mean greater parasympathetic tone, or differential signaling of pathways such as those involving oxytocin or dopamine? Likewise, it is not known whether stress factors act in a relatively linear dose–response fashion or whether there are thresholds for stress/depression/social isolation that determine physiological trajectories that will influence the clinical course of cancer. These kinds of data will help us better understand who will most benefit from behavioral or pharmacological interventions to reduce adrenergic signaling or stress response states - for example, what levels of stress/distress are necessary at the outset for an intervention to make a difference. Moreover, the use of discrete interventions is useful for mechanistic research purposes, but it is possible that multifaceted total lifestyle interventions that address stress factors, as well as nutritional and exercise lifestyle components, will be necessary to profoundly impact cancer growth. To date, research on multimodal interventions remains quite limited.

Additionally, the effects of biobehavioral pathways on recovery from specific cancer treatments such as HSCT, adoptive immunotherapy, surgical recovery, are important frontiers for future work. Understanding tumor and treatment effects on the central nervous system are equally important. As reported by some of the papers in this volume, we are just beginning to understand the relevant biology in post-chemotherapy fatigue and cognitive difficulties – this type of mechanistic understanding is critical before new treatments can be developed and tested.

Future directions also include determination of what are the most important intermediate outcome variables for biobehavioral cancer research. In addition to overall survival and progression-free survival, to what extent are gene signatures, metabolomics, and epigenetic changes important outcomes for this work? The research in this volume points to the dramatic discoveries that have been made in the last decade to define this field. Future research holds promise for discovery of novel biobehavioral signaling pathways that are relevant to cancer and a greater understanding of behavioral, pharmacologic, and complementary interventions that target these mechanisms.

In conclusion, we would be remiss if we did not thank lead authors and their authorship teams for contributing scientific advances relevant to this volume. These individuals and many others have worked quite tirelessly to improve methodological rigor, establish causation as appropriate, collaborate in the spirit of transdisciplinary team science, and move between different research designs to test and confirm experimental and clinical findings. We thank the many scholars who engaged in the peer review process to vet the invited mini-reviews and empirical papers that comprise this supplement. We acknowledge the inspiration and contributions of the National Cancer Institute Network on Biobehavioral Pathways in Cancer[6] and the invaluable support of the National Cancer Institute Division of Cancer Control and Population Sciences. Lastly, we thank the *Brain, Behavior, and Immunity* senior editorial staff for their support of this special issue.

[6] See http://cancercontrol.cancer.gov/brp/bbpsb/ncintwk-biopthwys.html.

Conflict of interest

The authors of this manuscript have nothing to declare.

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Nervous system regulation of the cancer genome

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ABSTRACT

Genomics-based analyses have provided deep insight into the basic biology of cancer and are now clarifying the molecular pathways by which psychological and social factors can regulate tumor cell gene expression and genome evolution. This review summarizes basic and clinical research on neural and endocrine regulation of the cancer genome and its interactions with the surrounding tumor microenvironment, including the specific types of genes subject to neural and endocrine regulation, the signal transduction pathways that mediate such effects, and therapeutic approaches that might be deployed to mitigate their impact. Beta-adrenergic signaling from the sympathetic nervous system has been found to up-regulated a diverse array of genes that contribute to tumor progression and metastasis, whereas glucocorticoid-regulated genes can inhibit DNA repair and promote cancer cell survival and resistance to chemotherapy. Relationships between socio-environmental risk factors, neural and endocrine signaling to the tumor microenvironment, and transcriptional responses by cancer cells and surrounding stromal cells are providing new mechanistic insights into the social epidemiology of cancer, new therapeutic approaches for protecting the health of cancer patients, and new molecular biomarkers for assessing the impact of behavioral and pharmacologic interventions.

1. Introduction

Cancer is fundamentally a disease of dysregulated gene function that originates from structural genomic damage such as chromosomal amplifications, deletions, mutations, and rearrangements. The biological consequences of that structural genomic damage in cancer cells interacts with dysregulated expression of physiologic genes in neighboring healthy "stromal" cells to facilitate the unchecked growth and survival of cancer cells and their metastatic dissemination to distant tissues (Hanahan and Weinberg, 2011; Kinzler and Vogelstein, 1998, 2004). The development of high-throughput molecular technologies for mapping the structure of the human genome (e.g., "next generation" DNA sequencing) and quantifying the expression of all ~21,000 human genes (e.g., RNA sequencing and gene expression microarrays) has revolutionized cancer biology. Molecular genetics now constitutes the primary paradigm through which cancer is understood as a biological phenomenon, and genomics-based analyses are playing an increasingly prominent role in clinical cancer diagnosis and treatment selection (Kim and Paik, 2010), and in mapping the molecular lesions that cause cancer (Tomlins et al., 2005). In this review, we consider another domain in which genomics-based approaches have begun to revolutionize our understanding of cancer – in mapping the biological pathways by which patient-level social and psychological processes can influence the development, progression, and treatment of cancer. The human genome has evolved a broad transcriptional sensitivity to hormones and neurotransmitters that convey information from the social and psychological realm into molecular biological alterations that help the body respond to both current and anticipated homeostatic challenges (Cole, 2009). As pathological derivatives of normal human cells, cancer cells are also sensitive to neural and endocrine regulation, as are the surrounding immune cells, blood vessels, and other stromal cells that interact with tumor cells within the "tumor microenvironment" (Antoni et al., 2006). Thus, the same gene regulatory programs that allow social and psychological processes to modulate healthy human genome function can also modulate the altered cancer genome, and thereby influence the development and progression of neoplastic disease.

2. Social and psychological regulation of the human genome

2.1. Gene programs

The potential for psychosocial regulation of human gene expression first emerged in the context of studies analyzing the

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effect of social stress on viral genomes such as Herpes Simplex viruses (Glaser et al., 1985; Jenkins and Baum, 1995; Kupfer and Summers, 1990; Leib et al., 1991; Padgett et al., 1998; Rasmussen et al., 1957; Schuster et al., 1991), Human Immunodeficiency Virus (HIV-1) (Capitanio et al., 1998; Cole et al., 1996, 1997; Sloan et al., 2007), Epstein–Barr virus (Glaser et al., 1985; Yang et al., 2010), Cytomegalovirus (Glaser et al., 1985; Prosch et al., 2000), and the Kapossi's Sarcoma-Associated Human Herpesvirus 8 (Chang et al., 2005). As obligate parasites of human host cells, viruses have evolved within a micro-environment structured by our own genome. If social factors can regulate the expression of viral genes, it stood to reason that our own complement of ~21,000 genes might also be regulated by social and psychological processes.

Over the past 5 years, a series of genome-wide transcriptional profiling studies has found that extended periods of psychological or social stress are often associated with a specific pattern of differential gene expression in human immune cells. Across several distinct types of adversity such as social isolation (Cole et al., 2007, 2011; Creswell et al., 2012), imminent bereavement (Miller et al., 2008b), low socioeconomic status (SES) (Chen et al., 2009, 2011), early life social deprivation (Miller et al., 2009b), late life social adversity (Cole et al., 2010), traumatic stress (O'Donovan et al., 2011), diagnosis with a life-threatening illness (Antoni et al., 2012; Cohen et al., 2012), and experimentally imposed social threat (Cole et al., 2010; Sloan et al., 2007, 2010), circulating leukocytes show a common pattern of transcriptional alteration involving increased expression of genes involved in inflammation (e.g., *IL1B*, *IL6*, *IL8*, *TNF*) and decreased expression of genes involved in innate antiviral responses (*IFNB*, *IFIs*, *MX*, *OAS*) and antibody production (particularly the IgG1 isotype) (Cole, 2009, 2010; Irwin and Cole, 2011; Miller et al., 2009a). Each type of adversity is also associated with other transcriptional alterations that are relatively unique to that condition. However, this core pattern of pro-inflammatory and anti-antiviral transcriptome shift emerges much more consistently across diverse types of adversity than would be expected by chance, and similar patterns also emerge in response to experimentally imposed adversity in animal models of social instability, low social rank, and social threat or defeat (Cole et al., 2010, 2012; Irwin and Cole, 2011; Tung et al., 2012). With the statistical challenges of multiple hypothesis testing across ~21,000 genes, these studies rarely find identical sets of differentially expressed genes (although all studies apply standard False Discovery Rate analyses to limit the rate of false positive findings). Consistent patterns are most apparent in subsequent bioinformatic analyses extracting common functional themes from the lists of 10s–1000s of differentially expressed genes (e.g., Gene Ontology annotations regarding shared biological functions and analyses of transcription control pathways regulating expression of multiple genes) (Cole, 2010). The recurrence of these core pro-inflammatory/anti-antiviral biological themes across both different adverse environments and different mammalian species suggests that there may exist a conserved transcriptional response to adversity (CTRA) which is triggered whenever individuals experience extended periods of stress, threat, or uncertainty (Antoni et al., 2012; Irwin and Cole, 2011). This general transcriptional program may be expressed somewhat variably at the level of individual gene transcripts depending upon specifics of individual history, genetic background, and particulars of the current environment (Cole, 2010). A key role for psychological experience in triggering the CTRA dynamic is suggested by results from several small randomized controlled experiments showing that stress-reducing interventions can reverse CTRA transcriptional dynamics in human immune cells (Antoni et al., 2012; Black et al., 2012; Creswell et al., 2012).

CTRA transcriptional dynamics appear to represent an evolutionarily adaptive "defensive program" that redeploys transcriptional resources to counter the changing patterns of microbial exposure historically associated with changing life circumstances (e.g., increased risk of wound-related bacterial infection during periods of acute threat vs. increased risk of viral contagion during extended periods of close social contact) (Cole et al., 2011; Irwin and Cole, 2011). Because antiviral and pro-inflammatory gene modules are to some extent mutually exclusive (Amit et al., 2009), the immune system must "choose" which gene module to favor at any given time. The CTRA dynamic suggests that that choice is informed in part by the broader physiological and environmental conditions surrounding the individual (i.e., organism-level adaptive fitness) (Cole et al., 2011; Irwin and Cole, 2011). However, when the CTRA defensive program is chronically stimulated, the resulting pro-inflammatory/anti-antiviral shift in leukocyte transcriptional equilibrium may promote the complex pattern of "modern mortality" diseases involving both up-regulated immune function (e.g., inflammation-related diseases such as heart disease, neurodegenerative diseases, and some types of cancer) and down-regulated immune function (e.g., impaired response to vaccines and viral infections) (Finch, 2007). At the level of gene regulation, the CTRA profile underscores the fact that stress is not broadly immunosuppressive, but instead selectively suppresses some groups of immune response genes (e.g., Type I interferons and some immunoglobulin genes) while simultaneously activating others (e.g., pro-inflammatory cytokines) (Irwin and Cole, 2011).

Adverse social conditions can also regulate gene expression in a wide variety of other tissues besides circulating leukocytes, including the central nervous system (Karelina et al., 2009; Karssen et al., 2007; Weaver et al., 2006) and peripheral lymphoid organs such as the lymph nodes and spleen (Cole et al., 2010; Sloan et al., 2007). Given the much smaller number of social genomics analyses targeting solid tissues, and the relative difficulty in ascertaining the functional significance of specific transcriptional alterations outside the well-charted territories of immune response, it is not yet clear what specific "gene programs" are being activated in these other tissue contexts (e.g., are these tissue "defensive programs" analogous to the leukocyte CTRA, or do they represent some other type of functional adaptation specific to the organ system involved?).

2.2. Signal transduction

The widespread penetrance of social and psychological conditions into gene regulatory dynamics in diverse tissue sites implies that there must exist some specific transcription control pathways that are sensitive to socio-environmental conditions. Pharmacologic and molecular dissection of the leukocyte CTRA dynamics has provided a prototype for mapping such "social signal transduction" pathways (Irwin and Cole, 2011). Biologists have traditionally construed "signal transduction" as the set of events that translates extracellular biochemical signals, such as hormones or neurotransmitters, into changes in gene expression through the activation of protein "transcription factors" which bind to DNA and flag it for transcription into RNA (Fig. 1). "Social signal transduction" extends this analysis to include the upstream neural dynamics that translate social conditions into specific systemically distributed signaling molecules (e.g., glucocorticoids from the hypothalamus–pituitary–adrenal (HPA) axis or catecholamines from the sympathetic nervous system (SNS)), and to include the specific downstream gene modules that are activated by a given transcription factor. For example, when norepinephrine is released from the SNS during fight-or-flight stress responses, cells bearing beta-adrenergic receptors translate that signal into activation of the transcription factor CREB (cyclic 3′–5′ adenosine monophosphate response element-binding protein) (Sanders and Straub, 2002).

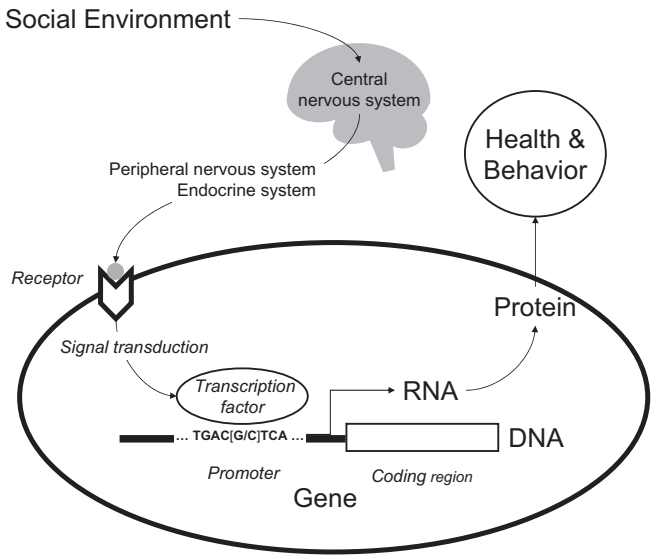

Fig. 1. Social signal transduction. Socio-environmental processes regulate human gene expression by activating central nervous system processes that subsequently influence hormone and neurotransmitter activity in the periphery of the body. Peripheral signaling molecules interact with cellular receptors to activate transcription factors, which bind to characteristic DNA motifs in gene promoters to initiate (or repress) gene expression. Only genes that are transcribed into RNA actually impact health and behavioral phenotypes. Individual differences in promoter DNA sequences (e.g., the [G/C] polymorphism shown here) can affect the binding of transcription factors, and thereby influence genomic sensitivity to socio-environmental conditions.

Activated CREB proteins can up-regulate the transcription of hundreds of cellular genes (Zhang et al., 2005). Which genes can be activated by CREB is determined by the nucleotide sequence of the gene's promoter – the stretch of DNA lying upstream of the coding region of the gene that is transcribed into RNA. For example, CREB binds to the nucleotide motif TGACGTCA, whereas the pro-inflammatory transcription factor NF-κB targets the motif GGGACTTTCC. These two transcription factors are activated by different receptor-mediated signal transduction pathways, providing distinct molecular channels through which extra-cellular signaling molecules, and by extension, their upstream environmental triggers, can regulate intracellular genomic responses. The distribution of transcription factor-binding motifs across our ~21,000 genes' promoters constitutes a "wiring diagram" that maps specific types of environmental processes (e.g., infection vs. a fight-or-flight stress response) onto a specific pattern of genome-wide transcriptional response (e.g., CREB vs. NF-κB target genes). In that sense, each transcription factor can be said to represent some type of evolutionarily significant characteristic of the environment outside the cell (e.g., CREB = "threat or stress", NF-κB = "microbe or damaged cell"), and the distribution of transcription factor-binding motifs across our 21,000 genes can be understood as an evolved "wisdom of the genome" regarding which genes should be activated to optimally adapt to that environment.

Signal transduction research initially analyzed the role of physicochemical or microbial stimuli in activating transcription factors, but studies of social signal transduction have begun to highlight an additional role for subjective psychological perceptions in regulating gene expression (Cole, 2009, 2010; Irwin and Cole, 2011). Several studies suggest that activation of the leukocyte CTRA is more closely linked to subjective perceptions of social threat than to objective characteristics of the social environment (Chen et al., 2009; Cole, 2009; Cole et al., 2007, 2011; Irwin and Cole, 2011; Miller et al., 2008b), and CTRA transcriptome skewing can be reversed by psychological interventions that reduce perceived threat (Antoni et al., 2012) or isolation (Creswell et al., 2012). These subjective psychological experiences may also be transduced into gene expression via different receptor systems than are microbial or chemical stimuli (e.g., through activation of the glucocorticoid receptor (GR) by the HPA-axis or activation of β-adrenergic receptors by the SNS). The extensive cross-talk among post-receptor signal transduction pathways within the cell may also allow stress-responsive mediators such as the SNS and HPA-axis to modulate other signaling pathways that are more classically activated by physicochemical or microbial stimuli. For example, β-adrenergic signaling can up-regulate activity of immune response transcription factors such as NF-κB (Bierhaus et al., 2003) and GATA family factors (Cole et al., 2010), and inhibit activity of Interferon Response Factors (IRFs) (Collado-Hidalgo et al., 2006). In contrast, activation of the GR inhibits all 3 of those pathways (Irwin and Cole, 2011). The contrasting gene regulation programs of the SNS/β-adrenergic and HPA/GR pathways suggests that different types of psychological adversity may elicit different transcriptional responses by triggering different profiles of neural or endocrine responses (Cole, 2010; Frankenhaeuser et al., 1980; Henry, 1992; Lundberg and Frankenhaeuser, 1980).

Identification of the specific transcription factors mediating social and psychological influences on gene expression has been greatly accelerated by bioinformatics analyses that make use of gene promoter sequence data to infer functional activation of transcription factors based on patterns of differential gene expression derived from genome-wide transcriptional profiles (Cole, 2010). This approach scans the promoters of activated genes for transcription factor-binding motifs that are highly over-represented relative to their prevalence across the genome as a whole, and might thus indicate which transcription factor is structuring the observed differences in gene expression (Cole et al., 2005). For example, the genes up-regulated in tissues from people experiencing extended social adversity often bear a high prevalence of CREB target sequences (Cole et al., 2007; Lutgendorf et al., 2009), which is consistent with CREB's role in mediating transcriptional effects of β-adrenergic receptor activation (Sanders and Straub, 2002). In the context of the leukocyte CTRA, promoter bioinformatics have implicated increased NF-κB activity in the up-regulated pro-inflammatory gene component and decreased IRF activity in its anti-antiviral component (Cole, 2009, 2010; Irwin and Cole, 2011). Several studies have also linked chronic stress to reduced (not increased) expression of GR target genes despite the presence of stable or increasing levels of glucocorticoid hormones (Cole et al., 2007; Miller et al., 2008b, 2009b; O'Donovan et al., 2011). That paradoxical effect appears to stem from a stress-induced functional desensitization of the GR, which renders the leukocyte transcriptome partially deaf to glucocorticoid signaling (Cole et al., 2007; Miller et al., 2008b). A similar "glucocorticoid desensitization" dynamic has been observed in mice repeatedly exposed to social threat and appears to be mediated by increased SNS activation of β-adrenergic signaling pathways (Hanke et al., 2012).

2.3. Limitations and prospects

Although much has been learned in the past 5 years regarding how social and psychological processes can potentially regulate human gene expression programs, this literature is still nascent and much remains to be clarified regarding when and how these dynamics actually operate in the context of human health and disease. Most "social genomics" studies have focused primarily on mRNA levels and involve limited, if any, assessment of their downstream impact on protein expression, cellular function, and clinical health outcomes. Human social genomics studies of risk factors such as social isolation, bereavement, PTSD, and low SES generally

involve observational designs subject to potential confounding or reverse causation (Chen et al., 2009; Cohen et al., 2012; Cole, 2008; Lutgendorf et al., 2009; Miller et al., 2008b; O'Donovan et al., 2011; Segman et al., 2005). However, the predicted reversal of CTRA dynamics by positive psychological interventions in randomized experiments (Antoni et al., 2012; Creswell et al., 2012) and the parallel impact of experimentally imposed social adversity in animal models (Cole et al., 2010, 2012; Irwin and Cole, 2011; Tung et al., 2012) both suggest that the human observational associations could potentially reflect, at least in part, causal effects of social conditions on gene expression. The first generation of human social genomics studies also involved relatively small sample sizes (Chen et al., 2009; Cohen et al., 2012; Cole, 2008; Lutgendorf et al., 2009; Miller et al., 2008b; O'Donovan et al., 2011; Segman et al., 2005), as does the currently available stock of human experimental intervention studies (Antoni et al., 2012; Black et al., 2012; Creswell et al., 2012). It is promising, however, that some of those initial observational studies have now been replicated in more robust study samples (Chen et al., 2011; Cole et al., 2011; Miller et al., 2009b), that relatively similar patterns of results provide some measure of conceptual replication across the existing set of small human intervention studies (Antoni et al., 2012; Black et al., 2012; Creswell et al., 2012), and that similar CTRA dynamics are observed in experimental animal models (Cole et al., 2010, 2012; Irwin and Cole, 2011; Tung et al., 2012). It is also important to recognize that, although there are some core similarities in the nature of the gene programs regulated across different types of adversity (i.e., the CTRA), most social environmental risk factors are also associated with a distinctive set of transcriptional alterations not observed in other settings. Those distinctive profiles may be mediated by objective physicochemical, microbial, or behavioral exposures associated with those environments (e.g., social network influences on transmissible disease exposure (Cole et al., 2011), sleep disruption (Irwin et al., 2006), adiposity, physical activity (Zieker et al., 2005), depression/fatigue (Bower et al., 2011; Landmark-Hoyvik et al., 2009; Ohmori et al., 2005), or medication exposures (Felger et al., 2012)). In addition, little is currently known about social and psychological influences on gene expression profiles in neural, reproductive, or other tissue systems in humans or other primates. Much also remains to be learned regarding the kinetics and molecular mechanisms of CTRA dynamics. Leukocyte transcriptome shifts can emerge within the course of one week of overt social conflict (Cole et al., 2010) or a few months of general social instability (Cole et al., 2012), and human intervention studies have shown that reversal of the CTRA pattern can occur within periods as short as 8 weeks (Black et al., 2012; Creswell et al., 2012) and persist for at least a year (Antoni et al., 2012). However, finer-grained time-course studies will be required to more precisely quantify the kinetics of CTRA development, persistence, and reversal. The developmental stage of the individual may also play a major role in determining how deeply and persistently socio-environmental conditions influence gene expression (Chen et al., 2011; Cole et al., 2012; Miller et al., 2009b). Pharmacologic interventions (e.g., the β-adrenergic antagonist Propranolol) can block some pro-inflammatory responses to social adversity in mice (Hanke et al., 2012), but the transcriptome-wide impact and human applicability of these effects remains untested. Despite those limitations, the emerging multi-level analysis of the leukocyte CTRA in terms of psychological processes, neural and endocrine mediators, transcription factor activity, and specific target gene programs has provided a general biological framework for understanding how psychological and social processes might affect gene expression throughout the rest of the body's tissue systems, including potential effects on cancer cells and the tumor microenvironment.

3. Social and psychological regulation of the cancer genome

3.1. Clinical studies of human cancer

Several studies have now linked individual psychosocial conditions to altered patterns of gene expression within tumor tissues and immune cells from human cancer patients. Some of these studies have taken a purely "discovery-based" approach to describe empirically how social risk factors or a biobehavioral intervention modulate gene expression profiles at a global level. Other studies have used gene expression analyses to test specific hypotheses regarding social signal transduction pathways and the role of the immune system in modulating effects. Although the results of these studies are broadly consistent with the possibility that patient-level biobehavioral processes might influence gene expression in human cancer, no study has yet provided a clear causal demonstration of that dynamic.

In a study of 30 low-risk prostate cancer patients undergoing an intensive lifestyle modification program, Ornish and colleagues (Ornish et al., 2008) carried out genome-wide transcriptional profiling of prostate cancer needle biopsies collected at baseline prior to the intervention and again 3 months later. Results showed significant reductions in expression of several metabolic and growth-related gene modules (e.g., RAS family oncogenes and *FLT1*) as well as a reduced expression of the matrix metalloproteinase *MMP9*, which plays a role in tumor metastasis. Although no control group was available in this study, the observed gene expression changes paralleled pre-to-post intervention improvements in psychological function, body mass index, blood pressure, and lipid profiles.

Lutgendorf and colleagues (Lutgendorf et al., 2009) surveyed genome-wide transcriptional profiles in 10 primary ovarian carcinomas and found that tumors from patients with high levels of biobehavioral risk factors (high depressive symptoms and low social support) showed alterations in the expression of 266 genes relative to grade- and stage-matched tumors from low-risk patients. Promoter-based bioinformatics analyses tested the hypothesis that the observed transcriptional differences might be shaped by β-adrenergic signaling (as observed in previous cell culture models and in vivo animal models of ovarian cancer (Landen et al., 2007; Lutgendorf et al., 2003; Nilsson et al., 2007; Sood et al., 2006; Thaker et al., 2006)). Results indicated increased activity of β-adrenoreceptor-regulated transcription factors from the CREB, NF-κB, STAT, and Ets families. Also consistent with potential β-adrenergic regulation was the observation that high biobehavioral risk was associated with elevated intra-tumor concentrations of norepinephrine (Lutgendorf et al., 2009, 2011).

Fagundes and colleagues (Fagundes et al., 2012) carried out targeted profiling of four immune response genes (*CD25*, *CD3E*, *ICAM1*, *CD68*) in 91 basal cell carcinoma biopsies and found reduced average expression of those transcripts in patients who reported both early life emotional maltreatment and a severe life event within the year prior to biopsy. All participants in this study had a previous history of basal cell carcinoma prior to the diagnosis and biopsy of the analyzed tumor specimen, which provided an unusual opportunity to examine tumor-related immune response gene dynamics as they evolved in the aftermath of their primary antigenic challenge.

As part of a larger study on biobehavioral risk factors for disease progression in 217 patients with metastatic renal cell carcinoma, Cohen and colleagues (Cohen et al., 2012) conducted genome-wide transcriptional profiling of peripheral blood leukocytes from 31 patients to determine whether depressive symptoms were associated with increased inflammatory gene expression. Patients with high levels of depressive symptoms showed both shorter survival times

in the total-sample analysis and up-regulated expression of genes involved in inflammation (including COX2/*PTGS2*, *IL1A*, *IL1B*, *IL6*, *TNF*), oxidative stress (*SOD2*), and immunologic activation (*CD69*, *HLA-DR*, *CD83*) in the gene expression sub-study. Promoter-based bioinformatics analyses implicated increased activity of pro-inflammatory transcription factors (NF-κB, STAT1) and transcription factors involved in myeloid cell differentiation and activation (EGR family factors, MEF2, MZF1) in structuring the gene expression alterations associated with high depressive symptoms. Histological analysis of primary tumor tissues confirmed leukocyte gene expression analyses in documenting increased density of tumor-associated macrophages and increased expression of pro-inflammatory and metastasis-related gene products. Together, these results suggest that systemic alterations in inflammatory and immune system homeostasis may mediate the relationship between biobehavioral risk factors and localized disease dynamics within the tumor microenvironment.

To determine whether a cognitive–behavioral stress management (CBSM) intervention might reverse leukocyte pro-inflammatory/CTRA dynamics in early stage breast cancer patients, Antoni and colleagues conducted genome-wide transcriptional profiling of peripheral blood mononuclear cells collected at baseline and at 6- and 12-month follow-ups from 79 stage 0-III patients randomized to a 10-week CBSM or active control condition (Antoni et al., 2012). At baseline, negative affect was associated with up-regulated expression of genes involved in inflammation (including COX2/*PTGS2*, *IL1A*, *IL1B*, *IL6*, *TNF*), oxidative stress (*SOD2*), and metastasis (*MMP9*). In analyses of pre-to-post-intervention changes in gene expression, CBSM showed significantly greater down-regulation of pro-inflammatory and metastasis-related genes and significantly greater up-regulation of type I interferon response genes compared to controls. Promoter-based bioinformatic analyses implicated decreased activity of NF-κB and GATA family transcription factors and increased activity of IRF transcription factors and the glucocorticoid receptor as potential mediators of CBSM's effects on gene expression. This randomized intervention study provides the first demonstration that a psychologically-targeted intervention can causally influence gene expression in cancer patients. No measures of tumor tissue gene expression were available in this study, but several of the transcriptome changes observed in circulating leukocytes paralleled those observed within primary tumor tissues in experimental animal models of stress effects on breast cancer (Sloan et al., 2010).

The studies reviewed above show that patient-level psychological, neural, and endocrine processes are associated with differences in tumor-level gene expression. However, the majority of these studies reflect cross-sectional or longitudinal associations, and no experimental data have yet definitively demonstrated that psychological or neural/endocrine dynamics causally influence tumor cell gene expression. Given the potential for tumor-produced inflammatory mediators to influence CNS-related psychological and behavioral parameters (Dantzer et al., 2008), the observed associations could potentially reflect a reverse causal dynamic originating from naturally occurring variations in tumor biology. To help resolve the causal relations and more clearly define their molecular mechanisms, laboratory experimental models of human cancer have provided valuable new insights into the pathways by which biobehavioral processes can regulate gene expression in cancer.

3.2. Experimental laboratory models of human cancer

Many aspects of human cancer can be modeled in xenograft mouse systems, in which human tumor cells are introduced into immunodeficient mice, or in syngeneic tumor models in which mouse cancer cells are introduced into immunocompetent mice. General results from this literature are reviewed elsewhere in this Special Issue (Armaiz-Pena et al., 2012), but some of these findings can be re-summarized specifically through the lens of gene expression. Many studies have shown that chronic stress can increase tumor development and/or disease progression (e.g., metastsis of solid tumors or dissemination of hematopoietic tumors). Pharmacologic and molecular dissection of these models has identified a diverse array of gene modules that appear to play a role in mediating such effects, including glucocorticoid-induced activation of the *SGK1* gene and associated inhibition of the key tumor suppressor p53 (Feng et al., 2012), and β-adrenergic induction of genes involved in macrophage recruitment and inflammation (Sloan et al., 2010), induction of pro-inflammatory cytokine genes such as *IL6* and *IL8* by tumor cells (Cole et al., 2010; Nilsson et al., 2007; Shahzad et al., 2010) and immune cells (Cole et al., 2010), *VEGF*-mediated increases in angiogenesis (Chakroborty et al., 2009; Thaker et al., 2006; Yang et al., 2006), matrix metalloproteinase-related increases in tissue invasion (Landen et al., 2007; Sood et al., 2006; Yang et al., 2006), tumor cell mobilization and motility (Drell et al., 2003; Lang et al., 2004; Palm et al., 2006), *FAK*-mediated resistance to anoikis/apoptosis (Sood et al., 2010), *BAD*-mediated resistance to chemotherapy-induced apoptosis (Sastry et al., 2007), and *RANKL*-mediated modulation of osteoclast function and bone metastasis (Campbell et al., 2012). Other studies have also shown that glucocorticoids can up-regulate a diverse array of genes involved in cell survival and resistance to chemotherapy (Kamradt et al., 2000a; Mikosz et al., 2001; Moran et al., 2000; Pang et al., 2006; Petrella et al., 2006; Wu et al., 2004, 2005), and activate oncogenic viruses such as Epstein–Barr Virus (EBV) (Cacioppo et al., 2002; Glaser et al., 1995; Yang et al., 2010) and Human Papilloma Viruses (Kamradt et al., 2000b; Mittal et al., 1993; Pater et al., 1988), and that β-adrenergic signaling can inhibit p53-mediated DNA repair (Hara et al., 2011), inhibit expression of Type I interferons (Collado-Hidalgo et al., 2006; Sloan et al., 2010) and interleukin 12 (Goldfarb et al., 2011), upregulate the *Her2* signaling pathway implicated in breast cancer (Gu et al., 2009; Shi et al., 2011), stimulate arachadonic acid signaling (Cakir et al., 2002), activate gene expression by tumor-promoting viruses such as HHV-8 (Antoni et al., 2006; Chang et al., 2005), and upregulate the *SNAI2* transcription factor regulating epithelial–mesenchymal transition (S. Cole, S. Lutgendorf, and A. Sood, personal communication). Each of the later molecular dynamics could plausibly mediate biobehavioral influences on tumor progression, but has not yet been shown to do so definitively through direct inhibition of in vivo tumor incidence or progression. However, it is clear that neural and endocrine dynamics can causally influence gene expression in cancer via both direct regulation of the cancer cell transcriptome and regulation of gene expression by other cells present in the tumor microenvironment such as macrophages, lymphocytes, and vascular cells.

Other studies have also documented stress effects on gene expression in tumor tissues without identifying specific neural or endocrine mediators. For example, stress can up-regulate the expression of pro-inflammatory chemokines and basal cell carcinoma development in the skin of UV-irradiated mice (Dhabhar et al., 2010; Parker et al., 2004; Saul et al., 2005) and modulate gene expression and tumor development in breast tissue (Hermes et al., 2009; McClintock et al., 2005; Williams et al., 2009).

Collectively, the data from experimental animal models of cancer suggest that tumor-level gene expression is shaped in significant ways by the broader physiological "macroenvironment" of the host (Antoni et al., 2006). Moreover, stress mediators such as glucocorticoids and catecholamines generally act to facilitate the progression of cancer (albeit with some exceptions (Cao et al., 2010)) because the molecular genetic "defense programs" they activate (e.g., inflammation, angiogenesis, cell survival,

proliferation, and epithelial-mesenchymal transition) are also those that are co-opted by cancer (Hanahan and Weinberg, 2011). As a result, pharmacologic antagonists of biobehavioral signaling pathways, such as the β-adrenergic receptor system, are often effective in blocking the effects of experimental stress on both tumor-level gene expression and macro-level measures of cancer incidence and progression in laboratory models of cancer (Cole and Sood, 2012).

3.3. Implications for cancer treatment

Given the generally salutary effects of non-selective β-adrenergic antagonists in laboratory experimental systems, there is growing interest in the potential translation of such approaches into human clinical oncology (Cole and Sood, 2012). A number of observational studies have found reduced disease progression rates and extended survival times in breast cancer, prostate cancer, liver cancer, and malignant melanoma patients who were incidentally receiving β-adrenergic antagonists at the time of cancer diagnosis (Barron et al., 2011; De Giorgi et al., 2011; Grytli et al., 2012; Lemeshow et al., 2011; Melhem-Bertrandt et al., 2011; Nkontchou et al., 2012; Powe et al., 2010) (though some studies fail to note such effects) (Shah et al., 2011). Epidemiologic analyses show little indication that β-antagonists can protect against the initial development of breast cancer (Bangalore et al., 2011) or most other types of cancer (Grossman et al., 2001), which is consistent with epidemiologic data suggesting that biobehavioral factors likely exert their greatest effects on the progression of incident cancer, rather than on the initial development of tumors (Antoni et al., 2006; Chida et al., 2008). Consistent with that observation, three studies have shown that psychosocial risk factors measured in patients at the time of diagnosis are associated with more adverse gene expression profiles in primary tumor tissues (Lutgendorf et al., 2009) and circulating immune cells (Antoni et al., 2012; Cohen et al., 2012), independently of established clinical pathology parameters such as tumor grade, stage, and other histological characteristics. Given these links between biobehavioral risk factors and adverse gene expression profiles, evidence of reduced disease progression in people receiving β-antagonists, and effects of CBSM in reversing adverse gene expression profiles in immune cells (Antoni et al., 2012) (as well as similar effects of behavioral interventions on leukocyte transcriptomes in non-cancer settings (Black et al., 2012; Creswell et al., 2012)), the time appears ripe for Phase II randomized controlled biomarker trials examining the effects of pharmacologic and/or CBSM interventions on gene expression profiles in tumor tissue and/or immune cells. Results of such studies would provide important proof-of-principle data to help rationalize larger Phase III trials gauging impacts on clinical outcomes (e.g., disease recurrence, survival, etc.), and help optimize interventions to achieve maximal biological impact. Further observational studies are not likely to decisively address such issues due to the confounding of biobehavioral risk factors (e.g., β-blocker indications such as cardiovascular disease, or socio-environmental risk factors such as depressive symptoms or isolation) with cancer-relevant host physiologic processes such as inflammation (Cole and Sood, 2012).

No human studies have examined associations between HPA-axis antagonists (e.g., RU-486) and clinical cancer outcomes or cancer-related gene expression, so the clinical relevance of that biobehavioral pathway remains poorly understood in humans. However, given the key role of endogenous glucocorticoids in inhibiting inflammation (an effect which should be salutary in the context of most cancers) and the key role of pharmacologic glucocorticoids in treating some types of cancer (e.g., hematological malignancies), pharmacologic antagonism of glucocorticoids would be a more challenging concept to advance. At the very least, much more pre-clinical research will be required to understand the causal relationship between HPA-axis function, gene regulation, and tumor biology before HPA-axis-targeted interventions are contemplated in humans.

Beyond direct effects on tumor biology, biobehavioral gene regulation may also contribute to the quality-of-life decrements that constitute some of the most profound burdens of cancer and its treatment (Miller et al., 2008a). Two genome-wide transcriptional profiling studies have implicated dysregulated expression of immune response genes in the development of cancer-related fatigue (Bower et al., 2011; Landmark-Hoyvik et al., 2009), which is one of cancer's most debilitating sequelae. One study identified up-regulated expression of B lymphocyte-related transcripts (Landmark-Hoyvik et al., 2009) and a second identified increased expression of genes regulated by the pro-inflammatory transcription factor NF-κB (Bower et al., 2011). The second study also indicated down-regulated expression of GR target genes, suggesting potential involvement of the leukocyte CTRA and its associated glucocorticoid insensitivity dynamic. Several studies have also linked cancer-related fatigue to promoter polymorphisms that up-regulate expression of the *IL1B*, *IL6*, and *TNF* genes (Saligan and Kim, 2012). In addition to shedding new light on the molecular etiology of cancer-related fatigue, these genomics-based analyses have identified specific transcription control pathways that may serve as targets for therapeutic intervention to improve quality of life in the aftermath of cancer.

3.4. Frontiers in cancer biology

In the decade that has passed since the initial sequencing of the human genome, molecular genomics analyses have vastly expanded our understanding of basic cancer biology and now provide a comprehensive mechanistic framework for mapping how psychological and social factors might influence those dynamics via neural and endocrine control of gene expression. Given recent developments in cancer genomics, we can anticipate several new areas in which our growing map of cancer's "genomic landscape" and the tumor microenvironment will intersect with our burgeoning understanding of social signal transduction in the broader "macroenvironment" of the human body (Antoni et al., 2006).

Perhaps the most significant opportunity lies in more precisely defining the specific molecular mechanisms by which social signal transduction interacts with the tumor genome. Major efforts are now underway to map the structural genomic landscape of cancer, and define the specific patterns of genomic damage (i.e., individual chromosomal deletions, amplifications, translocations, and mutations) the serve as causal drivers of tumor development and progression. As profiles of "usual suspect" genomic alterations become increasingly well-defined for specific types of cancer by The Cancer Genome Atlas and other projects (Cancer Genome Atlas Research Network, 2008; Wood et al., 2007), it will become increasingly apparent how those genomic alterations functionally affect cell growth, survival, metastasis, and other cancer-related biological dynamics. As a result of that convergence in structural and functional genomics, a broad range of new opportunities will arise to mechanistically determine when biobehavioral processes are most influential in the context of cancer (e.g., when their neural and endocrine representations physically interact with or functionally complement the genomic lesions that initiate a tumor) and when those processes may be comparatively unimportant (e.g., when a tumor has developed genomic alterations that mimic or supplant cancer-promoting effects previously supplied by the nervous or endocrine system). This potential can be understood as a Gene x Environment interaction in which social/psychological/neural/endocrine "environments" interact with genetic differences between healthy somatic cells and cancer cells in the same way

that biobehavioral signaling pathways have already been shown to differentially regulate alternative alleles of naturally occurring genes (Caspi and Moffitt, 2006; Cole et al., 2010). These developments open the possibility of extending the "personalized" diagnosis and therapy of cancer into the context of biobehavioral interactions to determine when and for which patients neural/endocrine or behavioral interventions might have the most clinical impact. Viewing biobehavioral influences on cancer genomes as a Gene x Environment interaction also raises the possibility that neural and endocrine dynamics might help shape the evolution of the tumor genome, for example, by selecting for genomic alterations that take advantage of the "ecological niche" supplied by stress biology. Combining our growing molecular portrait of biobehavioral influences and social signal transduction with the explosion of data on tumor genome sequences will revolutionize our ability to precisely specify how psychological and social dynamics influence the molecular pathogenesis of cancer.

Other areas in which genomics-based approaches will revolutionize biobehavioral cancer research include the increasing use of gene expression biomarkers to evaluate the impact of neural/endocrine or behavioral interventions on immune cells (Antoni et al., 2012; Cohen et al., 2012) and tumor tissue (Fagundes et al., 2012; Lutgendorf et al., 2009; Ornish et al., 2008), genomics-driven bioinformatics analyses to identify the basic biological "programs" that are subverted by cancer and modulated by the neural and endocrine systems (e.g., activation of the CTRA in leukocytes and the epithelial-mesenchymal transition in tumor tissues) and map the specific social signal transduction pathways mediating these effects (Cole, 2009; Irwin and Cole, 2011), as well as analysis of the gene expression dynamics in brain that mediate the conversion of socio-environmental stimuli into neural and endocrine influences on the tumor microenvironment (e.g., as in a pioneering analysis of hypothalamic *BDNF* activation dynamics in mouse models of melanoma and colon cancer) (Cao et al., 2010). Also critical will be more extensive studies of gene expression dynamics in tumor tissue itself, both in clinical human cancer samples and in experimental animal models, using in situ molecular mapping and genetics-based imaging strategies to provide both spatial/cellular resolution and longitudinal temporal resolution of the complex network of molecular transactions that develop between tumor cell populations and the cells of their surrounding microenvironments (Lamkin et al., 2012; Sloan et al., 2010). These developments will provide a much more comprehensive, integrative, and dynamic view of the interface between the biobehavioral macroenvironment of the human body and the complex molecular evolutionary dynamics of the tumor microenvironment. To the extent that these analyses can define some of the key regulatory forces that connect those two domains (e.g., particular transcription factors mediating biobehavioral influences), cancer patients may benefit from new therapeutic approaches that harness the "wisdom of the body" in the service of health.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Neuroendocrine influences on cancer progression

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ABSTRACT

During the past decade, new studies have continued to shed light on the role of neuroendocrine regulation of downstream physiological and biological pathways relevant to cancer growth and progression. More specifically, our knowledge of the effects of the sympathetic nervous system (SNS) on cancer biology has been greatly expanded by new data demonstrating how the cellular immune response, inflammatory processes, tumor-associated angiogenesis, and tumor cell invasion and survival converge to promote tumor growth. This review will summarize these studies, while synthesizing clinical, cellular and molecular research that has continued to unearth the biological events mediating the interplay between SNS-related processes and cancer progression.

1. Introduction

Biobehavioral factors, such as chronic stress, have long been thought to affect many health processes, including cancer (Antoni et al., 2006). To better understand the effects of chronic stress on human disease, it is imperative to study the biological mechanisms underlying this process. The past decade has produced a substantial amount of new work that has shed light on the role of neuroendocrine regulation of physiological pathways in cancer growth and progression. While there is mounting evidence suggesting that neuroendocrine stress mediators can enhance cancer progression, clinical data have remained largely inconsistent in their support of a relationship between behavioral risk factors and cancer initiation. Here, we summarize current knowledge about the potential influences of stress-induced neuroendocrine responses on key cellular and molecular processes that lead to cancer progression and highlight recent advances in this field (Fig. 1 and Table 1).

2. Stress response

The stress response is a complex process that is largely mediated by the sympathetic nervous system (SNS) and the hypothalamic pituitary adrenal (HPA) axis (Glaser and Kiecolt-Glaser, 2005). Once these compartments are activated, catecholamines and glucocorticoids are released into the bloodstream or directly by neurons into various organs. Epinephrine (Epi) and norepinephrine (NE) act as hormones and neurotransmitters that are able to control physiological responses while mediating the "fight-or-flight" stress response that is responsible for rapid elevation of blood pressure, increased heart rate and release of glucose from energy stores. Conversely, the third catecholamine, dopamine (DA), serves as a neurotransmitter that primarily functions to mediate the body's ability to sense pleasure and pain. Besides catecholamines, glucocorticoids are also involved in the stress response and can control many normal physiological activities such as circadian rhythms, immune function and restoration of homeostasis (Glaser and Kiecolt-Glaser, 2005). Although stress can be divided into many categories such as physical, mental, emotional, social, or biological, it is mainly grouped into two major types: acute and chronic. In this review, we will focus on chronic stress conditions that keep the body in a constant state of "overdrive", leading to deleterious downstream effects, deregulating the stress response and negatively affecting many organs systems (McEwen, 1998).

It is important to note that during chronic stress, NE and Epi remain elevated, while DA levels are quickly depleted following an initial increase in the acute phase (as reviewed in Moreno-Smith et al., 2010). This shift in catecholamine levels can result in a more conducive microenvironment for tumor growth (Antoni et al., 2006). For example, in the rat, it is well known that SNS-induced catecholamine release can play a role in the regulation of peripheral organs, such as the ovary, where stress hormones affect ovarian hormone production and menstrual cycle (Aguado and Ojeda, 1984; Ben-Jonathan et al., 1984). Interestingly, rat ovaries have significantly higher catecholamine levels than those found in

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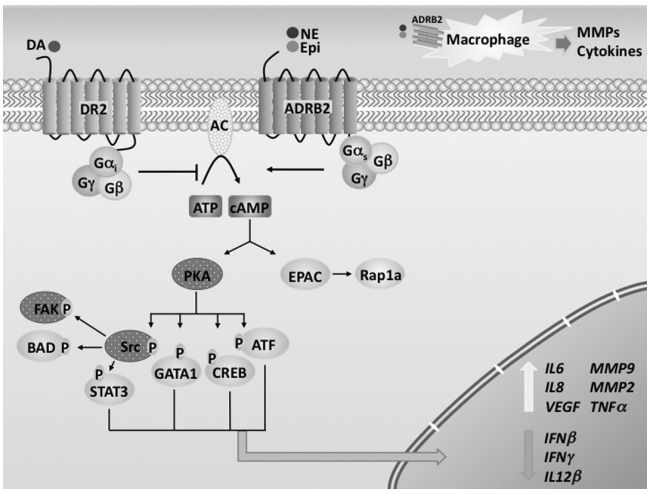

Fig. 1. Overview of catecholamine-mediated signaling pathways in cancer. Norepinephrine (NE) or Epinephrine (Epi) bind to beta adrenergic receptors (ADRB), resulting in increased cyclic AMP (cAMP) levels that induce Protein Kinase A (PKA) activity. PKA can then phosphorylate several proteins, such as CREB/ATF, GATA1 and Src leading to increased activity of several pro-tumoral proteins. Additionally, elevated levels of cAMP also result in the activation of Exchange Protein Activated by cAMP (EPAC) and its associated signaling cascade. Conversely, upon binding to dopamine receptor 2 (DR2), dopamine (DA) can inhibit adenylyl cyclase (AC) function leading to decreased cAMP and downstream pro-tumoral protein levels.

plasma (Ben-Jonathan et al., 1984). Furthermore, rat ovarian catecholamine levels are augmented in response to increased sympathetic activity and this elevation has been associated with the appearance of precystic follicles (Lara et al., 2000). Catecholamine levels are also elevated in the murine bone marrow, as nerve endings and bone marrow cells possess the ability to secrete them into the bone marrow microenvironment (Maestroni, 2000).

3. Effect of chronic stress on animal models of disease

Animal models of human disease are a powerful tool in our effort to understand and study the mechanisms by which biobehavioral factors can promote tumor growth and metastasis. Various approaches have been used to model disease (Frese and Tuveson, 2007). For example, in orthotopic tumor models, human cancer cells are implanted into the organ of origin where they grow and eventually disseminate. Additionally, tumors can be generated by exposure to carcinogens that lead to site-specific disease or they can arise from transgenic or mutant animals carrying insertions, deletions, or mutations in gene sites known to enhance the development of site-specific cancers. Animal tumor models are available for a wide range of cancers, including lung, colon, skin, bladder, mammary, prostate, head and neck, esophagus, ovary, and pancreas. The effect of neuroendocrine factors on cancer have been studied in several of these models by utilizing various types of stressors, including restraint stress, swim stress, surgical stress, social confrontation and hypothermia (Table 1). Restraint stress has been shown to increase norepinephrine, epinephrine and corticosterone levels in various murine-based models (as seen in Thaker et al., 2006), leading to increased inflammatory responses and depressed antiviral cellular immunity (as reviewed in Antoni et al., 2006). Restraint stress has also been consistently associated with elevated interleukin 6 levels (Nilsson et al., 2007). While most of these models have been used in the context of the effects of stress on the immune system, new evidence is suggesting that increased neuroendocrine activity can lead to increased tumor growth by immune independent mechanisms (Armaiz-Pena et al., 2009).

4. Chronic stress and cancer initiation

Several studies have reported that there is no relationship between stressful events (Duijts et al., 2003) or personality (Bleiker et al., 2008) and cancer incidence, while other reports seem to suggest that severe life stressors might induce cancer development (Lillberg et al., 2003; Price et al., 2001). Interestingly, viral infections have been noted to be important co-factors at the initiation stage of several human cancers. Central to this process is the fact that all major human tumor-associated viruses have been found to be responsive to beta-adrenergic receptor (ADRB) or glucocorticoid-dependent signaling cascades. For example, human herpesvirus 8, which induces Kaposi's sarcoma, possesses a cyclic adenosine monophosphate (cAMP) response element in the promoter of an important viral transcription factor (Chang et al., 2005). Additionally, ADRB stimulation of the viral host cell has been shown to increase cAMP response element binding (CREB)-mediated expression of viral oncogenes and growth factors that

Table 1
Representative studies on the effects of stress and stress-associated hormones on cancer.

Experimental condition	Species	Main biological effect	Cancer type	Effect on tumor growth	References
Immobilization stress	Mice	Increased angiogenesis	Ovarian	Increased growth	Thaker et al. (2006)
Enriched environment	Mice	Decreased proliferation	Skin, colon	Decreased tumor growth	Cao et al. (2010)
Restraint stress	Mice	Anchorage-independent survival	Ovarian	Increased growth and metastasis	Sood et al. (2010)
Force swim, surgical stress	Rat	Suppressed NK cell activity	Leukemia, breast	Increased growth	Ben-Eliyahu et al. (1999)
Restraint stress	Mice	Suppressed immune function	Skin	Increased susceptibility to UV-induced disease	Saul et al. (2005)
Restraint stress	Mice	Increased macrophage infiltration	Breast	Increased metastasis	Sloan et al. (2010)
Dopamine administration	Mice, Rat	Decreased angiogenesis	Gastric	Decreased growth	Chakroborty et al. (2004)
Depression	Human	Increased cortisol, IL-6	Ovarian	n/a	Lutgendorf et al. (2008a–c)
Psychological intervention	Human	Increased immune activity	Breast	Improved survival	Andersen et al. (2010)
Social support	Human	Increased immune activity	Ovarian	n/a	Lutgendorf et al. (2005)
Beta-blocker Administration	Human	n/a	Breast	Decreased mortality	Barron et al. (2011)
Stressful life events	Human	n/a	Breast	No association with cancer risk	Duijts et al. (2003)
Personality	Human	n/a	Breast	No association with cancer risk	Bleiker et al. (2008)
Stressful life events	Human	n/a	Breast	Association with cancer risk	Lillberg et al. (2003)
Stressful life events	Human	n/a	Breast	Association with cancer risk	Price et al. (2001)

are known to promote tumor growth, and Epstein-Barr virus and high-risk variants of the human papilloma virus are similarly subject to activation by glucocorticoids (Cacioppo et al., 2002; Glaser et al., 1995). Furthermore, genetic factors have been implicated in stress-mediated cancer initiation. One study identified the genetic status at the interleukin 6 (*IL6)* locus as an important factor in ADRB activation of the transcriptional factor GATA1, potentially explaining how social adversity can affect health risk (Cole et al., 2010; Webster et al., 2002). Even though there are emerging data supporting the role of neuroendocrine factors in cancer initiation, knowledge in this area remains limited and needs further investigation.

5. Effects of chronic stress on the metastatic cascade

The majority of cancer-related deaths are caused by metastases resistant to current treatment regimens. Metastasis is a sequential process in which a cancer cell has to overcome and complete multiple steps in order to colonize distant tissues. For tumors to grow beyond 1 mm, recruitment of new blood vessels has to occur (Fidler, 2003). This new vasculature is needed to deliver the nutrients required to support further growth and to provide a route for the dissemination of tumor cells to distant sites. For this dissemination to occur, the tumor cell must have the capacity to invade through the basement membrane and embolize into the bloodstream. While traveling through the bloodstream, it must then adhere and arrest in capillary beds and extravasate into parenchymal tissues. Once tumor cells have colonized a new site, they must interact with the local microenvironment to grow and develop their own blood supply. Tumor cells must perform these steps while evading immune system surveillance, and the cells that fail in any of these tasks cannot metastasize (Fidler, 2003). New evidence points toward the stress response playing an important role in many of the steps required for cancer metastasis (Armaiz-Pena et al., 2009). In the following subsections, we will summarize the current knowledge regarding these interactions and the underlying mechanisms mediating these effects.

5.1. Tumor growth

Once a tumor is established at the primary site, its growth is determined primarily by nutrient and oxygen diffusion. However, once tumor cells establish a metastatic site, its growth is dependent on the net balance of a multitude of autocrine, paracrine and endocrine signals. Several studies have shown that the effect of stress hormones on cancer cell proliferation varies according to the specific hormone influences and the tumor type studied (as reviewed in Antoni et al., 2006). For example, in a rat model of breast cancer, ADRB activation was associated with increased tumor growth (Marchetti et al., 1991). Downstream mediators of ADRB signaling, such as CREB, have been shown to promote stress hormone-induced tumor cell proliferation, migration and angiogenesis and to inhibit apoptosis in animal models of human disease (Cole and Sood, 2012; Duijts et al., 2003). Conversely, in human melanoma and neuroblastoma cells, catecholamines appear to inhibit tumor cell proliferation, a process that may be mediated by alpha adrenergic receptors (ADRA) (Bleiker et al., 2008; Lutgendorf et al., 2002; Pifl et al., 2001; Scarparo et al., 2004). Additionally, much of the data on the effects of catecholamines on normal cell proliferation indicates that catecholamines are suppressors of cell proliferation, as it has been shown that stress hormones can delay wound healing, mainly by decreasing keratinocyte proliferation (Flaxman and Harper, 1975; Merritt et al., 2008).

It has been shown that NE can inhibit neuroblastoma cell growth, primarily in cells that express the DA transporter (Pifl et al., 2001). Furthermore, in cells that can uptake DA, the proportion of non-dividing cells was significantly increased after treatment with NE. These data point towards a role for DA in inhibiting cell proliferation and counteracting NE-mediated effects. Interestingly, in human prostate carcinoma cells, Epi treatment resulted in epithelial prostate cancer cells acquiring neuroendocrine characteristics (Cox et al., 1999). This process has been associated with poor prognosis in prostate cancer patients. Although these cells have minimal proliferative activity, they provide pro-proliferative paracrine stimuli for nearby cells. Furthermore, it is well known that among epithelial tumors, some decrease in proliferation may be reflective of a more invasive phenotype.

5.2. Angiogenesis

One key to tumoral progression is the development of a new blood supply that can sustain rapid growth and distant metastases. Neovascularization is a multifactorial, complex process that requires the activation of many signaling pathways to induce endothelial cell proliferation and migration. There are several proteins known to induce and promote angiogenesis, including vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), interleukin 8 (IL-8), transforming growth factor alpha and beta (TGF-α and β) and tumor necrosis factor alpha (TNF-α) (Moreno-Smith et al., 2011). Recent studies have examined the relationship between alterations in biobehavioral states and increases in pro-angiogenic factors. Data now demonstrate a clear link between social support and elevated serum VEGF levels in ovarian cancer patients (Lutgendorf et al., 2002). Furthermore, poor social support was associated with higher tumor VEGF levels as well. These results were validated in murine brown adipocytes and human ovarian cancer cells *in vitro* that showed that norepinephrine and the beta agonist isoproterenol were both capable of inducing VEGF expression and that this effect was largely mediated by the ADRB/cAMP/Protein Kinase A (PKA) signaling axis (Fredriksson et al., 2000; Lutgendorf et al., 2003). We have demonstrated that tumor-bearing mice exposed to chronic restraint stress had higher tissue catecholamine levels, as well as greater tumor burden and more invasive disease, than mice not exposed to restraint stress (Thaker et al., 2006). Additionally, it was shown that NE and Isoproterenol increased VEGF levels in mice undergoing chronic stress and that this effect was abrogated by the use of a beta-blocker, which validates the role of ADRB in this setting. Furthermore, restraint stress resulted in increased tumoral microvessel density via the elevated VEGF production (Thaker et al., 2006).

It is well known that IL-6 is a cytokine that plays an important role in tumor progression by inducing angiogenesis, among other functions. Interestingly, ovarian cancer patients who experience distress and poor social support have elevated IL-6 levels, which have been associated with shorter survival in epidemiologic studies (Costanzo et al., 2005; Lutgendorf et al., 2008c). On the other hand, patients with stronger social support had lower IL-6 levels in serum and ascites. We have shown that NE can induce IL-6 production in ovarian cancer cells through a mechanism mediated by the Src kinase (Nilsson et al., 2007).

Other factors are also known to be key mediators in the induction of angiogenesis by chronic stress. Chronic stress can result in the activation of signal transducer and activator of transcription 3 (STAT-3), which increases tumor-associated angiogenesis in an orthotopic model of ovarian cancer (Landen et al., 2007). More specifically, it was demonstrated that NE and Epi can induce STAT-3 activation, leading to its translocation into the nucleus and subsequent binding to DNA to promote transcription of genes associated with angiogenesis, among other processes (Landen et al., 2007).

Additionally, tumor-bearing mice undergoing chronic stress had increased levels of the pro-angiogenic factor, interleukin 8 (IL-8) that were mediated by FosB (Moreno-Smith et al., 2010; Shahzad et al., 2010). Elevated levels of IL-8 were associated with increased microvessel density, all of which were completely abrogated by IL-8 gene silencing (Moreno-Smith et al., 2010; Shahzad et al., 2010).

Alternatively, *in vivo* mouse and *in vitro* studies have shown that DA can abrogate VEGF-induced angiogenesis by reducing the downstream activity mediated by one of its receptors, VEGFR-2 (Basu et al., 2001). Furthermore, studies performed on DA receptor 2 (DR2) knockout mouse models validate these findings as they exhibit more tumor burden, which is associated with increased angiogenesis (Basu et al., 2004). Our group has utilized a mouse model of chronic stress, in which tumoral DA levels are significantly decreased, to show that chronic stress results in increased tumor growth, angiogenesis and metastasis (Moreno-Smith et al., 2011). Recently, we have demonstrated that DA restoration counteracts the effect of chronic stress on tumor growth and angiogenesis through activation of DR2 (Moreno-Smith et al., 2011). Taken together, these studies support a major role for neuroendocrine factors in tumor angiogesis while opening the door for beta-blockers and DR2 receptor antagonists for the abrogation of stress-induced effects on cancer.

5.3. Adhesion

Tumor cell adhesion to the extracellular matrix within the microenvironment is key to the invasion of cancer cells and their spread to distant organs. The extracellular matrix is largely composed of collagen, laminin, fibronectin and other glycoproteins. Tumor cells adhere to these proteins mainly through the interaction of integrins on the tumor cell surface with the extracellular matrix. Even though the mechanisms underlying adhesion to matrix components are not clearly understood, it is well known that cAMP/PKA-mediated pathways are involved in the regulation of guanosine triphosphate (GTP) hydrolysis, Ras homolog gene family member A (RhoA) and Rac, which are important in the cell adhesion process (Mercurio and Rabinovitz, 2001). Recently, it was shown that cAMP, besides targeting PKA, can also signal through exchange protein activated by cAMP (EPAC), a widely expressed exchange factor for the small GTPases Ras-related protein (RAP) 1 and 2 (Mercurio and Rabinovitz, 2001). More importantly, it is well known that EPAC can control a number of cellular processes that were once thought to be exclusively regulated by PKA. For example, cAMP controls cell adhesion through an Epac–Rap1-mediated pathway. Furthermore, it has been demonstrated that isoproterenol can promote ovarian cancer cell movement and adhesion via laminin-5 through an Epac1 dependent pathway (Bos, 2006). Another *in vitro* study reported that isoproterenol can stimulate cell adhesion to fibronectin in a cAMP-mediated Epac–Rap1 pathway (Rangarajan et al., 2003). Taken together, the data demonstrate that stress hormones can promote the attachment of cancer cells to the matrix. Understanding this process is paramount, especially for certain cancers, such as ovarian cancer, in which the cancer cells shed off from the primary tumor and implant at multiple peritoneal sites.

5.4. Invasion

As part of the metastatic cascade, cancer cells have to separate from the primary tumor site, move through the basement membrane, survive the journey through the blood supply, and invade distant organs. Stress hormones can increase the invasiveness of cancer cells, mainly by increasing matrix metalloproteinase protein production and acting as chemoattractants to induce cell migration (Armaiz-Pena et al., 2009). Several *in vitro* and *in vivo* studies have demonstrated that NE can increase the migratory and metastatic potential of several types of cancers by inducing the activation of ADRB-mediated signaling cascades (Voss and Entschladen, 2010). More specifically, we have shown that NE can increase the *in vitro* invasive potential of human ovarian cancer cells and that a beta blocker completely abrogated this effect (Sood et al., 2006). Additionally, several *in vivo* and *in vitro* studies have shown that stress hormones can significantly increase the production of matrix metalloproteinase (MMP) 2 and 9 by ovarian cancer cells through ADRB-mediated pathways (Sood et al., 2006; Thaker et al., 2006). This effect was recapitulated by the use of isoproterenol, which significantly increased tumor cell infiltration into normal tissue, while beta-blockers abrogated this effect. Experiments using human head and neck cancer cells have shown similar results, demonstrating that MMP levels were substantially elevated in response to NE treatment in these cell lines (Yang et al., 2006). Additional studies have provided evidence that pro-tumoral macrophages and other stromal cells in the tumor microenvironment are partially responsible for the release of MMPs. Orthotopic mouse models of breast cancer have shown that chronic stress did not induce primary tumor growth, but did lead to a 30-fold increase in the rate of metastasis (Sloan et al., 2010). Subsequently, it was found that these effects were mediated by ADRB signaling and increased infiltration of protumoral macrophages into the primary tumor site. Furthermore, in human studies, our group has shown that ovarian cancer patients with depressive symptoms and high levels of stress had increased MMP9 in tumor associated macrophages and incubation of monocyte derived macrophages with that hydrocortisone or NE resulted in increased levels of MMP9 (Lutgendorf et al., 2008b). These data now support a role for neuroendocrine factors in the potentiation of tumor cell invasiveness.

5.5. Tumor cell survival and anoikis

To complete the metastatic process, a tumor cell has to evade apoptotic signals. There is now evidence that catecholamines may make cancer cells less sensitive to apoptotic factors (Armaiz-Pena et al., 2009). For example, a recent study has shown that Epi can reduce apoptosis sensitivity in breast and prostate tumor cell lines through activation of ADRB2-mediated pathways. More specifically, when cancer cells were exposed to Epi, ADRB2 activation resulted in the induction of PKA signaling pathways, which led to the phosphorylation of B-cell lymphoma-2 (Bcl-2) associated death promoter (BAD) (Sastry et al., 2007). BAD is known to have pro-apoptotic functions in its unphosphorylated form, but once phosphorylated it releases Bcl-2 and B-cell lymphoma-extra large (Bcl-xL), thereby inhibiting the apoptotic process (Sastry et al., 2007). Even though emerging data support the role of stress hormones in avoiding apoptosis, there are still some models in which NE and DA can induce apoptosis, such as neuroblastoma cells, but this effect was not seen in lung cancer cells (as reviewed in Moreno-Smith et al., 2010). Because DA might largely mediate these effects, our group has examined its role in apoptosis and found that DA can induce *in vivo* and *in vitro* ovarian cancer cell apoptosis through dopamine receptor 2 (DR2) (Moreno-Smith et al., 2011). Furthermore, it has been shown that glucocorticoids can work in concert with catecholamines to aid tumor growth (Herr et al., 2003). In lung cancer cells, cortisol treatment increased membrane-bound ADRB, while potentiating the cellular effects of interleukin-1α, interleukin-1β, and tumor necrosis factor-α (Nakane et al., 1990).

Another process affected by catecholamines in the context of cancer biology is avoidance of anoikis. Anoikis is a cellular process by which normal cells undergo apoptosis once they are detached from the extracellular matrix and neighboring cells. We found that

NE and Epi can protect ovarian cancer cells from anoikis by inducing the activation of focal adhesion kinase (FAK) through an ADRB/PKA/Src dependent pathway (Sood et al., 2010). Furthermore, we showed that NE-induced FAK activation was required for chronic stress-induced tumor growth in a murine orthotopic ovarian cancer model. Moreover, in ovarian cancer patients, our data suggested that both depression and tumor norepinephrine levels were associated with increased FAK activation, which was, in turn, associated with decreased survival (Sood et al., 2010). Collectively, these studies suggest that stress hormones can increase tumor cell survival by increasing the ability of cancer cells to avoid apoptotic processes.

6. Cancer-related immunity

Glucocorticoids and catecholamines have a profound effect on the immune response. In the setting of chronic stress, altered release of glucocorticoids and catecholamines will result in suppression of the immune system, enabling tumor cells to avoid elimination (as reviewed in Antoni et al., 2006). More specifically, it has been shown that natural killer cell function is compromised in tumor bearing animals and cancer patients that underwent surgery. (Ben-Eliyahu et al., 1999; Pollock et al., 1991). Animal models of disease have shown that tumor incidence and progression can be exacerbated by chronic stressors, mainly through the reduction of type 1 cytokines and T cell and natural killer cell activity resulting in impaired antigen presentation and higher numbers of regulatory T cells (Webster et al., 2002). For example, mice exposed to ultraviolet radiation and undergoing chronic stress had an increased incidence of squamous cell carcinoma resulting from the reduction of type 1 cytokines and protective T-cell infiltrates (Saul et al., 2005). While the role of the SNS was not investigated, it is conceivable that such dynamics could play a role in the stress effects on skin immune cell infiltration.

In humans, it is known that states such as chronic stress, loneliness and depression can decrease immune function via mechanisms including adrenergic and glucocorticoid mediated pathways (Antoni et al., 2006). Furthermore, breast cancer patients that underwent surgery demonstrated immune alterations such as lowered T cell mediated secretion of T_H1 versus T_H2 cytokines, a poorer T-cell response to mitogen stimulation and reduced natural killer cell activity (Andersen et al., 1998; Blomberg et al., 2009; Thornton et al., 2007). Advanced breast cancer patients diagnosed with clinical depression exhibit decreased cellular immunity (Sephton et al., 2009). Moreover, in ovarian cancer patients, distress at the time of surgery was associated with decreased natural killer cell activity in tumor infiltrating lymphocytes (TIL) and lower T cell secretion of T_H1 and T_H2 cytokines in peripheral blood mononuclear cells and TIL (Lutgendorf et al., 2005). Conversely, higher social support was associated with increased natural killer cell activity (Lutgendorf et al., 2008a). In summary, there is a substantial amount of research related to the effects of chronic activation of the stress response on the immune system that can lead to increased tumor growth. These effects are mainly due to the disruption of the delicate balance among the central nervous system (CNS), endocrine and immune systems.

7. Clinical studies

Human studies investigating the effects of biobehavioral influences on cancer have focused on two distinct strategies: psychosocial interventions and pharmacological approaches. Although there are promising data relating psychosocial interventions to survival (Andersen et al., 2010, 2008), additional work is needed to understand the efficacy of such interventions and the physiological mechanisms mediating such results. These data are discussed in further detail in another article in this issue.

ADRBs are the key components of the stress signaling pathway, and data now suggest that they also play a major role in protumoral signaling pathways (Cole and Sood, 2012). This convergence allows for the use of clinically available beta-blockers as a viable strategy for disrupting the effects of altered biobehavioral states on tumor growth and progression. At the time of this review, most of the data related to clinical efficacy of beta-blockers have been obtained from non-randomized observational studies. However, some studies have started to shed light on the involvement of ADRBs in cancer risk and survival. For example, an epidemiological study found that patients who used beta-blockers had a reduced risk of developing prostate cancer, which suggests that ADRBs play a role in the development of prostate cancer (Perron et al., 2004). A second study found that the use of beta-blockers by patients with cardiovascular disease significantly reduced their risk of cancer (Algazi et al., 2004). Furthermore, recent studies have reported that beta-blocker use can improve the outcomes of breast cancer patient (Barron et al., 2011; Melhem-Bertrandt et al., 2011; Powe and Entschladen, 2011). We recently analyzed the US Food and Drug Administration's Adverse Event Reporting System (262,262 patients) to examine whether the use of beta-blockers by patients affected cancer related mortality. Our analysis revealed that mortality was reduced by an average of 17% across all major cancer types if patients were treated with beta-blockers (Armaiz Pena et al., 2011). Even though these data suggest a potential role for beta-blockers as adjuvant therapy for cancer patients, additional studies are needed to further examine their protective effects.

8. Summary

In summary, there is strong and growing evidence to suggest that altered biobehavioral states can promote tumor growth and progression, whereas data supporting their role in cancer initiation remain weak. In this light, studies such as, a recent report indicating that chronic stress, through ADRB2-mediated signaling, leads to the accumulation of DNA damage in murine and human cell lines (Hara et al., 2011) suggest that new research strategies may shed light on the question of whether and/or under what conditions biobehavioral states, such as depression and chronic stress, might contribute to cancer initiation. Despite the significant progress seen in the past decade linking biobehavioral factors and tumor progression, more research is required to completely understand how stress hormones affect the many steps of the metastatic cascade. Such research efforts may lead to the development of new behavioral and pharmacological alternatives for the treatment of cancer patients. Animal models of disease have shown that beta-blockers can reduce or substantially eliminate many of the deleterious effects of chronic stress related to cancer. Human studies are now starting to support a protective role for beta-blockers in cancer patients. We have also identified other targetable proteins, such as STAT-3, IL-6, VEGF and FAK that are activated in response to SNS activation. Some of these, such as VEGF and FAK, are already being targeted with drugs either approved for use in the clinic or being evaluated in clinical trials. Furthermore, a rationale exists for the use of antidepressants, owing to their known anti-inflammatory effects in the context of several types of cancer (Moreno-Smith et al., 2010). Another interesting approach could be the use of dopamine analogues since dopamine replacement abrogates chronic stress-induced cancer growth (Basu et al., 2004; Moreno-Smith et al., 2011).

It is well known that chronically elevated stress hormone levels, besides affecting tumor cells and their microenvironment, can alter a variety of important physiological processes that may play a role

in determining the efficacy of chemo- or immuno-modulatory therapy. Additionally, we need to better determine the roles of environmental factors and social support in cancer progression. One study demonstrated that in animal models of colon and skin cancer, an enriched environment decreased tumor growth, and increased tumor remission. Furthermore, this study suggests that hypothalamic brain-derived neurotrophic factor can be selectively upregulated by the enriched environment, while proposing that genetic or environmental activation of this brain-derived neurotrophic factor-leptin axis may have therapeutic significance for cancer (Cao et al., 2010).

Cancer therapy is rapidly moving toward individualized regimens based on parameters that are distinct in each patient. Because of the complexity and inherent variability of the human biobehavioral response, it will be crucial to define the behavioral and/or pharmacological interventions that are most likely to benefit individual patients.

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Conflict of Interest

The authors of this manuscript have nothing to declare.

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Invited Minireview

The influence of glucocorticoid signaling on tumor progression

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ABSTRACT

The diagnosis of cancer elicits a broad range of well-characterized stress-related biobehavioral responses. Recent studies also suggest that an individual's neuroendocrine stress response can influence tumor biology. One of the major physiological pathways altered by the response to unrelenting social stressors is the hypothalamic–pituitary–adrenal or HPA axis. Initially following acute stress exposure, an increased glucocorticoid response is observed; eventually, chronic stress exposure can lead to a blunting of the normal diurnal cortisol pattern. Interestingly, recent evidence also links high primary tumor glucocorticoid receptor expression (and associated increased glucocorticoid-mediated gene expression) to more rapid estrogen-independent breast cancer progression. Furthermore, animal models of human breast cancer suggest that glucocorticoids inhibit tumor cell apoptosis. These findings provide a conceptual basis for understanding the molecular mechanisms underlying the influence of the individual's stress response, and specifically glucocorticoid action, on breast cancer and other solid tumor biology. How this increased glucocorticoid signaling might contribute to cancer progression is the subject of this review.

1. Introduction

The human biobehavioral response to stressors includes physiological changes that are initiated through an individual's interaction with the social environment. In response to these environmental stressors, including social stressors, well-defined physiological changes occur at the organismal level. These changes can be buffered by social support networks, thereby mitigating the deleterious effects of the stress response. However, when life's stressors are unrelenting and social support or other resources (e.g. financial resources) are insufficient, neuroendocrine pathways can become deregulated. It is this deregulation of physiological pathways that underlies the mechanisms whereby psychosocial stressors are hypothesized to influence the biology of chronic disease.

Cardiovascular and immune-related diseases have long served as examples of the stress response-disease relationship (Black and Garbutt, 2002; Stojanovich and Marisavljevich, 2008). More recent studies have begun to explore connections between psychosocial factors and cancer biology. Indeed, recent clinical, epidemiological, and animal-based studies suggest there is a biobehavioral influence on tumor progression (Armaiz-Pena et al., 2009; Costanzo et al., 2011). However, evidence for the impact of psychosocial factors on cancer initiation (rather than progression) has been less consistent (Costanzo et al., 2011). Nevertheless, it is well-established that neuroendocrine hormones (e.g. glucocorticoids and noradrenaline) can and do influence cancer biology (Armaiz-Pena et al., 2009). Thus, the fact that significant stress exposure can lead to deregulation of the neuroendocrine axis has led to further investigation into the effects of psychosocial factors on cancer biology as outlined below.

There are several neuroendocrine cell signaling mechanisms, executed downstream of both the adrenal and sympathetic systems, which could contribute to cancer growth. Recent reviews have outlined some neuroendocrine–cancer relationships and have extensively outlined neuroendocrine influences on the immune system (Armaiz-Pena et al., 2009; Costanzo et al., 2011). The immune system has well-established and important roles in the progression of some cancer types (e.g. melanoma and renal cell carcinoma); however, its role in other cancers is less well understood. The overall impact of the immune system on cancer biology [reviewed in (Grivennikov et al., 2010)] and specifically, the social stress-induced modulation of immunity are reviewed extensively elsewhere (Armaiz-Pena et al., 2009; Costanzo et al., 2011).

This review instead focuses specifically on glucocorticoids (GCs), steroid hormones that are either secreted from the adrenal gland during exposure to acute and chronic stressors or administered pharmacologically to reduce inflammation, and the role of GC signaling in epithelial cancer biology. We discuss potential mechanisms through which endogenous GCs (cortisol in humans and corticosterone in rodents) may influence cancer progression. These data suggest that the routine pharmacological use of

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synthetic GCs in some cancer treatment may not be optimal. Furthermore, we explore how psychosocial mechanisms might intersect with both systemic and tumor microenvironmental GC action to increase tumor progression.

2. Stress signaling: cortisol and the glucocorticoid receptor

2.1. Cortisol

The active GC in humans, cortisol, is produced and secreted by the adrenal cortex. Cortisol release into the circulation has important systemic roles in modulating metabolic and immune processes; cortisol also elicits cell-type-specific effects, some of which are discussed in detail below. Release of cortisol from the adrenal gland is regulated by the hypothalamic–pituitary–adrenal (HPA) axis, a biological circuit capable of integrating human experience with physiological signaling. In the stress response, specific neurons within the hypothalamus secrete corticotrophin-releasing hormone (CRH). In turn, CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary, which subsequently acts on the adrenal cortex to promote cortisol release. A negative feedback loop completes the HPA circuit resulting in cortisol suppressing the production of CRH and ACTH through feedback to the hypothalamus and pituitary. The HPA axis is further linked to the Circadian clock thereby resulting in regulation of GC levels in a diurnal pattern (Chung et al., 2011).

The biological effects of cortisol are in part mediated by the average concentration of circulating cortisol over a certain time period; however, cortisol levels within specific tissues also play an important role in its cell- and tissue-specific effects (Draper and Stewart, 2005). Two isozymes are responsible for regulating local cortisol levels within specific tissues, 11β-Hydroxysteroid dehydrogenase (11β-HSD) type I, which converts inactive cortisone to active cortisol, and 11β-HSD type II, which is responsible for the reverse reaction that inactivates cortisol. Accurately measuring tissue and intracellular concentrations of human cortisol remains challenging. However, transgenic mice with adipose tissue-specific overexpression of the gene encoding 11β-HSDI exhibit increased local corticosterone production and develop the metabolic syndrome, clearly demonstrating that a tissue-specific (rather than systemic) increase in active GCs can dramatically affect whole animal physiology (Masuzaki et al., 2001).

2.2. The glucocorticoid receptor

The most important determinant of cortisol action is its cognate binding protein, the glucocorticoid receptor (GR), which is a member of the nuclear receptor family. The GR's primary action is as a ligand-dependent transcription factor regulating gene expression. Prior to cortisol binding, the GR is cytoplasmic, where it exists in a complex with heat-shock protein 90 (Hsp90) and several immunophilins (Lewis-Tuffin and Cidlowski, 2006). Ligand binding by GC results in dissociation of the GR-Hsp90 complex, GR homodimerization, and nuclear translocation of the dimer (Lewis-Tuffin and Cidlowski, 2006). Within the nucleus, the GR regulates the expression of target genes, either directly through interacting with glucocorticoid response elements (GREs) or indirectly through interacting with other transcription factors that in turn bind to DNA.

Through GR activation, cortisol regulates numerous biological processes including metabolism, behavior, growth and cellular apoptosis. As mentioned previously, the specific response to GR activation is often dependent on the target cell or tissue type. How a single hormone-receptor interaction can result in such divergent effects in different cell types is still under active investigation and is likely to involve multiple mechanisms. For example, the GR exists as multiple transcriptional and translational isoforms (Oakley and Cidlowski, 2011). In addition, isoforms of the GR are each subject to post-translational modifications including ubiquitination, SUMOylation, acetylation, and methylation that have been shown to modulate the stability and/or function of the receptor (Duma et al., 2006; Oakley and Cidlowski, 2011). The GR can also undergo ligand-dependent phosphorylation at several serine residues, events that regulate its transcriptional activity (Duma et al., 2006). Notably, in rats subjected to the chronic stressor of social isolation, phosphorylation of the GR in the brain may regulate GR's transcriptional activity independently of elevated serum corticosterone levels (Adzic et al., 2009). Thus, the diverse effects of glucocorticoids in particular cellular contexts are likely due to the presence and proportion of specific GR isoforms and their post-translational modifications, as well as to the concentration of active GCs (Oakley and Cidlowski, 2011).

3. Pharmacological glucocorticoids and the role of GR activation in cancer

The potential for divergent GR activity in different cell types is striking when comparing GC effects on lymphocytic malignancies versus epithelial cell-derived cancers. In the former case, synthetic GCs, such as dexamethasone (DEX), are routinely used to induce apoptotic cell death in malignant lymphoid cells (e.g. lymphoma). Conversely, in epithelial (i.e. "solid") tumors, several reports suggest that GCs have the opposite effect: GCs stimulate anti-apoptotic gene expression and antagonize the ability of cancer cytotoxics to effectively induce cell death (Zhang et al., 2007). Despite mounting evidence in tumor models suggesting GC-mediated antagonism of therapy-induced tumor cell apoptosis, GCs are routinely administered before, during, and after epithelial cell tumor-chemotherapy to mitigate nausea and allergic reactions. This section will provide a brief historical perspective, focusing on the relatively recent data suggesting GCs antagonize the effectiveness of cancer cytotoxic therapy and will then highlight studies that have begun to identify the molecular mechanisms through which GCs (either endogenous or pharmacologically administered) influence solid tumor biology. Finally, we will discuss data that suggest careful reconsideration of GC use in cancer patients and underscore the potential negative impact of increased endogenous stress-induced GCs on effective cancer treatment.

3.1. Laboratory studies and model systems

One of the earliest reports of the cell survival effect by GCs was observed in immortalized human mammary epithelial cells (Moran et al., 2000). Using serum-free media and specific growth factor supplementation, Moran et al. identified novel antiapoptotic pathways in the breast epithelial MCF10A cell line and its derivative line, MCF10A-Myc. When plated under serum-free conditions, both cell lines displayed high levels of cell death. Upon addition of the GC hydrocortisone, cells were protected from apoptosis independently of activating the antiapoptotic PI3-K and Akt signaling pathways (Moran et al., 2000). In an extension to this study, GR activation in a panel of several breast cancer cell lines protected from serum deprivation-induced apoptosis (Mikosz et al., 2001). GR activation was associated with the rapid induction of the serum and glucocorticoid-regulated kinase-1 (SGK1), a protein kinase encoded by a direct GR target gene. This induction of *SGK1* expression was required for much of the GR-mediated protection from cell death usually induced by serum withdrawal (Mikosz et al., 2001; Wu et al., 2004).

In concurrent studies, another group of investigators attempted to define mechanisms by which paclitaxel (an anti-mitotic chemotherapy) induces cell death and reported an interesting result: GCs also inhibit apoptosis induced by paclitaxel in breast, ovarian and cervical cancer cells (Huang et al., 2000). This observation allowed Huang and colleagues to identify a role for NF-Kappa-B activity in paclitaxel-mediated cytotoxicity, because GCs altered its activity.

For other investigators, however, the observed GC-mediated antagonism of paclitaxel was the first evidence suggesting that synthetic GCs (and potentially high levels of endogenous cortisol) could inhibit the effectiveness of chemotherapy treatment by blocking tumor cell death (Herr et al., 2003; Wu et al., 2004). Wu et al. investigated GC effects on both paclitaxel- and doxorubicin-induced apoptosis in breast cancer cell lines (Wu et al., 2004). Using MCF-7 and MDA-MB-231 breast cancer cell lines, DEX treatment resulted in the inhibition of apoptosis induced by either commonly used breast cancer chemotherapy. As part of this study, microarray analyses were performed to better understand the global GR-mediated gene expression changes associated with cell survival. In addition to *SGK1*, mitogen-activated protein kinase phosphatase-1 (*MKP1*/*DUSP1*) and *IκBα* (encoding a negative regulator of the NFκB transcription factor), were identified among the top genes upregulated by DEX treatment. As had been observed with *SGK1* during serum deprivation (Mikosz et al., 2001), anti-apoptotic effects of DEX in breast cancer cells treated with paclitaxel or doxorubicin required either *MKP1*/*DUSP1* or *SGK1* induction (Wu et al., 2004). Thus, *SGK1* and *MKP1*/*DUSP1* were identified as GR transcriptional target genes whose protein products have important roles in GC-mediated cancer cell survival.

The first *in vivo* report of pharmacologic doses of GCs antagonizing chemotherapy came from Herr and colleagues (Herr et al., 2003). In this study, the effect of DEX on cancer chemotherapy-mediated apoptosis in human lung and cervical carcinoma cells grown in tissue culture and as xenografted tumors was investigated. Compared to animals treated with cisplatin alone, strong antiapoptotic tumor cell effects were observed when DEX was added to the animals' drinking water. Analogous results were observed in cell culture and these effects were associated with the negative regulation of pro-apoptotic genes of the death receptor and the mitochondrial apoptosis pathways. When the apoptosis-initiating caspases, caspase-8 and caspase-9, were introduced into cells, DEX-induced apoptosis resistance was attenuated (Herr et al., 2003). More recently, Zhang et al. reported a remarkably comprehensive analysis of GC effects on the chemotherapeutic cytotoxicity index of a large number of solid tumor cells (Zhang et al., 2007). In this study, cells of human malignant solid tumors derived from surgical specimens or established cell lines were examined in cell culture or as tumor xenografts, using several cytotoxic treatments and several different GCs. GC-induced resistance toward cytotoxic therapy was observed in 89% of over 150 analyzed tumor samples. Chemotherapy resistance was common, irrespective of the specific cytotoxic treatment or GC used, and occurred in association with inhibition of apoptosis, and/or promotion of cell cycle progression (Zhang et al., 2007).

Taken together, these data provide strong evidence suggesting that exogenous GCs and subsequent tumor cell GR activation inhibit cancer cell death pathways to promote cell survival in the setting of cancer therapy. In epithelial cell cancers, GR activation and the ensuing gene induction (*SGK1*, *MKP1*, and *IκBα*) and gene repression (death receptors and mitochondrial apoptosis genes) appear to have principal roles in GC-induced therapy resistance (Wu et al., 2004; Herr et al., 2003). These effects observed in epithelial tumors contrast dramatically with the GC-mediated cell death observed in lymphoid malignancies. Although the mechanisms underlying the divergent actions of GCs on solid vs. lymphoid cancers are not yet known, it is likely that cell context-specific transcriptional networks exist.

3.2. Clinical studies

To date, no prospective analysis of patient samples has assessed the effect of synthetic GCs on the growth or chemotherapy sensitivity of solid tumors; however, several retrospective analyses suggest GC administration induces chemotherapy resistance in cancers of the breast and lung, and enhances the risk of skin cancer and perhaps lymphoma (Herr and Pfitzenmaier, 2006). Additionally, a correlative study evaluated anti-apoptotic gene expression in tumor samples from patients randomized to normal saline or DEX (Melhem et al., 2009). DEX administration to patients was associated with reproducible and rapid up-regulation of known GR target genes (*SGK1* and *MKP1*/*DUSP1*) that are associated with antagonizing chemotherapy-induced ovarian cancer cell apoptosis (Melhem et al., 2009). More recently, in a meta-analysis of primary breast tumor gene expression, high expression of the gene encoding the GR (*NR3C1*) was found to correlate with a significantly shorter relapse-free survival of patients with early stage estrogen receptor negative (ER−) tumors either treated or untreated with adjuvant chemotherapy (Pan et al., 2011). Interestingly, in ER+ breast cancer patients, a high level of primary tumor GR expression was instead associated with a better outcome relative to low GR expression (Pan et al., 2011). GR-mediated upregulation of estrogen sulfotransferase, an enzyme involved in estrogen deactivation (Gong et al., 2008), may help to explain why GR activity has differential effects on ER+ and ER− breast cancer. It is also possible that a more complex crosstalk exists between the GR and ER, although both these hypotheses have yet to be formally tested. In summary, mounting clinical evidence suggests that GR activation by GCs could induce therapy-resistance in specific subsets of solid tumors. Specifically, in breast cancer, GR expression appears to differentially influence patient outcome depending on whether or not ER is expressed. The current data identify a need for well-designed prospective clinical trials to determine the effect of GC administration on solid tumor progression and patient outcome.

4. Endogenous glucocorticoids, stress response, and cancer progression

"Transdisciplinary" approaches apply expertise in two or more disciplines to a complex problem (Gehlert et al., 2010). For example, both psychology and cancer biology disciplines were recently applied to develop animal models that allow for in-depth analyses of stress responsiveness and tumor biology (Williams et al., 2009). This approach is beginning to uncover the molecular underpinnings linking the psychosocial stress response to cancer. For example, female Sprague–Dawley rats have an inherited genetic predisposition for spontaneous mammary tumor formation; McClintock and colleagues reported altered systemic GC regulation following exposure to the chronic stress of social isolation that was associated with a significantly increased mammary tumor burden (Hermes et al., 2009). In a related study, the transgenic SV40 T-antigen mouse mammary tumor model found that social isolation was associated with abnormal GC regulation in response to a superimposed acute stressor and also associated with increased mammary tumor growth (Williams et al., 2009). More recently, a human breast cancer xenograft model demonstrated that chemotherapy efficacy is significantly improved with concurrent transient systemic inhibition of the GR using the GR antagonist mifepristone (Skor and Conzen, unpublished data). In addition to supporting the hypothesis that psychosocial stressors contribute

to increased tumor growth through GR signaling, these *in vivo* studies also suggest that altered endogenous GC activity plays a mechanistic role linking stress to cancer biology. Additional animal models have observed effects of altering the social environment on cancer growth (Cao et al., 2010). Future studies focusing on genetic and pharmacologic manipulation of specific neuroendocrine components will help tease out the most influential neuroendocrine effectors for specific cancers.

In cancer patients, alterations in cortisol regulation have been observed. For example, women with metastatic breast cancer frequently have flatter than normal diurnal cortisol patterns, and the degree of diurnal variation may predict earlier breast cancer mortality (Sephton et al., 2000). While the flattened cortisol levels in metastatic patients might be related to tumor burden, the diagnosis of cancer can also elicit a broad range of patient-specific psychological stress-related responses. For example, a recent study reported that some recently diagnosed breast cancer patients with avoidant coping mechanisms also had flattened diurnal cortisol rhythms (Dedert et al., 2012). Whether altered cortisol regulation plays a direct role in human tumor progression at the cellular level has yet to be determined; however, available laboratory evidence cited above suggesting a role for GR signaling in cancer supports the importance of evaluating the psychological stress response and tumor recurrence.

Based on the hypothesis that an intervention that reduces the stress response might also lower GC levels and slow tumor growth by preventing cancer cell survival pathways, a more recently reported prospective clinical trial evaluated the effect of supportive-expressive group therapy (SEGT) on survival of patients with metastatic breast cancer. Researchers found no effect in patients with ER+ breast cancers, but a positive effect of therapy on reducing tumor progression within the subset of patients with ER− breast cancer (Spiegel et al., 2007). However, another randomized trial of SEGT plus relaxation therapy versus relaxation therapy alone found that patient survival was not prolonged with the addition of SEGT, regardless of tumor ER status (Kissane et al., 2007). More recently, Andersen and colleagues reported a prospective clinical trial in which patients with recently treated early stage breast cancer were randomized to psychological intervention plus assessment or assessment-only cohorts (Andersen et al., 2008). Patients in the intervention arm had a reduced risk of both recurrence and breast cancer-related death. Interestingly, patients with cancer recurrence exhibited higher cortisol levels 17 months prior to relapse compared to patients that remained disease free (Andersen et al., 2008). Clearly, the impact of effective intervention to significantly reduce the psychological stress response in breast cancer patients requires further investigation with careful analysis of breast cancer subtype and diurnal cortisol levels.

5. Cancer-promoting effects of GCs in the tumor microenvironment

GC-mediated mechanisms influencing solid tumor progression are not limited to the effects of GCs directly on tumor cells. Tumor progression involves simultaneous interactions between the cancer cell, the microenvironment that supports the cancer cell's proliferation, the host and the individual's environment (Fig. 1). Neuroendocrine pathways have diverse targets. Indeed, nearly every mammalian tissue is believed to express the GR. Thus, attempting to integrate stress physiology with aspects of cancer progression also requires examining the tumor-microenvironment and an individual's systemic physiology to fully understand how the stress response influences tumor progression.

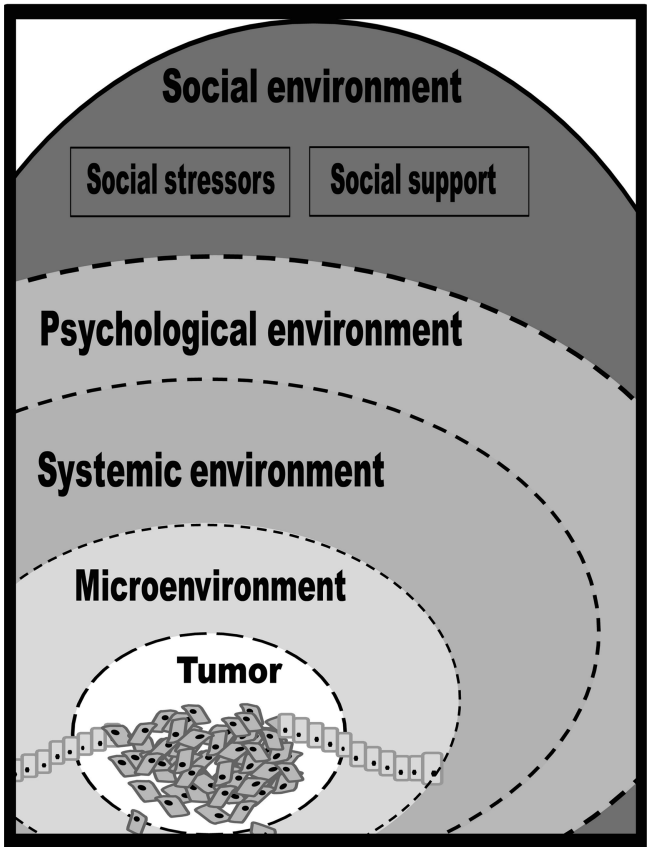

Fig. 1. The multi-layered environments of tumor growth within an individual. The malignant cell environment exists within a nested series of environments capable of varying levels of reciprocal communication. The stressors and support systems of an individual's social environment (dark gray) can interact with an individuals psychological environment (dark pink) to impact the physiology of the systemic environment (pale gray). These physiological changes can promote tumor progression by directly affecting tumor biology or indirectly though the microenvironment (pale pink). Transdisciplinary research considers all five environments through the coordinated experiments of both social and biological scientists using unifying model systems.

5.1. GCs in the tumor microenvironment

Genetic alterations can result in the uncontrolled growth and proliferation of otherwise normal cells. Given the proper time and conditions, cells lacking normal growth and proliferative controls can progress to become cancerous lesions. However, tumor progression is only partially dependent on cell-autonomous programming. Signals extrinsic to the tumor cell, including circulating systemic neuroendocrine and paracrine factors as well as those factors derived from cells in close proximity to the tumor cells (e.g. stromal cells), can influence overall tumor growth dramatically [reviewed in (Liotta and Kohn, 2001)]. Indeed, stromal signals from cells in the tumor microenvironment are increasingly appreciated as significant factors influencing tumor progression. Importantly, the tumor-stroma "crosstalk" is not unidirectional, but instead a dialogue involving enzymes, metabolites, growth factors and other cytokines on both compartments. Notably, both cancer cells and stromal cells can express the GR and are therefore likely influenced by variations in GC signaling.

The GR is expressed to varying degrees in almost every cell in the body. This includes the most commonly studied stromal constituents: fibroblasts, macrophages, adipocytes and immune cells. Developing systems that allow for accurate measurement and identification of relevant tumor-stroma interactions continues to

be challenging. However, gene expression profiling studies have compared tumor-associated stroma to normal stroma and suggest that cancerous cells can dramatically alter stromal mRNA expression (Finak et al., 2008; Smith et al., 2007). Using semi-quantitative PCR, Smith and colleagues analyzed mammary gland stromal mRNA expression of several nuclear receptors, including the GR (Smith et al., 2007). Significant differences in expression between cancer-associated stroma and control tissues were seen for the GR and the progesterone receptor (PR). GR showed increased expression in the stroma from later stage patient samples, whereas PR was decreased in expression compared to control stroma (Smith et al., 2007). Although this study was correlative and did not determine how or if increased GR expression contributed to tumor progression, it does suggest that GC signaling in the tumor microenvironment could be altered by paracrine- or endocrine-associated upregulation of GR expression.

The specific cells of the tumor microenvironment that display altered tumor-promoting signals in response to GCs have yet to be determined. The immune cell-modulating effects of GCs within the cancer microenvironment are one example whereby GCs could affect stromal cell populations. Notably, cancer-associated fibroblasts, which have established roles in cancer growth, invasion and migration, have been reported to have altered GR-mediated transcriptional activity (Hidalgo et al., 2011). Additionally, increased GCs can induce insulin resistance in adipocytes, a major component of the mammary microenvironment. Insulin-resistant adipocytes secrete pro-inflammatory cytokines and growth factors, many of which have been implicated in tumor progression (Park et al., 2011). Adipocytes also express high levels of 11β-HSD1, the enzyme responsible for generating active GC from its inactive precursor, and could influence GR tumor signaling through upregulating local GC levels (Masuzaki et al., 2001). Although indirect (e.g. stroma-mediated) influences by GCs on tumor progression are likely, much work remains to be done to better delineate how the tumor microenvironment is affected by increased GR signaling.

5.2. Systemic cancer-promoting effects of GCs

Systemic GCs can also influence tumor progression indirectly through their effects on the immune system and sytemic metabolism. Because of the role of the stress response in immune-modulation and GC-induced apoptosis of lymphoid-derived tumor cells, much of the biobehavioral research linked to cancer biology has focused on immune system function (Armaiz-Pena et al., 2009; Costanzo et al., 2011). Contrary to GC's role in immune function, GC-mediated metabolic modulation in relation to cancer biology is much less studied. However, strong associations exist between metabolic disorders, such as obesity and the metabolic syndrome, and the increased incidence and natural history of several types of cancer (Kaidar-Person et al., 2011). The underlying mechanisms establishing these links are unclear; however, elevated serum glucose and insulin levels, abnormal lipid profiles, and systemic inflammation are examples of likely mechanisms linking altered metabolism to cancer biology. Indeed, the high-energy demand of rapidly proliferating cancer cells is in line with increased energy substrates and growth factor availability of various metabolic disorders that facilitate pro-tumorigenic effects. Notably, neuroendocrine stress pathways and metabolic pathways are known to have important overlap. Indeed, GCs have potent effects on all of these potential metabolic links to cancer. Elevations in GCs can cause adipocytes, predominantly in visceral fat depots, to favor energy storage (Masuzaki et al., 2001) and accumulation of visceral fat has deleterious effects on systemic metabolism. For example, the best predictor of adverse consequences associated with obesity is the amount of visceral fat rather than total body fat mass (Masuzaki et al., 2001).

The effects of elevated GCs on adipose tissue accumulation also involve interactions within the CNS. Animal models suggest that during stressful experiences increased circulating GCs and insulin increase the drive for calorically dense foods (Dallman et al., 2005). Interestingly, consumption of calorically dense foods seems to attenuate the overall stress response, leading some investigators to conclude that increased consumption of high-calorie "comfort" food is a physiological means to cope with unrelenting stressors (Finger et al., 2011). In man, dietary changes are a common behavior seen in those individuals responding to overwhelming stressors (Wallis and Hetherington, 2009). However, some individuals consume more calories under stress, while others eat less. In those individuals who consume more food, GC action in peripheral tissues (i.e. visceral adipose tissue) and GC action in the CNS may act in a feed forward loop to promote weight gain (Pecoraro et al., 2004). Chronic stressors and the ensuing alterations in endogenous GC secretion are associated with behavioral changes that can disrupt whole-body metabolism. Through exacerbating the onset of obesity, the metabolic syndrome, and the associated hyperglycemia and hyperinsulinemia, increased endogenous GCs, secondary to the biobehavioral stress response, might also provide indirect mechanisms to facilitate cancer growth.

5.3. Closing remarks

Cancer patients diagnosed with solid tumors face emotional and social stressors that can be associated with significantly disrupted endogenous cortisol production. Furthermore, pharmacologic GC therapy is frequently administered to cancer patients to reduce the associated side effects of chemotherapy. The extent to which endogenous and synthetic GCs overlap and influence epithelial cancer biology remains poorly understood; however, recent evidence suggests that both may promote tumor growth. Anti-apoptotic mechanisms in tumor cells may be important determinants of synthetic GC-mediated chemotherapy resistance, although high endogenous GCs also likely initiate increased GR-mediated anti-apoptotic signals in malignant epithelial cells. In addition, even prior to a cancer diagnosis, deregulated endogenous GC signaling that occurs during exposure to unrelenting stressors may have important direct effects on promoting pre-malignant tumor growth and on the tumor microenvironment. Finally, associated metabolic alterations following exposure to unrelenting social stressors could synergize with GC deregulation to generate a pro-tumorigenic host environment.

Much work is still needed to determine the mechanisms through which GCs influence tumor progression in specific tumor cell types. There is also a need for prospective clinical trials to evaluate the effect, if any, of administering synthetic GCs on patient outcome. Exposure to unrelenting stressors and the resulting alteration in GC levels in cancer patients may also contribute to tumor growth, suggesting the importance of identifying such patients for intervention. The recently reported benefits in subsets of cancer patients randomized to psychosocial-intervention suggest that modulating the neuroendocrine stress response deserves further investigation. In addition to psychosocial and behavioral intervention, targeting the neuroendocrine effector pathways in tumors cells (e.g. the use of GR antagonists and GR selective modulators) may benefit those cancer patients with specific tumor cell or tumor microenvironment characteristics make them particularly susceptible to GC signaling effects – for example tumors demonstrating relative overexpression of the GR. In summary, understanding the influence of glucocorticoid signaling in human cancer biology will require coordinated efforts from both the cancer and behavioral researchers whose common goal is to optimize patient care.

Conflict of Interest

Dr. Conzen has a patent application pending proposing the use of glucocorticoid receptor antagonism in breast cancer. There is no financial conflict of interest.

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Surgery and stress promote cancer metastasis: New outlooks on perioperative mediating mechanisms and immune involvement

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ABSTRACT

Surgery for the removal of a primary tumor presents an opportunity to eradicate cancer or arrest its progression, but is also believed to promote the outbreak of pre-existing micrometastases and the initiation of new metastases. These deleterious effects of surgery are mediated through various mechanisms, including psychological and physiological neuroendocrine and paracrine stress responses elicited by surgery. In this review we (i) describe the many risk factors that arise during the perioperative period, acting synergistically to make this short timeframe critical for determining long-term cancer recurrence, (ii) present newly identified potent immunocyte populations that can destroy autologous tumor cells that were traditionally considered immune-resistant, thus invigorating the notion of immune-surveillance against cancer metastasis, (iii) describe *in vivo* evidence in cancer patients that support a role for anti-cancer immunity, (iv) indicate neuroendocrine and paracrine mediating mechanisms of stress- and surgery-induced promotion of cancer progression, focusing on the prominent role of catecholamines and prostaglandins through their impact on anti-cancer immunity, and through direct effects on the malignant tissue and its surrounding, (v) discuss the impact of different anesthetic approaches and other intra-operative procedures on immunity and cancer progression, and (vi) suggest prophylactic measures against the immunosuppressive and cancer promoting effects of surgery.

1. The perioperative period as a critical timeframe for metastatic progression

In cancer patients, surgical removal of the primary tumor is commonly the first and most important step toward abrogating the disease or controlling its progression. While this treatment has been utilized in cancer patients for several millennia (starting with the ancient Egyptians), its shortcomings have become clearer in the last decades. An epidemiological historical study (Demicheli et al., 2001) had compared two databases of breast cancer patients, showing that while untreated patients exhibited only one peak of mortality 3–4 years after diagnosis, operated patients showed an additional distinct peak at 7–8 years after surgery, suggesting that beside its important beneficial outcomes, surgery may indeed have long-term deleterious effects. Given that this notion cannot be directly tested in cancer patients, researchers and clinicians have to rely on animal models and human correlative or indirect findings in determining the potential role of surgery in metastatic progression.

Starting at mid-20th century, using various animal models, researchers have shown that surgery or various stress responses can increase susceptibility to experimental and spontaneous metastases of both solid and hematological tumors (Glasner et al., 2010; Goldfarb et al., 2011; Inbar et al., 2011; Kinsey, 1961). In the following years, animal and human studies have proposed several underlying mechanisms for this phenomenon. First, in humans, it had been repeatedly shown that surgery increases shedding of malignant cells into the blood and lymphatic circulations due to mechanical manipulations of the tumor and its vasculature (Eschwege et al., 1995; Weitz and Herfarth, 2001; Yamaguchi et al., 2000). Second, surgery was shown to increase malignant cell proliferation and resistance to apoptosis: for example, post surgical sera of cancer patients were reported to stimulate *in vitro* tumor proliferation (Kirman et al., 2002). Third, surgery was found to potentiate invasion capacity and motility of free malignant cells by inducing the release of matrix metalloproteinases (MMP) (Kirman et al., 2006), and by enhancing adhesion-molecule expression on tumor cells (Reviewed in (van der Bij et al., 2009). Fourth, factors related to tumor vascularity were also shown to be affected by surgery. Specifically, removal of the primary tumor was reported to cause a drop in levels of tumor-related anti-angiogenic factors (e.g. angiostatin and endostatin) (O'Reilly et al., 1997, 1994), and resulted in increased levels of pro-angiogenic factors (e.g. VEGF) (Svendsen et al., 2002), thus "turning on" the angiogenic switch in latent preexisting micro-metastases. Finally, tissue damage

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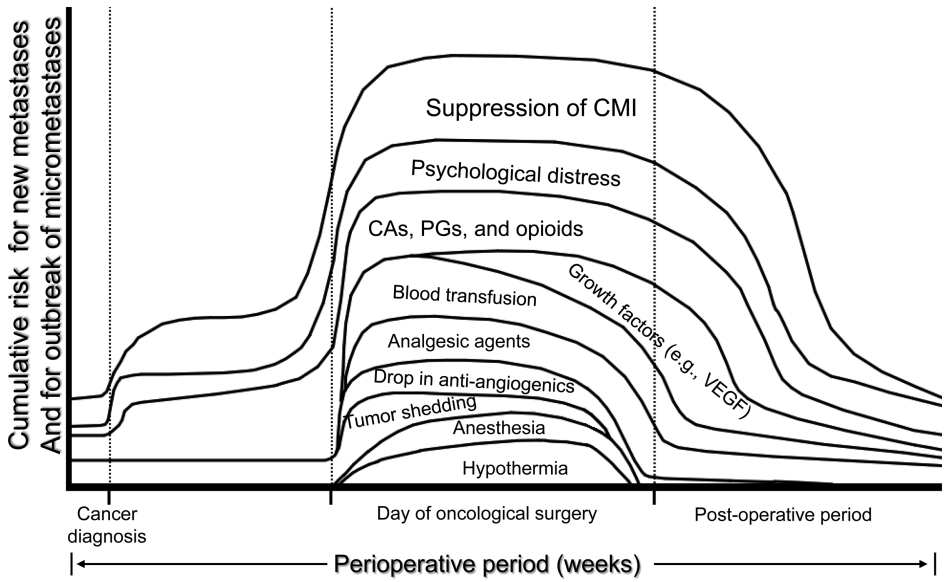

Fig. 1. A schematic representation of the cumulative kinetics of several perioperative risk factors for the initiation of new metastases and the outbreak of preexisting micrometastases in cancer patients (reviewed in Section 1). Each risk factor is represented by a horizontal layer, whose height at different time points along the perioperative period signifies its theoretical contribution to the overall risk. *Not indicated are the direct effects of many of the soluble factors, including catecholamines (CA), prostaglandins (PG), and opiates/opioids on malignant tissue proliferation, invasion capacity, secretion of VEGF, etc, which are reviewed in Sections 3 & 4.

caused by surgery, and specifically the subsequent local pro-inflammatory and wound-healing responses, were shown to increase levels of growth factors (e.g. EGF) (Abramovitch et al., 1999; Pascual et al., 2011), endorsing local and distant recurrence.

Additional aspects inherent to the surgical setting may also play a role in metastatic progression. Anesthetic and analgesic agents, nociception, and pain, were all shown to markedly suppress several aspects of immunity and to promote cancer progression. These effects are discussed below at length. Additionally, perioperative blood transfusions were causally linked, in animals (Atzil et al., 2008) and humans, to greater recurrence rates. Specifically, a recent meta-analysis, combining seven randomized controlled trials (RCTs) in colorectal cancer patients, had re-confirmed this finding and indicated a 42% percent increased risk for recurrence (Amato and Pescatori, 2006). Severe hypothermia was shown in animal studies to increase susceptibility to metastasis (Ben-Eliyahu et al., 1999), although milder hypothermia, which is more common in cancer patients, was not associated with cancer recurrence (Yucel et al., 2005).

An often disregarded additional perioperative risk factor for cancer recurrence is psychological distress: starting with cancer diagnosis, throughout and following surgical and adjuvant treatments, patients experience anxiety, stress, and depression, which translate, among others, to activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis (Seok et al., 2010; Thornton et al., 2010), and the consequent release of stress hormones. Importantly, psychological stress was reported to down-regulate cellular immune indices, including NK and CTL activity, and macrophage motility and phagocytosis (Ben-Eliyahu et al., 2000; Li et al., 2005; Palermo-Neto et al., 2003; Stefanski, 2001). Stress hormones, specifically catecholamines, opioids, and glucocorticoids, were repeatedly shown in animal models to causally promote metastatic progression through various mechanisms, immunological and non-immunological (Benish et al., 2008; Goldfarb et al., 2009; Inbar et al., 2011; Lee et al., 2009; Page et al., 1998; Shahzad et al., 2010; Shakhar and Ben-Eliyahu, 1998; Shavit et al., 2004; Thaker et al., 2006). In fact, it was shown in animals that even a single exposure to stress or stress hormones during a critical period of tumor progression, could increase cancer mortality (Inbar et al., 2011).

Lastly and importantly, it is well acknowledged that surgery itself profoundly suppresses cell-mediated immunity (CMI) (Shakhar and Ben-Eliyahu, 2003). In patients, surgery and its associated neuroendocrine and paracrine responses were shown to increase secretion of immune suppressing hormones (e.g. cortisol), decrease numbers and activity of NK, Th1 and CTL cells, and reduce the pro-CMI type-1 cytokines (e.g. IL-12 and IFN-γ) (Bartal et al., 2010; Greenfeld et al., 2007). These phenomena commence even before surgery, are exacerbated following surgery, and dissipate during the few post-operative days or weeks (Faist et al., 1996; Greenfeld et al., 2007). The role of CMI, and its recently discovered unique lymphocyte populations, in controlling minimal residual disease (MRD), is extensively discussed below, providing the rationale for considering immunosuppression as a significant perioperative risk factor for cancer recurrence.

Taken together, the risk factors described above, which are all common in oncological surgery, occur simultaneously during the short perioperative period. Specifically, shedding of malignant cells, increased tumor-cell proliferation, excess release of pro-angiogenic/pro-invasive factors, accelerated spreading of tumor cells, abundant release of growth factors, psychological distress, and suppression of CMI, may act in synergy to render the patient temporarily vulnerable to metastases which could have been controlled otherwise. Therefore, the short perioperative period seems to have a non-proportionally high impact on long-term recurrence rates (Fig. 1), and thus presents an important and unexplored window of opportunity to improve prognosis.

2. Newly-acknowledged tumor-controlling leukocyte populations, and evidence from cancer patients, invigorate the notion of anti-metastatic immune-surveillance

The ability of the immune system to prevent cancer and control metastasis had been originally hypothesized by Paul Erlich more than a century ago. Fifty years later, Burnet & Thomas have coined the term *immune surveillance* to describe the ability of the immune system, especially CMI, to recognize and destroy transformed cells (Burnet, 1967), and numerous studies in animals have supported this notion. For example, it was repeatedly shown that depletion

of NK cells dramatically increased tumor load and metastatic formation of some syngeneic malignancies, while adoptive transfer of large granular lymphocytes (NK cells) restored normal tumor resistance (Barlozzari et al., 1985, 1983; Shakhar and Ben-Eliyahu, 1998); in mice, anti-IFN-γ treatment, IFN-γ deficiency, or RAG-2 knock-out (preventing T, B, and NK cell-genesis) promoted spontaneous tumor development and metastasis (Smyth et al., 2001). However, animal tumor models, including those based on human malignancies implanted in immune-deficient mice, have been justifiably criticized (Shakhar and Ben-Eliyahu, 2003) for not comprehensively simulating the initiation, immune-editing, and progression of human cancer, and for being based selectively on immune-sensitive tumor lines.

However, new evidence based on studies conducted in cancer patients, have since emerged, and have clearly indicated the role of immune-surveillance in cancer progression. Firstly, numerous immune-escape mechanisms revealed in human malignancies indicate a profound immune-tumor interaction, and tumor destruction and selection by the immune system (reviewed in (Kim et al., 2006)). The prevalence of escape mechanisms are greater in metastatic foci than in the primary tumor, indicating a higher selection pressure during the metastatic process (See (Shakhar and Ben-Eliyahu, 2003)). Secondly, in operated cancer patients, an indication for pre-existing immune-tumor interaction in the form of *in vitro* mixed lymphocyte responses against the excised autologous tumor, was reported to predict long-term survival rates even better than tumor stage and grade (McCoy et al., 2000; Uchida et al., 1990). Third, there is an increased frequency of certain malignancies, and a dramatic increase in metastatic progression in immune-compromised patients, including those receiving immunosuppressant therapy (Detry et al., 2000; Penn, 1993), patients with AIDS (Bernstein and Hamilton, 1993), and patients carrying anti-lymphocyte antibodies (Decaens et al., 2006). Lastly, and despite prior disappointing results, recent advances in immunomodulatory therapy also support the role of immunity in tumor resistance. For example, the newly FDA-approved CTLA-4 receptor blocker, ipilimumab, which enhances T-cell mediated anti-tumor immunity, was recently shown to increase survival time of patients with metastatic or unresectable melanoma, adding to the known benefits of recombinant IL-2 therapy in such patients (Postow et al., 2011). Taken together, these findings unequivocally indicate interactions of immunocytes with autologous malignancies in cancer patients, including cancer cell destruction, and a significant control over the metastatic process.

Still, despite the *in vivo* clinical evidence described above, for many years scientists had failed to directly demonstrate significant *in vitro* immune cytotoxic activity against many autologous tumors, in humans or in animals (Melamed et al., 2005). This apparent contradiction has elicited the hypothesis that yet undiscovered unique leukocyte populations that control MRD do exist *in vivo*. Indeed, in recent years, modern harvesting, phenotyping, and sorting techniques have led to the identification of several new leukocyte populations (some also found in humans), which display a unique ability to lyse "immune-resistant" autologous tumor cells. These populations are described below, and functionally resemble *in vitro* activated lymphocytes (e.g., by various Th1 cytokines), which had long been shown to exhibit superior and distinct tumor-lysing capabilities (Rosenberg and Lotze, 1986).

Marginating pulmonary (MP) leukocytes are defined as white blood cells adhering to the endothelium of the lung vasculature. These cells have been discovered and studied in rats (Melamed et al., 2005), and more recently also in mice (Unpublished data from our lab). When compared with circulating leukocytes, MP leukocytes naturally exhibit a continuous state of activation. Specifically, MP-leukocytes show a twofold higher cytotoxicity against xenogeneic tumor lines, and three- to tenfold increased cytotoxicity against syngeneic allegedly "NK-resistant" tumor lines (Melamed et al., 2010b, 2005; Shakhar et al., 2007). Morphologically, the proportion of large NK cells in the MP compartment is threefold higher than in the blood and spleen (Shakhar et al., 2007), and the MP cellular composition is characterized by a twofold greater proportion of "innate" leukocytes (granulocytes, monocytes, and NK cells) (Melamed et al., 2010b). Finally, MP leukocytes exhibit an increased production of IL-1β, IL-6, IL-10 and TNF-α in response to immunostimulation by poly I:C, CpG, and LPS (Melamed et al., 2010a). Additionally, specific leukocyte subsets within the MP compartment exhibit several characteristics of activation, including (i) significantly higher percentage of intracellular IFN-γ positive NK cells, (ii) elevated CD11b expression on NK cells, granulocytes and monocytes, (iii) elevated CD161 (also known as NKR-P1/NK1.1) on monocytes, (iv) twice as many CD80 positive dendritic cells (DCs), and (v) a significantly lower CD4/CD8 T-cell ratio (Melamed et al., 2010b).

Notably, the MP-population was reported to be very susceptible to immunosuppression following surgery or behavioral stress, or following exposure to corticosterone, catecholamines, or prostaglandins (Ben-Eliyahu et al., 2010; Benish et al., 2008; Inbar et al., 2011; Melamed et al., 2005). However, this population was also found to be highly responsive to *in vivo* immune stimulation with poly I:C (Rosenne et al., 2007; Shakhar et al., 2007) or CpG-C (Goldfarb et al., 2011), which enhanced tumor-lysis by MP leukocytes, and increased lung tumor-resistance.

Liver pit cells are activated hepatic NK cells with a potential wide range of anti-metastatic activity. These cells constitute a relatively rare population (approximately one tenth of Kupffer cells), and inhabit the liver sinusoids, adhering to the endothelial cells. Pit cells were initially described in rats in 1976 by (Wisse et al., 1976), and later also in mice (Luo et al., 2000) and humans (Hata et al., 1990). However, their potential significance to tumor resistance was only lately acknowledged. Pit cells are considered NK cells as they express high levels of NKR-P1, and specific patterns of CD2, CD18, and CD54, which are identical to those of circulating NK cells. Notably, all pit cells are CD8 positive, as opposed to only 40% of blood NK cells, and none of the NKT cells (Luo et al., 2000). Interestingly, and similarly to MP leukocytes, when compared to circulating/spleen NK cells, pit cells demonstrate characteristics of immune activation. These cells exhibit (i) a greater number of intra-cellular granules, (ii) a larger size, (iii) an increased NK activity against xenogeneic cells and syngeneic-NK resistant tumor cells, (iv) an elevated expression of the NK-activation markers gp42, CD25, and ANK44 antigen, and (v) high mRNA expression levels of perforin, granzymes, INF-γ, and tumor necrosis factor (TNF)-α (Luo et al., 2001, 2000). Pit cells are not a homogenous NK population, and can be divided to high-density (HD) and low-density (LD) pit cells, the latter demonstrating an even greater NK cytotoxicity and increased levels of activation-related mRNAs (i.e., perforin, granzymes, INF-γ, and TNF-α). It is believed that pit cells originate as blood NK cells, and when reaching the specific micro-environment of the liver sinusoids differentiate into HD, and later into LD pit cells (Vanderkerken et al., 1993).

We have recently studied the marginating hepatic (MH)-leukocyte population in its entirety, which contains pit cells and other leukocytes. Compared to circulating leukocytes, and much like MP leukocytes (Melamed et al., 2010a), MH-leukocytes exhibited greater cytotoxicity against xenogeneic and syngeneic tumor cells, and also greater levels of mRNA and induced-production of IL-1β, IL-6, IL-10, and TNF-α (manuscript in preparation).

Type 1 NKT cells, also known as invariant or classical NKT cells, are a subset of NKT lymphocytes with anti-tumor capabilities, extensively studied during the recent years (reviewed in (Hegde et al., 2010)). Initially, NKT cells had been defined as T cells

expressing NK markers (CD161 and/or CD56), but functionally this definition was found to be neither inclusive of all NKTs, nor exclusive of other populations. In recent years NKTs have been re-defined as T cells expressing CD1d, a non-classical MHC-I molecule. NKT cells are subdivided into two distinct populations: type1 NKT cells, which express an invariant Vα14 (in mice)- or a Vα24 (in humans)-T cell receptor (TCR), and bind to the α-GalCer glycolipid; and Type2 NKTs (non-classical NKTs) which express a variant TCR, and do not bind to α-GalCer. Type 1 NKT cells in humans typically comprise only 0.01–0.1% of peripheral blood mononuclear cells (PBMCs), ~1% of liver lymphocytes, and ~10% of lymphocytes in the omentum (Berzins et al., 2011). In recent years, type 1 NKT cells had been shown to secrete IFN-γ, promote IL-12 secretion by DCs, promote DC maturation, and, similarly to NK cells, to directly lyse tumor cells via the perforin, FasL, and/or TRAIL pathways (Seino et al., 2006) following recognition of specific glycolipids (Metelitsa et al., 2001, 2003). Interestingly, in numerous studies, defects in type 1 NKT cells were causally linked in mice (and associated in humans) to the promotion of both solid and hematological cancers (Berzins et al., 2011). Several phase-I clinical studies have already began to utilize α-GalCer injections, or adoptive transfer of type 1 NKT cells or of α-GalCer-loaded DCs in cancer patients (Motohashi and Nakayama, 2009).

Dendritic Epidermal T cells (DETC) are skin-specific γδ T-cells which express an invariant canonical Vγ3 Vδ1 TCR (Macleod and Havran, 2011), as well as the NKG2D activating receptor for tumor killing (Ebert et al., 2006). DETCs were discovered and mainly studied in mice, and their presence was also confirmed in humans. Their primary role appears to be in maintaining epidermal homeostasis – balancing keratinocyte proliferation and apoptosis. DETCs were shown to secrete TNF-α, IFN-γ, and CCL1 in response to stimulation, to produce intra-cellular perforin and to exhibit significant cytotoxicity against melanoma cells, similarly to skin NK cells and in contrast to the more abundant skin αβ T cells (Macleod and Havran, 2011).

Killer dendritic cells are a bi-phenotypic population of DCs found in mice, rats, and humans, which can express typical markers of DCs (e.g., MHC-II, CD11c) and NK cells (e.g., NK1.1). These cells have been identified in the spleen, lymph-nodes, thymus, liver, and lungs. Based on their unique traits, these cells were termed NKDCs or, by a different group, interferon-producing killer DCs (IKDC) (Larmonier et al., 2010). This population is unique in its ability to transform, after lysing tumor cells, from a naïve NK-like state (with up-regulated NKG2D, TRAIL, and killing capabilities) to a mature DC-like antigen-presenting cell state (with up-regulated MHC-II and co-stimulatory molecules). The *in vivo* significance of NKDCs in controlling tumor progression has rarely been studied, though they were recently shown to delay the development of the syngeneic B16-melanoma (Larmonier et al., 2010).

Taken together, these unique leukocyte populations (and other yet undiscovered) with anti-tumor capacities can explain the discrepancy between the *in vivo* evidence for anti-metastatic immune-surveillance and the *in vitro* apparent inability to lyse some malignant cells. Importantly, most of these populations inhabit strategic locations, specifically lung and liver capillary vasculature, fostering tight interactions with all circulating aberrant cells, and constituting an important barrier against metastatic dissemination. Overall, the discovery of these populations suggests a greater role than previously assumed for CMI in controlling circulating malignant cells and other aspects of MRD, even though immunity had failed to prevent the development of the primary tumor. It is also noteworthy that the removal of a primary tumor often terminates malignancy-related immunosuppression (Serafini et al., 2006), potentially allowing improved post-operative immune activity against MRD.

3. Catecholamines and prostaglandins are key mediators suppressing anti-metastatic immunity and acting directly on MRD to promote metastatic progression

Despite the removal of the primary tumor, and despite the ability of CMI to restrict or eliminate MRD, many patients exhibit cancer recurrence. Given the above-discussed significance of the perioperative period in determining long-term prognosis, and the marked paracrine, endocrine, and immunological perturbations that occur during this period, it is our hypothesis that certain surgery-related stress responses (i) reduce patient immune resistance to MRD, and (ii) directly facilitate MRD capacity to survive and progress, synergistically increasing the risk of cancer recurrence. While similar hypotheses have been suggested years ago, specific soluble factors and mechanisms have only recently been identified, and include catecholamines, prostaglandins, glucocorticoids, various cytokines, pro-angiogenic factors, and opioids. Indeed, human and animal studies have reported that a variety of physiological and psychological stressors perturb immune indices, including cytokine levels and their induced production, number and distribution of leukocyte subtypes, and cellular and humoral immune functions (Ben-Eliyahu, 2003; Maes et al., 1998; Segerstrom and Miller, 2004; Stefanski, 2001; Viswanathan and Dhabhar, 2005). A substantial amount of research has focused specifically on catecholamines and prostaglandins, which also mediate the secretion of most of the other pro-tumor and anti-CMI compounds described above (Giguere and Labrie, 1983; Glass and Ogawa, 2006; Rettori et al., 2009). Lastly, excess release of catecholamines and prostaglandins can be safely targeted pharmacologically in the perioperative context, and we propose that such an intervention may constitute a novel and easy approach to reduce recurrence rates in oncological patients.

3.1. Prostaglandins - direct effects on malignant tissue and its microenvironment

Ample scientific evidence implicates prostaglandins, especially prostaglandin E_2 (PGE_2), in promoting neoplastic progression. COX-2, a member of the cyclo-oxygenase enzyme family that produces prostaglandins (mostly PGE_2), is usually undetectable in most healthy human tissues (Reader et al., 2011). However, this enzyme is upregulated in many human malignant and pre-malignant tumors (Howe, 2007), especially colorectal and mammary carcinomas (Reader et al., 2011). Transgenic mice over-expressing the PGE_2 receptor, EP1, were reported to be significantly more prone to malignant skin tumors. PGE_2 administration was shown to facilitate macrophage differentiation toward the pro-tumoral M2 phenotype (Sica et al., 2006), contributing to tumor angiogenesis (Brecht et al., 2011). In colorectal cancer patients, tumor COX-2 expression levels (but not COX-1) were associated with tumor size, stage, depth of invasion, lymph node metastasis, blood vessel invasion, recurrence, and overall survival rates (Soumaoro et al., 2004). Blocking the COX-2 pathway in patients or animals was shown to facilitate tumor cell apoptosis (Cao et al., 2000; Roche-Nagle et al., 2004; Sinicrope and Gill, 2004; Zha et al., 2004), to reduce levels of pro-angiogenic agents (Jones et al., 1999; Sinicrope and Gill, 2004; Wei et al., 2004), to decrease tumor microvascular density (Roche-Nagle et al., 2004), and to lower neoplasm vascular invasive capacity by reducing local inflammation and vascular permeability (Condeelis and Pollard, 2006; Goswami et al., 2005).

3.2. Catecholamines - direct effects on malignant tissue and its microenvironment

The following and additional direct effects of catecholamines are comprehensively reviewed elsewhere (Cole and Sood, 2012) (also

see in this volume). Shortly, several lines of evidence demonstrate that activation of tumor β-adrenoceptors can promote malignant progression by facilitating tumor survival, angiogenesis, migration, proliferation, and resistance to anoikis (Antoni et al., 2006; Bernabe et al., 2011; Sood et al., 2010, 2006; Thaker et al., 2006; Wong et al., 2011). A pioneering study (Schuller et al., 1999) had shown that the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) promoted murine pulmonary tumor-cell DNA synthesis and proliferation by stimulating tumor β1 and β2 adrenoceptors. Other studies have shown that norepinephrine enhances *in vitro* production of several metastatic promoting factors, including VEGF, MMP2/9, IL-6, and IL-8, by a variety of human tumor lines (Bernabe et al., 2011; Sood et al., 2006; Thaker et al., 2006; Wong et al., 2011; Yang et al., 2009) – effects that were blocked by the β-antagonist propranolol (Lutgendorf et al., 2003). In mammary tumors, activation of β-adrenoceptors was linked to accelerated tumor growth (Antoni et al., 2006), and in a colon carcinoma cell line, norepinephrine was found to induce *in vitro* locomotion in a β_2-adrenoceptor-dependent manner (Masur et al., 2001). Lastly, a blockade of beta-adrenergic receptors had induced apoptosis of several human and animal carcinoma cell lines (Liao et al., 2010; Zhang et al., 2009).

3.3. Catecholamines and prostaglandins: effects on anti-tumor CMI

In addition to their direct effect on malignant tissue and its micro-environment, catecholamines and prostaglandins have been repeatedly shown to suppress many aspects of CMI *in vitro* (Hellstrand and Hermodsson, 1989; Koren and Leung, 1982), and *ex vivo* (Benish et al., 2008; Inbar et al., 2011; Levi et al., 2011; Shakhar and Ben-Eliyahu, 1998). Most lymphocytes express receptors for catecholamines and prostaglandins (Landmann, 1992; Uotila, 1996), and the intracellular cascades triggered by these substances that lead to immunosuppression have been extensively studied, and are mainly based on the cAMP-PKA pathway (Masera et al., 1989; Torgersen et al., 1997; Whalen and Bankhurst, 1990). Our studies in animals clearly indicate that administration of catecholamines (Ben-Eliyahu et al., 2000; Shakhar and Ben-Eliyahu, 1998) or prostaglandins, at presumably physiological levels, or the endogenous excess release of these compounds by stress or surgery, suppress NK activity *in vivo* (Benish et al., 2008; Melamed et al., 2005; Yakar et al., 2003). Furthermore, we provided causative evidence that this immunosuppression can compromise resistance to experimental metastasis (Shakhar and Ben-Eliyahu, 1998; Yakar et al., 2003). Last, our findings also support a role for this immunosuppression in reducing long-term survival rates in animals undergoing primary tumor excision (Benish et al., 2008; Glasner et al., 2010; Goldfarb et al., 2011; Inbar et al., 2011; Melamed et al., 2003). Catecholamines and prostaglandins are also known to shift the Th1/Th2 balance toward the anti-CMI Th2 dominance (Elenkov and Chrousos, 2002; Kalinski, 2012), and to increase ACTH and glucocorticoid levels(Giguere and Labrie, 1983), potentially suppressing several aspect of CMI through these responses (also see below). Lastly, specific anti-tumor leukocyte populations (described above), including MP-leukocytes (Benish et al., 2008; Melamed et al., 2005), DETCs (Martinet et al., 2009), and type 1 NKTs (Prigione et al., 2009), were all shown to be suppressed by β-adrenergic and/or prostanoid stimulation.

3.4. Synergistic effects of catecholamines and prostaglandins

In addition to the beneficial effects of blocking either catecholamines or prostaglandins on immunity and on resistance to tumor progression, recent studies emphasize the synergistic effects of blocking both factors. For example, only a combined treatment with a β-blocker and a COX-2 inhibitor attenuated the NK suppressive effects of surgery (Benish et al., 2008), and in two models of spontaneous metastasis only the combination of the two blockers, but none alone, improved survival rates following the removal of a primary metastasizing tumor (Benish et al., 2008; Glasner et al., 2010). We ascribe this synergism to the fact that both catecholamines and prostaglandins are elevated during the perioperative period, and that they can each alone cause immunosuppression and/or promote metastasis through non-immunological mechanisms described above. Indeed, both catecholamines and prostaglandins independently activate the same cAMP-PKA intracellular pathways on immune, malignant, and other host relevant cells, eventually promoting metastasis. Thus the blockade of only one receptor system could be ineffective.

3.5. Glucocorticoids: impact on immunity and tumor progression

Traditionally, glucocorticoids were considered as major mediators of the deleterious effects of stress on anti-tumor immunity. Indeed, glucocorticoids are potent *in vitro* suppressors of many aspects of CMI (Ashwell et al., 2000), including NK activity (Cox et al., 1983), and pharmacological doses of glucocorticoids in patients often lead to immunosuppression (Oehling et al., 1997). Like others before us (Tseng et al., 2005), we too observed *in vitro* and some *ex vivo* suppressive effects of exogenous and surgery-induced elevated glucocorticoid levels on NK activity (Shakhar and Blumenfeld, 2003). Nevertheless, our studies in rats have provided evidence that the *in vivo* role of glucocorticoids in the NK-suppressive and tumor-promoting effects of acute stress or surgery is rather limited. Physiologically relevant doses of corticosterone (3–9 mg/kg in rats) did not increase susceptibility to MADB106 metastasis or CRNK-16 leukemia (Inbar et al., 2011; Shakhar and Blumenfeld, 2003), although both models indicated significant impacts of other stress hormones (Inbar et al., 2011; Shakhar and Ben-Eliyahu, 1998). Correspondingly, interventions that did not markedly affect the HPA-axis responses almost completely abolished the *ex vivo* and *in vivo* effects of stress and surgery on NK activity and on tumor resistance (Benish et al., 2008; Glasner et al., 2010). Taken together, we suggest that an acute *in vivo* exposure to physiological high levels of glucocorticoids in rats is not sufficient to suppress levels of NK activity *in vivo*, and some studies in humans had reached a similar conclusion (Bodner et al., 1998). One hypothesis as to the apparent contradiction between the *in vitro* and *in vivo* findings addresses a potential difference in the effective concentrations of glucocorticoids used in the two approaches, and the fact that approximately 95% of glucocorticoids are bound *in vivo* to glucocorticoid binding globulins (CBGs), which further decrease their effective *in vivo* levels (Henley and Lightman, 2011).

On the other hand, it seems that longer *in vivo* exposures to elevated glucocorticoids can decrease Th1 cytokines, and through this mechanism induce a delayed reduction in CMI functioning. For example, we recently found that various prolonged stress paradigms reduced plasma IL-12 levels, beginning 5–10 h after stress initiation, and that this reduction was mediated through the release of adrenal corticosterone and activation of the GR receptors (Shaashua et al., 2011). However, it is worthy to note that *in vivo* high levels of catecholamines and prostaglandins can increase glucocorticoid levels (Giguere and Labrie, 1983; Rettori et al., 2009), and that their blockade perioperatively was shown to reduce delayed surgery-induced elevation in corticosterone levels (i.e., at 12, but not at 2 h post-operatively) (Glasner et al., 2010). Thus, the blockade of prostaglandins and catecholamines may also reduce delayed immunosuppressive effects of glucocorticoids that are secondary to catecholamine and prostaglandin release. Last, employing two models of prolonged stress and comparing the relative contribution of corticosterone to those of catecholamines and prostaglandins in causing *in vivo* suppression of NK activity, we

found that the blockade of corticosterone had a smaller effect than the blockade of catecholamines and prostaglandins. When adding corticosterone blockade to the two other interventions, no improvement was evident (Ben-Eliyahu et al., 2010). Overall, we suggest that elevated levels of glucocorticoids are of minor significance in suppressing NK activity *in vivo* relative to other responses to stress and surgery, and that prophylactic measures should focus on catecholamines and prostaglandins, which can also lead to reduced glucocorticoid levels, and are more feasible for clinical use during the perioperative period.

4. Anesthesia, analgesia, and pain: impact on immunity and tumor progression

Inherent to almost every surgery is the use of various anesthetic and analgesic agents administered through various approaches. It is now becoming clear that some common anesthetic and analgesic approaches are associated with an increase in cancer recurrence rates, as was shown regarding colorectal (Gupta et al., 2011), breast (Exadaktylos et al., 2006), melanoma (Schlagenhauff et al., 2000), ovarian (de Oliveira et al., 2011; Lin et al., 2011), and prostate (Biki et al., 2008) cancer. Generally, most of these studies reported that the common approach of employing general anesthesia combined with an opiate-based analgesia (the "GA approach") was linked to a poorer prognosis compared to various approaches which are exclusively based on, or include, regional or local blockade of nerve conduction (RA). Interestingly, supporting the clinical significance of the perioperative period, is the finding that epidural anesthesia (in addition to GA) in ovarian cancer patients was associated with improved recurrence-free survival, but only when administered intra-operatively, and not post-operatively (de Oliveira et al., 2011). Additionally, and as elaborated below, many of the deleterious effects of various aspects of anesthesia are based on mechanisms described above, including neuroendocrine responses, immunosuppression, and direct effects on the malignant tissue. Notably, a cautionary note is needed - all the above clinical studies are retrospective, and in few studies the adjustment for prognostic factors had eliminated significant differences between the anesthetic approaches (Melchi et al., 1995). In the only prospective study that was conducted, no significant differences were detected, but the study had a markedly limited statistical power (Myles et al., 2011).

The differences between the GA and the RA approaches can hint at specific factor(s) and mediating mechanisms underlying the alleged differences in long-term cancer outcomes. The GA approach commonly involves an induction phase (usually with thiopental or with propofol), and a maintenance phase utilizing a volatile anesthetic (e.g., sevoflurane or halothane), combined with the use of analgesics to relieve intra- and post-surgical pain, which are most commonly opiates. On the other hand, the RA approach employs administration of a local anesthetic (e.g. lidocaine or bupivicaine) in specific anatomic regions and in small quantities, to block peripheral or spinal nerve conduction (e.g., neuroaxial, paravertebral, or epidural block). This approach efficiently prevents nociception and pain, while, unlike the GA approach, also halting ascending neural transmission to CNS nuclei that otherwise may initiate HPA and sympathetic responses.

Animal studies had pointed at all factors differentiating between the two approaches as potential contributors to the poorer prognosis seen in patients subjected to GA. These include the utilization of specific induction agents, volatile anesthetics, opiate analgesics, and the centrally-mediated stress responses to nociception and pain. These factors, or the stress responses they elicit, eventually affect tumor progression either by impairing anti-tumor immunity, or directly by impacting the malignant tissue. For example, in a rat model of experimental metastasis, thiopental, ketamine, and halothane were all shown to reduce NK cytotoxicity, and to increase susceptibility to metastasis, some through activation of β-adrenoceptors (Melamed et al., 2003). Additionally, several volatile anesthetics, including isoflurane and desflurane, were shown to directly activate hypoxia inducible factors (HIFs) in tumor cells, increasing their resistance to cell death under hypoxic stress, partly by inducing secretion of VEGF and other angiogenic factors (Tavare et al., 2012). Opiate administration, and endogenously secreted opioids in response to nociception, were shown to facilitate tumor proliferation, promote tumor angiogenesis, and enhance tumor blood supply through nitric-oxide (NO) release (Gach et al., 2011; Gupta et al., 2002). Opiates were also shown to suppress NK and phagocytic activity, the production of antibodies, and the release of pro-CMI cytokines (Vallejo et al., 2004). Notably, at much lower doses, opiates are known to have central beneficial effects, reducing anxiety and pain, and were shown to actually attenuate postoperative stress responses and improve resistance to metastasis (Page et al., 2001). Therefore, pain alleviation and stress management, which are not based on systemic high-dose opiate administration, may be advantageous. Accordingly, some studies point at centrally-mediated mechanisms underlying beneficial effects of RA. Specifically, in two studies employing animal models of experimental metastasis, mice or rats were subjected to laparotomy under GA. Adding a spinal block resulted in a diminished deleterious effect on the IFN-γ/IL-4 ratio (reflecting the Th1/Th2 balance), on NK activity, and on the numbers of experimental liver or lung metastases (Bar-Yosef et al., 2001; Wada et al., 2007).

Human prospective and retrospective studies concur with the above causative findings. For example, several experimental studies in humans have recently shown that the GA approach as a whole can directly affect the malignant tissue and promote its growth. In two studies, breast cancer patients were randomly assigned to undergo either GA or RA. Only the GA approach (which includes opiate administration) was shown to directly increase serum levels of VEGF (Looney et al., 2010), MMP-3, and MMP-9 (Deegan et al., 2010). In another study, sera taken from patients who were randomly allocated to undergo GA, rather than RA, promoted the *in vitro* proliferation of a breast cancer cell line (Deegan et al., 2009). Other studies have reported that the use of RA had resulted in a reduced perioperative stress response, and spared postoperative immunity (reviewed in (Kurosawa and Kato, 2008)). For example, in patients undergoing hysterectomy, GA, but not RA, had resulted in a 3-day long lymphopenia and reduced NK activity, accompanied by an increased glucocorticoid and sympathetic responses (Tonnesen and Wahlgreen, 1988). Most exciting, two retrospective studies in cancer patients reported a marked improvement in survival when regional anesthesia was added to GA. Breast and prostate cancer patients that during surgery also received paravertebral or epidural analgesia (respectively), had shown a more than twofold higher long-term recurrence-free survival (3–5 years postoperatively) (Biki et al., 2008; Exadaktylos et al., 2006). These findings most likely reflect a cumulative effect of many potential mediating mechanisms, immunological and non-immunological, and their significance stems from the important clinical outcome of recurrence rates.

However, despite the extensive evidence suggesting that the anesthetic/analgesic approach can influence long term cancer recurrence, so far no randomized clinical trial has shown a causative effect on measures of survival. Thus, RCTs, including ongoing studies (e.g., (Sessler et al., 2008)) are required to provide direct evidence that the anesthetic/analgesic approach can affect long-term cancer prognosis.

5. Conclusions

The premise that immunosuppression during the perioperative period can increase long-term cancer recurrence rates is based on

empirical findings in animal studies, and on indirect evidence and argumentations based on findings from human studies. Prominent among these are (i) the recent identification of new leukocyte populations that exhibit uniquely potent cytolytic activity against autologous tumor cells that were traditionally considered "immune-resistant", (ii) the accumulation of evidence in cancer patients indicating *in vivo* immune control over the progression of cancer metastasis, (iii) the notion that the short perioperative period is markedly influential in determining long-term cancer recurrence, given the many risk factors that occur simultaneously and act synergistically during this period, including suppression of anti-metastatic CMI, (iv) the evidence that variations in surgical procedures, including anesthetic approaches and blood transfusion, affect tumor metastasis in animal models and apparently in cancer patients, through immunological and non-immunological mechanisms, and (v) the recent identification of neuroendocrine, paracrine, and cytokine mediators of the immunosuppressive and metastasis promoting effects of stress and surgery, of which we believe that catecholamines and prostaglandins are key players. Given that both catecholamines and prostaglandins are abundant during the perioperative period, are involved both in immunosuppression and in direct facilitation of malignant tissue progression, and can be pharmacologically controlled during the perioperative period, we believe that their simultaneous blockade presents an unexplored opportunity to limit long-term cancer recurrence employing a short and safe perioperative intervention during this critical timeframe.

Conflict of Interest

The authors of this manuscript have nothing to declare.

Acknowledgments

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Review

Psychosocial stress and inflammation in cancer

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ABSTRACT

Stress-induced immune dysregulation results in significant health consequences for immune related disorders including viral infections, chronic autoimmune disease, and tumor growth and metastasis. In this mini-review we discuss the sympathetic, neuroendocrine and immunologic mechanisms by which psychosocial stress can impact cancer biology. Both human and animal studies have shown the sympathetic and neuroendocrine responses to psychosocial stress significantly impacts cancer, in part, through regulation of inflammatory mediators. Psychosocial stressors stimulate neuroendocrine, sympathetic, and immune responses that result in the activation of the hypothalamic–pituitary–adrenal (HPA)-axis, sympathetic nervous system (SNS), and the subsequent regulation of inflammatory responses by immune cells. Social disruption (SDR) stress, a murine model of psychosocial stress and repeated social defeat, provides a novel and powerful tool to probe the mechanisms leading to stress-induced alterations in inflammation, tumor growth, progression, and metastasis. In this review, we will focus on SDR as an important model of psychosocial stress in understanding neural-immune mechanisms in cancer.

1. Introduction

Psychosocial stressors impact many physiological and pathological disease outcomes, including cancer. In multiple clinical and epidemiological studies, tumor growth, progression, and metastasis have been correlated with reports of stress, anxiety, poor coping behaviors, depression, lack of social support, and numerous other psychological and behavioral abnormalities (Lillberg et al., 2003; Price et al., 2001; Spiegel and Giese-Davis, 2003). The majority of studies that examine the impact of stress on malignant tissue have focused on suppressed immune responses to tumors and stress-induced alterations in the tumor microenvironment. More recently, stress-mediated immune modulation of lymphoid and myeloid cells and chronic inflammation mediated by cytokines such as interleukin (IL)-6 have been implicated as predictors of cancer progression, metastasis, and recurrence (Chung and Chang, 2003; Mundy-Bosse et al., 2011; Pierce et al., 2009; Salgado et al., 2003). By using mouse models of stress, significant progress has been made in determining the mechanisms behind stress-induced alterations in inflammatory immune status. For example, studies using a mouse model of repeated social defeat, termed social disruption (SDR) stress, have shown that stress alone can trigger the generation, egress, and trafficking of immature, inflammatory myeloid derived-cells that are glucocorticoid (GC) insensitive (Curry et al., 2010; Engler et al., 2004a, 2005). In addition, these GC insensitive cells produce high levels of IL-6 and other inflammatory cytokines and chemokines (Powell et al., 2009; Stark et al., 2002; Wohleb et al., 2011). As a consequence, these stress-induced changes at the cellular level translate to significant immune (enhanced inflammatory responses and immunity to microbial, viral, and allergen challenge) and behavioral (prolonged anxiety-like behavior) changes (Bailey et al., 2007, 2009b, 2009a; Dong-Newsom et al., 2010; Kinsey et al., 2007; Mays et al., 2010, 2012; Powell et al., 2011; Wohleb et al., 2011). Indicative of the important role of the SNS in stress-induced immune alteration, these changes are reversed by the blockade of sympathetic signaling prior to stressor exposure (Wohleb et al., 2011).

Studies discussed in this mini-review highlight evidence that stress and the resulting modifications in behavior, immune status, and production of stress hormones and neurotransmitters significantly influence tumor growth, progression, and metastasis. Specifically, we focus on the stress response and its mediators (i.e., GC, catecholamines, and cytokines) between the nervous, endocrine, and immune system interactions. The discussion will be extended to include the impact that stress-induced alterations in immunity and inflammation have on cancer. We discuss how psychological stressors modulate cellular immune function and tumor biology. Within the scope of this paper, we review the clinical and animal literature that focus on the effect that stress has on the im-

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mune response, which, ultimately, can prevent or promote malignant disease. Taken together, SDR and other mouse models of stress known to impact immune function are ideal platforms that can be used to explore of the overall impact of stress and inflammation on cancer biology.

2. The stress response

The stress response results from internal or external stimuli that activate "fight or flight" and defeat/withdrawal responses associated with the SNS and HPA activation. It is well established that specific central nervous system (CNS) pathways act to translate social stimuli into peripheral biological signals that regulate inflammatory responses. For instance, stress activates neuroendocrine and autonomic pathways like the HPA axis, and the SNS resulting in the release of GC, catecholamines, and pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. The release of these sympathetic, neuroendocrine, and immune factors has a profound influence on immunity, behavior, and physiology in both humans and rodents and triggers peripheral biological responses that, in turn, signal back to the CNS to complete a bi-directional communication circuit. This is evident in models of repeated social defeat, like SDR, that enhance immune responses to microbial, viral, and allergic challenges and promote and prolong anxiety-like behavior in rodents (Kinsey et al., 2007; Bailey et al., 2009a,b; Mays et al., 2010). Social disruption stress-induced prolonged anxiety-like behavior coincides with a unique pattern of c-Fos activation in brain regions associated with fear and threat appraisal. For example, SDR causes increased c-Fos activation in the prefrontal cortex, amygdala, hippocampus, paraventricular nucleus, bed nucleus of the stria terminalis and the lateral septum (Wohleb et al., 2011). As a result of the activation of this circuitry and the release of neurotransmitters and hormones, compensatory physiologic changes occur that impact behavior and immunity. In humans, chronic or repeated exposure to stress is associated with increased expression of inflammatory biomarkers, worsened disease states, and affective disorders (Glaser and Kiecolt-Glaser, 2005; Gouin et al., 2012). In clinical studies, stressed individuals have reduced anti-inflammatory GC regulation and increased inflammatory nuclear factor (NF)-κB signaling (Miller et al., 2008). As such, a stressor constitutes a challenge to homeostasis that is interpreted by sensory pathways which manifest as physiologic alterations (Glaser and Kiecolt-Glaser, 2005).

3. Interactions among the nervous, endocrine, and immune systems

The stress-induced release of catecholamines and GC has been associated with modulation of immune function including impaired antigen presentation, decreased T cell proliferation, and dampened humoral and cell mediated immunity (Glaser and Kiecolt-Glaser, 2005). Bi-directional communication between the neuroendocrine and immune systems is facilitated by receptor/ligand interactions. Glucocorticoids and catecholamines are capable of binding to receptors on and within immune cells. Specifically, GC interacts with immune cells by binding to intracellular receptors. The activated GC receptor translocates to the nucleus where it binds to GC response elements in gene promoter regions. As such, activated GC receptors can inhibit the actions of transcription factors, like NF-κB, and in turn inhibit the production of inflammatory cytokines (Padgett and Glaser, 2003).

Leukocyte activation, proliferation, and migration are known to be regulated by GC. Interestingly, GC has also been shown to affect tumor cells through GC-specific suppression or activation of target genes. While GC typically induce apoptosis in leukocytes through active or passive suppression of survival genes, recent reports in experimental models of stress have shown that GC can protect tumor cells that have been treated *in vitro* and *in vivo* with chemotherapeutic agents through GC-specific activation of survival genes (Wu et al., 2004). GC have also been shown to down-regulate tumor suppressor genes (Antonova and Mueller, 2008; Dickinson et al., 2011). In a study by Antonova et al., GC treatment of murine mammary non-tumorigenic Ras-transformed EPH4 cells induced a down-regulation of BRCA1, a tumor suppressor gene that when mutated contributes to familial breast cancer (Antonova and Mueller, 2008). GC is also known to inhibit cellular immune responses, thereby decreasing immune defenses against cancer. Thus, GC has both direct and indirect effects on tumor biology.

The catecholamines norepinephrine (NE) and epinephrine (EPI) mediate their effects on target immune cells via stimulation of two major receptor subtypes: alpha(α)- and beta(β)-adrenergic receptors (ARs) (Madden et al., 1995; Sanders, 1995). Both primary and secondary lymphoid organs are innervated by the SNS (Felten et al., 1985). Most studies suggest that splenic innervation is predominantly sympathetic, with splenic nerves being comprised of approximately 98% sympathetic fibers (Felten et al., 1985; Reilly et al., 1979; Williams et al., 1981). β-AR are G-protein coupled receptors on the surface of many cells, including immune and tumor cells. Upon interaction with its ligand(s), β-ARs signal to the nucleus to activate cyclic adenosine monophosphate (cAMP). cAMP is a second messenger that is activated upon binding of the nuclear cAMP responsive binding element (CREB), a transcription factor that is initiated by multiple signal transduction pathways in response to multiple biologic factors, including stress hormones. Activation of β2-ARs in the absence of immunological stimuli increases pro-inflammatory cytokine production by various cell types (Tomozawa et al., 1995; Tan et al., 2007). For example, activation of β2-ARs with the specific agonist salmeterol up-regulates IL-6 and IL-1β mRNA and protein levels in macrophages, which results in amplified immune responses (Tan et al., 2007). Activation of the SNS can also profoundly impact behavioral responses in a β-AR-specific manner (Wohleb et al., 2011).

While immunological and neurological changes can result as a consequence of activation of the stress response, recent compelling data argue for a critical role of the β-AR directly and indirectly influencing malignant tissue. For example, β-AR is present on breast and ovarian cells (Badino et al., 1996; Sood et al., 2006). To date, the CREB family of proteins has been implicated in various aspects of tumor biology including metastasis, growth, angiogenesis, and cell survival (Jean and Bar-Eli, 2000; Lang et al., 2004). In several studies, cAMP activation has been correlated with increased rate of tumor growth in mammary tissue. For example, a report by Sastry et al. showed that EPI protected androgen-sensitive human prostate adenocarcinoma cells (i.e., LNCaP cells) from apoptosis through a cAMP-dependent inactivation of pro-apoptotic proteins, downstream of β2-AR signaling (Sastry et al., 2007). In addition, studies have shown that NE modulates the migration of tumor cells, including breast, colon and prostate tumor cells, which can be blocked by β2-AR antagonists (Lang et al., 2004; Drell et al., 2003; Masur et al., 2001). Further, a study by Palm et al., describes that this effect can be replicated *in vivo* and pre-treatment with β-blockers prevents NE-driven metastasis in a model of prostate cancer in Balb/c mice (Palm et al., 2006). Coupled with the effects of GC on tumors, these studies highlight the emerging role that stress-associated factors play in tumor biology.

4. Cancer, inflammation, and immunity

There are multiple factors that impact tumor biology beyond stress and stress-associated molecules. Some are intrinsic factors

within the tumor and the tumor microenvironment, which include increased proliferative capacity and replicative immortality, resistance to suppressive signals which impair cell growth and/or promote apoptosis, and enhanced reactivity or exposure to molecules that promote angiogenesis, tissue invasion, and metastasis (Hanahan and Weinberg, 2011). In addition, the cellular composition of the tumor microenvironment is thought to significantly influence the biology of tumors. The cell subsets comprising the tumor microenvironment include both cancer-associated cells and normal immune cells that collectively enable tumor growth and progression. Common immune cells such as inflammatory macrophages, natural killer (NK) cells, and cytotoxic T-cells (CTL) can be found in the core of primary tumors and within the invasive and metastatic tumor microenvironments (Hanahan and Weinberg, 2011).

NK cells and CTL, which are known to influence tumor progression, are also known to be regulated by stress. The importance of NK cells in cancer biology is highlighted by the unique ability to lyse tumor targets without prior sensitization. Additionally, one of the hallmarks of tumor biology is the capability of taking over the genetic machinery of major histocompatibility complex (MHC) class I genes, ultimately reducing the efficacy of cytotoxic T cell recognition. As a primary way of activation, the capabilities of NK cells to recognize infected/tumor cells by the lack of MHC class I expression becomes crucial in tumor progression and metastasis (Bubenik, 2004). Once NK cells and CTL become activated, the release of perforins, granzymes A and B, and FasL cause apoptosis of the infected cell. Additionally, activated NK cells and CTL increase anti-viral/tumor interferon (IFN) production which directly inhibits tumor growth and coordinates anti-tumor innate and adaptive immunity.

Currently, there is a growing importance placed on inhibitory receptor expression on NK cells and CTL and cancer modulation (Carena et al., 1997; Hanke et al., 1999; Yu et al., 1996). Expressed both in humans and rodents, inhibitory receptors found on NK cells and CTL regulate activation pathways that lead to anti-viral and tumor mechanisms. The use of antibodies targeting NK cell inhibitory receptors are currently being used in clinical trials lending to promising novel approaches to immune-based anti-tumor therapies (Kradin et al., 2001; Vahlne et al., 2010; Yu et al., 1996). Stress has been shown to modulate these inhibitory receptors, potentially mediating the effect of NK and CTL on cancer progression. Several studies have shown that restraint stress diminishes NK cell function, cytokine secretion, and enhances viral replication, which was shown to be partially mediated by stress-induced glucocorticoids (Hunzeker et al., 2004; Tseng et al., 2005). In a study by Andersen et al., the lytic potential of NK cells from patients with invasive breast cancer and higher stress levels was significantly reduced (Andersen et al., 1998). In addition, NK cells from cancer patients with higher reported levels of stress responded poorly to treatment with recombinant IFN-γ *in vitro* as evidenced by a sustained decrease in lytic potential. However, stress may also cause beneficial effects on NK activity by enhancing migration and function (Schedlowski et al., 1993). Several studies have shown that subcutaneous administration of EPI or exposure to stress increases NK cell trafficking from the bone marrow, into the blood and peripheral organs (Engler et al., 2004b; Kradin et al., 2001). In addition, stress-induced increases in NK cell trafficking was blocked with propranolol or nadolol treatment, lending support to the importance of SNS signaling in stress-related immune alterations.

Similar findings were reported with CTL, which are also known to be affected by stress, and are known to target virally-infected and tumor cells. As such, the effect of stress on latent virus activation and virus-associated tumors has been explored. It has been shown that stress promotes latent virus reactivation and that there is a potential causal relationship between stress and the onset of Epstein bar virus (EBV)-associated tumors. Moreover, stress may facilitate the onset and progression of such tumors because stress is known to impair the lytic activity and cytokine secretion of CTL and NK cells (Glaser et al., 1994; Levy et al., 1987; Sieber et al., 1992). Stress can also shift cytokine profiles of CTL and other immune cells to favor virus replication, subsequently promoting tumor formation (Glaser and Kiecolt-Glaser, 2005). It is important to note, however, that stress is also known to enhance the generation of primary and memory CTL in response to an infection, enhance inflammatory and anti-viral cytokine secretion, and enhance killing (Mays et al., 2010, 2012). The impact that stress-enhancement of CTL and NK numbers and function have on malignant disease have not been fully elucidated.

It has been established that infiltrating cells from the immune system (e.g., CTL and NK cells) can stunt tumor growth and block tumor progression. Recently, however, inflammatory immune cells have been shown to enhance those factors responsible for tumor progression at the cellular and molecular level. Interestingly, a subset of immature, undifferentiated inflammatory myeloid cells that co-express the macrophage lineage marker CD11b and the neutrophil lineage marker Gr1 in mice, or co-express CD33 and CD11b and lack lineage specific markers in humans, have been identified (Ostrand-Rosenberg and Sinha, 2009). These tumor-infiltrating myeloid cells, termed myeloid-derived suppressor cells (MDSCs), can be found in normal, inflamed, and malignant tissues alike; in addition, MDSCs can actively suppress CTL and NK cell anti-tumor mechanisms at the tumor site (Ostrand-Rosenberg and Sinha, 2009). In a randomized clinical trial by Mundy-Bosse et al., more frequent stressful life events and higher stress levels after surgery were reported to correlate with an overall increase in MDSCs (Mundy-Bosse et al., 2011). This increase was coupled with higher, but not significant levels of interleukin-1 receptor antagonist, interferon gamma-induced protein 10, granulocyte colony stimulating factor, and IL-6. In addition to changes at the cellular level, inflammatory cells in the tumor microenvironment can contribute to all phases of tumor progression through multiple mediators, including cytokines and reactive oxygen species. For example, inflammatory immune cell release of reactive oxygen species (ROS), a characteristic product of inflammatory myelomonocytic cells, can promote tumorigenic factors in neighboring cancer cells. MDSCs isolated from tumor-bearing mice produced significantly more ROS than control MDSCs. In addition, tumor cells themselves can secrete ROS that lead to enhanced angiogenesis and tumor growth (Xia et al., 2007).

Chronic inflammation has also been linked to several types of cancers, and the immune system at the cellular, molecular, and/or genetic level has been shown to mediate the effect of stress-induced inflammation on cancer. In a study of breast cancer patients three years post-treatment, elevated levels of stress-inducible acute phase proteins correlated with an increase in morbidity and mortality in the experimental cohort (Pierce et al., 2009). Pro-inflammatory cytokines secreted as a consequence of stressful interactions (e.g., IL-6) have been described as playing an important role in the growth and progression of tumors (Kossakowska et al., 1999). Furthermore, circulating levels of IL-6 have been reported as prognostic indicator of survival and metastasis in human cancers (Chung and Chang, 2003; Salgado et al., 2003). Genetic analyses have identified IL-6 gene polymorphisms as biomarkers of breast cancer progression (DeMichele et al., 2003; Snoussi et al., 2005). In studies of mammary tumors, a single nucleotide polymorphism in the IL-6 promoter enhances gene transcription in response to β-AR signaling. At the cellular level, IL-6 gene expression is regulated by β2-AR signaling and is increased in circulation after stressor exposure. Taken together, evidence linking inflammation and stress-induced SNS activation to cancer progression provide insights into the importance of regulation of

cancer-related genes (e.g., IL-6) that appear to modulate the effects of stress on cancer.

5. Animal models of stress and cancer

Rodent models of stress have provided significant insight into the mechanisms of neuroendocrine and sympathetic-mediated regulation of immune cells and inflammatory products involved in the biology of cancer, as well as sympathetic regulation of tumors. Animal models of stress have provided important insights into the changes in immune status that can predict regulation/promotion of tumor growth and progression. Significant changes in the effector function of immune cells that are known to regulate tumor progression, including macrophages, NK cells, and T-cells, are evident after exposure to a variety of different stressors. A study using a rodent model of cancer showed that mice that were socially isolated had decreased macrophage activity and increased tumor growth (Palermo-Neto et al., 2003). Additional studies using a mouse restraint stress model showed decreased numbers of T cells, suppressor cells, and cytokine/chemokine secretion following stress exposure in breast cancer, and skin and squamous cell carcinoma models (Saul et al., 2005; Steplewski et al., 1985). β-adrenergic agonists in rodents have been shown to influence breast cancer cell metastatic activity resulting in lung metastases (Sloan et al., 2010). In contrast, blocking the stress-induced activation of the SNS with β-antagonists ameliorates stress-associated increases in lung metastasis. Interestingly these effects were amplified when β-antagonist treatment was coupled with the anti-inflammatory drug indomethacin. In a study by Sloan et al., pharmacologic and stress-induced β2-AR signaling in macrophages and cancer cells was shown to serve as a metastatic switch in primary breast cancer (Sloan et al., 2010). In this study, β-AR signaling mediated a significant increase in the infiltration of differentiated macrophages into the primary tumor microenvironment. At the level of the tumor itself, β-AR signaling didn't have an effect on the size of the tumor, but did increase the rate of metastasis to distant tissues. Interestingly, macrophage infiltration and tumor metastases were blocked when β-ARs were antagonized prior to stressful interactions (Sloan et al., 2010). In this study, the infiltrating macrophage itself was identified as a key intermediate in the effects of stress on tumor cell metastasis, as specifically targeting and suppressing macrophage populations in stressed mice with primary tumors reversed the stress effect on tumor metastasis. This example, as well as others, point to the importance of the intricate and complex relationship between stress, immune function, behavior, and disease.

An area largely unexplored is the effect of psychosocial stress on tumor growth, progression, and metastasis. Social disruption stress has been an effective animal model used to predict the impact of stressful interactions on disease and behavior. For example, several studies in which mice experienced repeated social defeat prior to an influenza infection indicated that repeated social defeat significantly influenced CTL priming by dendritic cells, enhanced primary and memory T cell responses, and altered lung microenvironments in response to influenza infection (Mays et al., 2010, 2012; Powell et al., 2011). Anxiety-like behavior was also shown to be pronounced in mice exposed to SDR (Wohleb et al., 2011; Kinsey et al., 2007). SDR-induced tissue remodeling lends to heightened inflammation and prolonged anxiety-like behavior (Avitsur et al., 2002; Curry et al., 2010; Engler et al., 2005; Wohleb et al., 2011). In a study by Curry et al., stress alone was able to induce significant lung inflammation in mice experiencing SDR (Curry et al., 2010). Inflammation was marked by an increase in cellular infiltrates into the lung, including monocytes and neutrophils, and an increase in inflammatory cytokines such as IL-1β. Furthermore, increased monocyte and neutrophil chemotactic factors, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), and the IL-8 homologue keratinocyte chemoattractant, were reported following SDR. In addition, to cell specific factors, a more reactive endothelium was present in mice that experienced SDR (Curry et al., 2010). Resident microglia in the brains of SDR mice displayed a more reactive phenotype which coincided with an increase in infiltrating bone marrow-derived, primed myeloid cells (Wohleb et al., 2011). This activation of resident and infiltrating myeloid cells was coupled with a significant increase in gene expression of inflammatory cytokines, chemokines, and prolonged anxiety-like behavior. All of the stress-induced phenomena in the CNS were reversed when propranolol was administered prior to stressor exposure (Wohleb et al., 2011).

While these examples point to stress-induced tissue remodeling in non-immune organs, several reports have also described significant social stress-induced changes in immune organs, including the spleen and bone marrow, and in blood. Stress increases the size and the cellular composition of the spleen, primarily due to a significant increase in infiltrating $CD11b^+$ bone marrow-derived myeloid cells (Avitsur et al., 2002; Stark et al., 2002). Like the spleen, the bone marrow and blood also show significant increases in these myeloid cell populations (Engler et al., 2004a). Along with an increase in number, SDR impacts the GC sensitivity and effector function of bone marrow-derived myeloid cells, including differentiated macrophages and dendritic cells, and immature myeloid cells alike (Bailey et al., 2007; Powell et al., 2009). The important aspect of these findings is that the experience of repeated social defeat ramps up the production of "primed" immature myeloid populations in the bone marrow, which egress and traffic to peripheral and central tissues. Myeloid cells derived from SDR-treated mice display increased numbers of Toll-like receptors and co-stimulatory molecules and are resistant to the anti-apoptotic effects of high levels of GC, indicative of a primed state (Bailey et al., 2007; Powell et al., 2009). As a result, primed myeloid cells trafficking to the spleen and other organs of SDR-exposed mice secrete increased amounts of pro-inflammatory cytokines when exposed to microbial and allergen challenges, and inflammatory stimuli both *in vivo* and *in vitro* (Bailey et al., 2007, 2009a, 2009b; Dong-Newsom et al., 2010; Mays et al., 2010, 2012; Powell et al., 2011; Wohleb et al., 2011). The resultant tissue remodeling has profound behavioral and immunological effects on the outcome of subsequent inflammatory and infectious challenges. The ability of these cells to traffic to sites of inflammation and infection has been documented, but the role of inflammatory myeloid cells, primed by social stress, and their regulation of the tumor microenvironment remains relatively unexplored.

The stress-induced generation and trafficking of myeloid cells to the tumor site can have significant implication for tumor progression and metastasis. For example, inflammatory cells, including myeloid cells like macrophages and neutrophils, are typically present within and along the margin of invasive and metastatic tumors. These normal immune cells present in the tumor microenvironment have been shown to promote tumors through the production of pro-angiogenic factors, pro-inflammatory cytokines, and/or pro-invasive matrix degrading enzymes like matrix metalloproteinases (MMPs) (Hanahan and Weinberg, 2011). MMPs are enzymes secreted by tumor and immune cells that allow for progressive metastasis and penetration into tissues by breaking down the extracellular matrix. The production of MMPs, which has been shown to be an integral part of both local and distant tumor spread, is enhanced by both tumor and immune cells after stressor or stress hormone exposure (Sood et al., 2006; Yang et al., 2006). In a study by Sood et al. (2006), *in vitro* treatment of ovarian cancer cells with stress-levels of NE increased the levels of MMP-9 and MMP-2 which lent to increased invasive potential. Interestingly, high levels of MMPs and invasiveness of the tumor

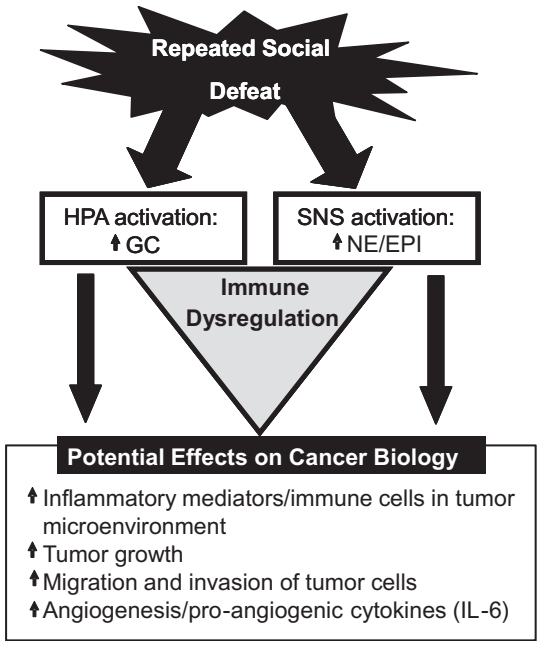

Fig. 1. The potential effects of repeated social defeat on cancer biology. This figure depicts the multiple ways that stress-induced immune dysregulation can impact cancer. Psychosocial stressors in clinical studies and animal models activate the HPA and SNS, thereby causing GC and NE/EPI mediated immune dysregulation. This change in immune status can increase various factors that lead to the morbidity and mortality associated with cancer.

cells were reversed after treatment with propranolol, highlighting the link between stress hormones and catecholamines and the invasive and metastatic potential of malignant tissue (Sood et al., 2006). Studies have also linked increases in MMPs to enhanced IL-6 production by immune cells within the tumor microenvironment (Kossakowska et al., 1999). Like MMPs, IL-6 can be produced by both tumor cells and macrophages that infiltrate the tumor site. Increased levels of IL-6, in animal and clinical studies alike, is a circulating biomarker indicative of immune changes in tissue and circulation. IL-6 is a characteristic cytokine that is secreted by stress-primed cells; in models of repeated social defeat, increased plasma IL-6 levels is a hallmark of the physiologic response to a stressor. In addition, stress-primed macrophages and dendritic cells from SDR mice produce more IL-6 at baseline and in response to Toll-specific stimuli (Bailey et al., 2007; Powell et al., 2009; Stark et al., 2002). This increase in IL-6 production was not abrogated *in vitro* by GC. This is relevant because these stressed-primed cells secrete cancer modulating cytokines that are induced by β-AR signaling (e.g., IL-6). Additionally, these cells are insensitive to the anti-inflammatory actions of GC, and have an increased capacity for survival and migration. Social defeat-induced activation of the HPA axis and SNS and the resultant immune alterations will likely impact factors that lead to the morbidity and mortality associated with cancer. This may include but is not limited to: enhanced inflammatory mediators and immune cells in the tumor microenvironment, increased tumor growth, increased migration and invasion of tumor cells, and enhanced angiogenesis (Fig. 1).

6. Conclusions

While there have been few reported studies describing causal relationships between stress and the onset of cancer, multiple epidemiologic studies have linked stress to enhanced progression of established malignant disease. Many of these studies, both highlighted within this review and described throughout the extant literature, specifically link stress-induced activation of the SNS, release of catecholamines, and signaling via β-AR receptors to primary tumor growth and metastasis and colonization of tumors in distant tissues. In addition, stress-inducible inflammatory factors and genes, such as IL-6, are regulated by β2-AR signaling and are increased in circulation after stressor exposure. Circulating levels of IL-6 as well as gene polymorphisms of IL-6 are prognostic indicators of tumor progression, metastasis, and survival in human cancers. Importantly, peripheral immune cells, which are responsive to β-AR signaling and secrete IL-6 and other regulatory factors, reside within and around the tumor microenvironment and can promote or prevent malignant disease. Inflammatory cell recruitment into the primary tumor microenvironment may serve as a biomarker for early detection of disease progression or serve as a prognostic indicator of therapeutic success and resolution of disease. The studies outlined in this review show that stress-induced alterations in immune status and tumor biology can shape the primary tumor microenvironment and can facilitate progression of malignant disease, highlighting the importance of a more refined understanding of the complex relationship between stress, inflammation and immunity, and cancer.

Given what is known about the significant impact that cellular and molecular factors altered as consequence of stress have on cancer, the SDR model of repeated social defeat is an ideal tool to study the impact of stress on cancer. Myeloid-derived immune cells from mice exposed to repeated social defeat demonstrate insensitivity to GC induced apoptosis and anti-inflammatory regulation; these changes are reversed by the blockade of β-AR signaling prior to stressor exposure. Moreover, repeated social defeat in mice leads to the generation, egress, and trafficking of immature myeloid-derived cells that are glucocorticoid insensitive and secrete higher levels of inflammatory cytokines, like IL-6, at baseline and upon stimulation. This is significant because these cells, deployed in response to a stressor, have an increased capacity to travel from the bone marrow (the site of genesis) to distant tissues. These cells traffic in a greater frequency and in a primed state to: (1) the spleen, where, under physiologic conditions, they can reside in reservoirs for future deployment, (2) the CNS, where they can contribute to stress-induced and prolonged anxiety-like behavior, re-enforcing the bi-directional communication between the neuroendocrine and immune systems, and (3) to inflamed, infected, and presumably malignant tissues where these stress-primed cells will further promote an inflammatory microenvironment. The contribution of stress-primed myeloid cells to the tumor microenvironment is an area that is currently being explored and may lead to development of novel, targeted therapeutic approaches and stress-reduction interventions that will limit cancer progression and minimize metastatic rates in malignant disease.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Inflammation and cancer-related fatigue: Mechanisms, contributing factors, and treatment implications

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ABSTRACT

Fatigue is one of the most common and distressing side effects of cancer and its treatment, and may persist for years after treatment completion in otherwise healthy survivors. Guided by basic research on neuro-immune interactions, a growing body of research has examined the hypothesis that cancer-related fatigue is driven by activation of the pro-inflammatory cytokine network. In this review, we examine the current state of the evidence linking inflammation and cancer-related fatigue, drawing from recent human research and from experimental animal models probing effects of cancer and cancer treatment on inflammation and fatigue. In addition, we consider two key questions that are currently driving research in this area: what are the neural mechanisms of fatigue, and what are the biological and psychological factors that influence the onset and/or persistence of inflammation and fatigue in cancer patients and survivors? Identification of the mechanisms driving cancer-related fatigue and associated risk factors will facilitate the development of targeted interventions for vulnerable patients.

1. Introduction

Fatigue is increasingly recognized as one of the most common and distressing side effects of cancer and its treatment (Lawrence et al., 2004). Prevalence estimates of fatigue during cancer treatment range from 25% to 99% depending on the sample, type of treatment, and method of assessment (Servaes et al., 2002; Lawrence et al., 2004). Energy typically improves in the year after treatment completion, although a significant minority of patients continue to experience fatigue for months or years after successful treatment (Bower et al., 2000; Cella et al., 2001). Studies of long-term cancer survivors suggest that approximately one-quarter to one-third experience persistent fatigue for up to 10 years after cancer diagnosis (Bower et al., 2006; Servaes et al., 2006). Fatigue has a negative impact on work, social relationships, mood, and daily activities and causes significant impairment in overall quality of life (Andrykowski et al., 1998; Bower et al., 2000; Broeckel et al., 1998). Fatigue may also be a predictor of shorter survival in cancer patients (Groenvold et al., 2007).

Qualitative reports suggest that cancer-related fatigue is more severe, more enduring, and more debilitating than "normal" fatigue caused by lack of sleep or overexertion and is not relieved by adequate sleep or rest (Poulson, 2001). In addition, cancer-related fatigue involves mental, physical, and emotional components. One definition that captures several of the key features of cancer-related fatigue describes it as "a subjective state of overwhelming and sustained exhaustion and decreased capacity for physical and mental work that is not relieved by rest" (Cella et al., 1998).

Studies conducted over the past decade have begun to elucidate the biological underpinnings of cancer-related fatigue, with a focus on inflammation. This research is motivated by basic research on neural-immune signaling, which indicates that pro-inflammatory cytokines can signal the central nervous system to generate symptoms of fatigue and other behavioral changes in animals and healthy humans (Dantzer et al., 2008). In the cancer context, inflammation may be induced by common cancer treatments, including radiation and chemotherapy, or by the tumor itself. Previous reviews of this literature have generally supported a link between inflammation and behavioral symptoms in cancer patients, including fatigue (Miller et al., 2008; Schubert et al., 2007; Seruga et al., 2008; Bower, 2007). This is a growing area of research that has seen important advances in methodological rigor (e.g., larger sample sizes, controls for confounders, advanced statistical methods) and examination of underlying mechanisms. In this review we will examine the current state of the evidence linking inflammation and cancer-related fatigue, drawing from recent human research and from experimental animal models probing effects of cancer and cancer treatment on inflammation and fatigue. We will

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then consider two key questions that are currently driving research in this area. First, what are the neural underpinnings of fatigue, and can they be discriminated from depression? Second, what are the biological and psychological factors that contribute to inflammation and fatigue during and after treatment? We conclude with implications for interventions and recommendations for future research.

2. Human and animal research on inflammation and cancer-related fatigue

We consider human and animal studies that have examined links between inflammation and fatigue at three stages of the cancer continuum: before, during, and after cancer treatment. The basic model guiding this area of research is that tumors and the treatments used to eradicate them can activate the proinflammatory cytokine network, leading to symptoms of fatigue via effects on the central nervous system (see Fig 1). In the pre-treatment period, the tumor itself may be a source for proinflammatory cytokines (Aggarwal, 2004; Coussens and Werb, 2002) while during treatment, cytokines may be produced in response to tissue damage from radiation or chemotherapy (Stone et al., 2003; Aggarwal et al., 2009). In addition to these direct effects, other processes may influence proinflammatory cytokine production and will be discussed in section 4.

2.1. Fatigue prior to cancer treatment

A few studies have investigated links between inflammatory markers and fatigue in cancer patients prior to treatment. In patients with newly diagnosed acute myelogenous leukemia or myelodysplastic syndrome, levels of several inflammatory markers were correlated with symptoms of fatigue (Meyers et al., 2005). In a sample of ovarian cancer patients assessed prior to surgery, those with advanced stage disease had higher levels of IL-6 as well as elevations in vegetative symptoms of depression (including fatigue) relative to those with early stage disease or tumors of low malignant potential (Lutgendorf et al., 2008). Moreover, elevations in plasma and ascites levels of IL-6 were correlated with fatigue and other vegetative depressive symptoms, but not with mood or affective symptoms of depression. On the other hand, a recent study of breast cancer patients assessed prior to surgery did not find elevated levels of CRP in those categorized as "fatigued" (Fagundes et al., 2012). It is possible that small, localized breast tumors may not produce elevations in systemic cytokine concentrations that are sufficient to induce symptoms of fatigue.

In rodents, fatigue has usually been measured by the amount of reduced spontaneous locomotor activity the animal exhibits (Kent et al., 1992). Recently, a mouse model of ovarian cancer was used to investigate the effect of tumor on inflammation and spontaneous locomotor activity (Lamkin et al., 2011). In this model, a syngeneic ovarian carcinoma cell line was injected into the peritoneum of the mice to grow over a period of two months. Congruent with what often happens in ovarian cancer patients, ascites (i.e., a build-up of fluid that forms around the tumor) forms in the peritoneum of the mice in this model and contains high levels of IL-6 and TNF-α. Consequently, levels of IL-6 and TNF-α as well as IL-17 and IL-10 are significantly elevated in systemic circulation as a result of tumor. We found reductions in home cage locomotion in tumor-bearing animals without any significant deficit in motor capacity (i.e., the animals were *able* to engage in spontaneous locomotion). Mediation analysis suggested that tumor-induced IL-6 in the circulation carried part of the effect of tumor on decreased locomotion, supporting a role for tumor-associated cytokines in the development of fatigue.

2.2. Fatigue during cancer treatment

Radiation therapy and chemotherapy are two of the most common types of cancer treatment, and both are associated with increases in fatigue (Donovan et al., 2004) and with elevations in certain inflammatory markers (Arpin et al., 2005; Mills et al., 2004). A handful of studies have examined the association between inflammation and fatigue during radiation therapy to test the

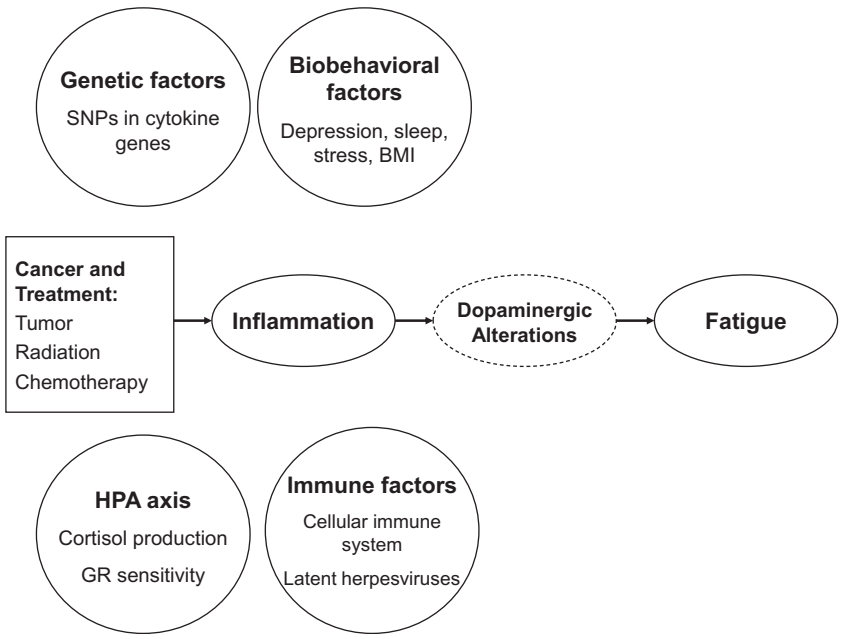

Fig. 1. Conceptual model linking cancer and cancer treatments to inflammation and symptoms of fatigue. These effects may be mediated by alterations in dopaminergic transmission; the dashed line around this mediator indicates that this pathway has not been fully elucidated. Host factors that may influence that onset and persistence of inflammation and fatigue in cancer patients are depicted in circles and include genetic polymorphisms, alterations in the hypothalamic–pituitary-adrenal (HPA) axis, alterations in the cellular immune system, and biobehavioral factors such as history of depression, sleep disturbance, early life stress, and body mass index.

hypothesis that inflammation may contribute to radiation-induced fatigue. Early reports on this topic were conflicting, possibly due to constraints of study designs (including use of non-standard measures to detect cytokine levels) and focus on cross-sectional associations between cytokine levels and fatigue (Greenberg et al., 1993; Wratten et al., 2004; Geinitz et al., 2001; Ahlberg et al., 2004). Our group examined within-subject relationships between inflammatory markers and fatigue before, during and after radiation therapy in patients with early-stage breast and prostate cancer (Bower et al., 2009). Results showed that changes in serum levels of inflammatory markers CRP and IL-1RA were positively associated with increases in fatigue symptoms; patients reported higher levels of fatigue on weeks when these biomarkers were elevated. Effects remained significant in analyses controlling for potential biobehavioral confounders including age, BMI, depression, and sleep disturbance. Of note, we found no evidence that serum concentrations of IL-6 or IL-1β were associated with fatigue in this study. These findings provide initial evidence that activation of the proinflammatory cytokine network may be a general mediator of radiation-induced fatigue, but require replication in larger samples. Further, these findings suggest that downstream markers of proinflammatory cytokine activity may be more reliably associated with cancer-related fatigue; similar results have emerged in our work with cancer survivors.

Investigators have also utilized animal models of ionizing radiation to study its effects on fatigue (e.g., King and Landauer, 1990). Likewise, researchers have used animal models to study the effects of radiotherapy on inflammatory processes, and have shown acute elevations in inflammatory responses following a single session of total body irradiation (TBI) (e.g., Haveman et al., 1998; Khayyal et al., 2009). However, very few researchers have looked at radiation-induced inflammation in conjunction with measures of fatigue. Van der Meeren and colleagues (2001) modeled the effects of radiotherapy in mice and found that TBI caused an increase in plasma levels of IL-6, serum amyloid A, Gro1, and granulocytes but no increase in IL-1β, TNF, or Scya5. Peak levels for these effects in circulation were observed at 6 h after TBI and had mostly resolved by 24 h. In a follow-up study, spontaneous locomotor behavior in the home cage was examined with automated telemetry (Van der Meeren and Lebaron-Jacobs, 2001). In accord with the acute rise in systemic inflammation, locomotion dropped significantly within a few hours of TBI. However, whereas inflammatory markers quickly returned to baseline levels, locomotion continued to decline until Days 13–17 before starting to recover by Day 25. Of note, the slow recovery of normal locomotion roughly parallels that seen with repeated radiation exposure in the clinical setting and may reflect late effects of radiation on irradiated tissues (Brush et al., 2007).

More recently, a study by York and colleagues (2012) examined the effect of TBI on spontaneous locomotion in mice at multiple time points during the first 24 h following exposure. Mice exhibited a decrease in locomotion by 6 h after exposure, but the effect waned afterward. Also at this time point, gene expression for TNF-α was significantly elevated in the whole brain, but not IL-1, IL-1RA, IL-6, or IFN-γ. Examination of cytokine gene expression in whole blood found evidence for IL-1 expression around this time as well, but not for TNF-α, IL-6, or IFN-γ.

Investigation into chemotherapy-associated inflammation and fatigue is complicated by the fact that there are numerous classes of antineoplastic drugs with unique mechanisms of action within and between each class (e.g., antimetabolites, antimicrotubule agents, alkylating agents, platinum agents, cytotoxic antibiotics, DNA topoisomerase I and II inhibitors) (Chabner and Longo, 2006). Nonetheless, clinical investigators have been interested in linkages between inflammation and fatigue during chemotherapy. Several early studies showed links between inflammatory markers and fatigue in cancer patients undergoing various chemotherapy regimens (Mills et al., 2005; Pusztai et al., 2004). More recently, Wang and colleagues intensively examined sickness symptoms and inflammatory markers in patients undergoing combined radiation and chemotherapy therapy for locally advanced colorectal, esophageal, and non-small cell lung cancer (Wang et al., 2010; Wang et al., in press). These investigators documented acute increases in markers of inflammation that were correlated with increases in fatigue and other prominent sickness symptoms. Similar effects were seen in a study of individuals undergoing allogeneic hematopoietic stem cell transplantation (which includes high-dose chemotherapy) for acute myelogenous leukemia and myelodysplastic syndrome (Wang et al., 2008).

Preclinical investigators have developed animal models of chemotherapy-induced fatigue using some of the more wide-spread antineoplastic agents. For example, a study by Wood and colleagues (2006) examined the effect of etoposide, a topoisomerase II inhibitor, on locomotion with voluntary wheel running. Etoposide caused a step-wise reduction in voluntary running that began after the first dose, resulting in an 80% reduction by the study's end. Examination of systemic IL-6 showed that a single administration of the drug induced an 18-fold increase compared to control mice, and levels at the end of the two-week administration schedule were still significantly elevated by more than 10-fold.

Malik and colleagues (2006) found that a single dose of the platinum-based drug, cisplatin, caused rats to exhibit decreased motor activity several hours after injection during their normally active nocturnal phase, and this decrease grew in magnitude until Days 3–4 before beginning to recover by Day 8. Although no differences in gene expression were found for specific proinflammatory cytokines examined, the investigators found that administration of the potent anti-inflammatory glucocorticoid, dexamethasone, inhibited the decrease of spontaneous motor activity in the model in a subsequent study (Malik et al., 2007), suggesting that yet undefined inflammatory mechanisms in the model may be at work.

One potential limitation of an investigation into the effect of cisplatin or other chemotherapeutic drugs on spontaneous locomotor activity is lack of information regarding motor capacity, because such drugs can cause peripheral neuropathy and, thus, contribute to decreased locomotion via this mechanism (Cavaletti et al., 1992; Mollman, 1990; Rowinsky et al., 1993). Recently, Ray and colleagues (2011) tested a model of paclitaxel-induced inflammation and fatigue where they examined motor capacity alongside spontaneous locomotion in mice. Results showed that paclitaxel reduced spontaneous home cage locomotion during the week of dosing and for an additional two weeks without substantially altering motor capacity. However, the investigators found no changes in a panel of 21 cytokines and chemokines in the blood at the conclusion of the week of dosing, including IL-1β, IL-6, and TNF-α, compared to saline-injected animals. Overall, these studies support an association between chemotherapy and fatigue, but provide less insight into how inflammatory mechanisms may underlie these effects.

2.3. Post-treatment fatigue in cancer survivors

Although fatigue typically abates in the year after cancer treatment, approximately 25–30% of cancer survivors report persistent fatigue that may last for 5–10 years post-treatment and beyond (Bower et al., 2006). In our earlier work, we documented consistent alterations in the pro-inflammatory cytokine network among breast cancer survivors with persistent post-treatment fatigue, including elevations in circulating markers of inflammation (Bower et al., 2002; Collado-Hidalgo et al., 2006) and elevated cytokine production after LPS stimulation (Collado-Hidalgo et al., 2006; Bower et al., 2007), controlling for potential demographic and

biobehavioral confounds. We have more recently shown an association between fatigue and elevations in plasma levels of the soluble TNF receptor type II (sTNF-RII), a downstream marker of TNF activity, in breast cancer survivors within one month after treatment; this association was particularly strong among women treated with chemotherapy (Bower et al., 2011b).

These findings have recently been replicated by other groups. For example, Alexander et al. found significant elevations in CRP in breast cancer survivors who met stringent criteria for cancer-related fatigue syndrome ($n = 60$) relative to non-fatigued controls ($n = 104$) (Alexander et al., 2009). Similarly, in sample of 299 disease-free survivors, Orre et al. found a positive association between CRP and fatigue that remained significant after controlling for age, BMI, depressive symptoms, sleep disturbance, medication use, and self-rated health (Orre et al., 2011). In a recent study by Alfano and colleagues, higher CRP was associated with increased odds of being classified as fatigued in a sample of 633 breast cancer survivors controlling for age, race, menopausal status, antidepressant/anxiolytic use, comorbidities, and BMI. (Alfano et al., 2012). This study also found a significant linear association between CRP levels and fatigue, particularly physical and behavioral dimensions of fatigue; however, these associations were attenuated to non-significant after controlling for use of antidepressants/anxiolytics, comorbidities, and BMI. A positive association between inflammatory markers and fatigue has also been documented in long-term survivors of testicular cancer (Orre et al., 2009).

Two recent studies have probed the molecular underpinnings of cancer-related fatigue by conducting genome-wide expression analyses on leukocytes from breast cancer survivors with persistent fatigue compared to non-fatigued survivors. A study conducted by our group focused on transcription of inflammation-related genes, particularly those responsive to the proinflammatory NF-κB transcription control pathway, guided by the hypothesis that peripheral inflammatory signaling may contribute to cancer-related fatigue (Bower et al., 2011a). Results showed that breast cancer survivors experiencing persistent fatigue showed increased expression of genes encoding proinflammatory cytokines and other mediators of immunologic activation. Further, promoter-based bioinformatic analyses indicated increased activity of proinflammatory NF-κB/Rel transcription factors in leukocytes from fatigued breast cancer survivors, which might structure the observed differences in the expression of inflammation-related genes. In contrast, an exploratory study by Landmark-Hoyvik et al. found that fatigued breast cancer survivors showed altered expression of genes involved in plasma or B cell pathways (Landmark-Hoyvik et al., 2009). These differences might be explained by differences in the populations studied, the microarray assay platforms, and the statistical methods used.

3. Convergence of inflammation on neural systems mediating fatigue

3.1. Lessons from research on sickness behavior and depression

To date, there is limited understanding of the neural processes that may mediate effects of peripheral inflammation on behavioral outcomes, including fatigue, in cancer patients. However, insight into these effects can be gleaned from the large array of information that has accrued over the last 25 years on inflammation-induced sickness behavior and major depressive disorder (MDD). For example, careful research into IFN-α treatment has shown that 30–50% of patients on this therapy can develop a full complement of the criteria used to make a diagnosis of MDD (Capuron and Miller, 2004). A landmark double-blind controlled trial in 2001 showed that development of MDD in these patients could be attenuated by administering the SSRI antidepressant, paroxetine, two weeks before beginning IFN-α and continuing the antidepressant for the duration of the 12-week IFN-α therapy (Musselman et al., 2001). Interestingly, however, the antidepressant was not able to attenuate significant increases in two of the symptoms that can constitute MDD: fatigue and loss of appetite (Capuron et al., 2002). Furthermore, when examining the temporal occurrence of symptoms in just the placebo group, researchers found that these more "neurovegetative" symptoms occurred early during treatment while depressive and anxious symptoms occurred later. Both of these results led the investigators to conclude that the effects of cytokine treatment on these two different symptom dimensions are mediated by different neural mechanisms. Similar results emerged from a randomized controlled trial of cancer patients undergoing chemotherapy, which found that paroxetine reduced depressive symptoms but not symptom of fatigue (Morrow et al., 2003).

Investigators using animal models have come to similar conclusions. One study showed that aged mice (80–96 weeks) exhibited normal locomotor activity by 72 h following intraperitoneal injection of the inflammatory endotoxin, LPS, but behavior in a measure known as the Tail Suspension Test (TST), which is considered representative of depression, was still significantly elevated compared to vehicle-injected controls (Godbout et al., 2008). Similar results were found in a report that showed younger mice (10–14 weeks) exhibited normal locomotor activity by 24 h after peripheral LPS injection but still manifested significantly higher levels of depressive-like behavior in the TST compared to vehicle-injected controls (O'Connor et al., 2009a). Based on these findings, it has been suggested that inflammation-induced increases in fatigue-like behavior during general locomotion and depressive-like behavior during the TST are mediated by partially different systems in the brain (Frenois et al., 2007).

3.2. The inflammation-dopamine hypothesis

Given the results of several studies looking at the relationship between inflammatory activity and dopamine alterations in the brain, it has been proposed that dopaminergic mechanisms may constitute the specific system underlying inflammation-induced neurovegetative symptoms like fatigue as well as anhedonic behavior that can follow from inflammation (Capuron and Miller, 2004; Miller, 2009). Indeed, a large and growing body of clinical and experimental research has now demonstrated that anhedonia is associated with functional alterations of dopaminergic neurons emanating from the ventral tegmental area (VTA) and terminating in the limbic system (Dunlop and Nemeroff, 2007). This system of neurons is known as the mesolimbic dopamine pathway and is seen as an essential component of how the perception of pleasure or reward is mediated by the brain. Dopaminergic neurons emanating from an area adjacent to the VTA, known as the nigrostriatal dopamine tract, are critical to how the brain mediates psychomotor function and are damaged in Parkinson's disease (Meyer and Quenzer, 2005). Capuron and colleagues (2007) showed that cancer patients undergoing IFN-α therapy exhibited a significant positive correlation between fatigue and glucose metabolism in both the putamen, which constitutes the terminal end of the nigrostriatal dopamine pathway, and the nucleus accumbens at the terminal end of the mesolimbic dopamine pathway. That study replicated a previous finding of increased glucose metabolism in the putamen following 12 weeks of IFN-α therapy in hepatitis C patients (Juengling et al., 2000).

Some investigators conclude that the nature of the dopaminergic alteration underlying symptoms of fatigue may be an overall reduction in dopaminergic neurotransmission (see review by Miller, 2009). Indeed, research on the effect of repeated IFN-α

administration on dopamine levels in the whole brains of mice suggests that such treatment causes a decrease in dopaminergic tone (Shuto et al., 1997). In contrast, chronic repeated administration in rats was found to *increase* levels of dopamine in specific areas of the brain, including the cerebral cortex, hypothalamus, and medulla oblongata but not the hippocampus or thalamus (Kumai et al., 2000). Also, when looking at synaptic dopamine levels specifically in the mesolimbic system, one study found that LPS-induced peripheral inflammation causes a significant increase in this area (Borowski et al., 1998).

Given what is known about dopaminergic signaling in decreased locomotor behavior, there is reason to believe that "too much" dopaminergic tone may exist specifically in the neural systems underlying fatigue. Dopaminergic neurotransmission is regulated in part by two major types of receptors (Meyer and Quenzer, 2005). Activation of D1-like receptors (i.e., D1 and D5) causes an increase in the rate of cyclic adenosine monophosphate (cAMP) production inside the cell, which results in an increase of dopamine synthesis inside the cell. Activation of D2-like receptors (i.e., D2, D3, D4) causes a decrease in cAMP activity with subsequent decreased production of dopamine. D2-like receptor activation can also increase the opening of potassium ion channels on the cell membrane, thus facilitating a hyperpolarization of the neuron and decreasing its excitability. These inhibitory D2-like receptors reside on both the presynaptic cell as an autoreceptor and on the postsynaptic cell (Dunlop and Nemeroff, 2007), and their role in psychomotor retardation is well-established. For example, quinpirole, a standard D2/D3 agonist used in experimental research since the early 1980s (see review by Levant et al., 1992), causes increased spontaneous locomotion. Conversely, full antagonism of D2 function can result in complete psychomotor retardation in rodents, as exemplified by high doses of the first generation antipsychotic drug and D2 receptor antagonist, haloperidol (Meyer and Quenzer, 2005). In accord with this finding, fatigue can often be the biggest side effect reported by patients taking haloperidol in clinical trials (Gothelf et al., 2003). Thus, it is conceivable that results showing increased activity in the nigrostriatal tract in patients undergoing proinflammatory immunotherapy and a correlation between this activity and fatigue in cancer patients (Capuron et al., 2007; Juengling et al., 2000) could be mediated by decreased function of D2-like receptors in that area of the brain, giving rise to an increase in dopaminergic tone. However, further research is needed to confirm D2-like receptor deficiency as a definite mechanism of inflammation-dopamine alternations in cancer-related fatigue.

4. Biobehavioral factors that influence inflammation and cancer-related fatigue

As described previously, the basic model underlying research on inflammation and cancer-related fatigue suggests that tumors and the treatments used to treat them activate pro-inflammatory cytokines, leading to fatigue. However, there is considerable variability in the extent of the inflammatory response and in the experience of fatigue in cancer populations. This is particularly evident in the post-treatment period, with some patients reporting high and persistent fatigue symptoms and other reporting very low levels of fatigue (Donovan et al., 2007). At this point, our understanding of factors that influence the severity and persistence of inflammation and associated symptoms of fatigue in cancer patients and survivors is extremely limited. Identification of these factors is important for advancing our understanding of this symptom and for improving detection and treatment of vulnerable patients. In this section, we examine biological factors that are known to regulate pro-inflammatory cytokine production and have been associated with fatigue in cancer survivors and/or other populations, including polymorphisms in inflammatory risk genes, alterations in the hypothalamic–pituitary-adrenal (HPA) axis, and alterations in the cellular immune system and reactivation of latent herpesviruses. We also consider psychological and behavioral factors that may contribute to cancer-related fatigue through inflammatory pathways, including depression, sleep disturbance, psychological stress, and physical activity/body mass index (BMI). These factors are illustrated in Fig 1. Of note, some of these factors (e.g., SNPs, early life stress) are present prior to cancer diagnosis and treatment, whereas others (e.g., cellular immune system, HPA axis) may also be influenced by treatment exposures. Also, these factors may influence the onset, the severity, and/or the persistence of cancer-related fatigue.

4.1. Polymorphisms in inflammation-related genes

Genetic factors play an important role in regulating proinflammatory cytokine production. Single nucleotide polymorphisms (SNPs) that influence quantitative gene expression levels have been identified in the promoters of *IL1B* (−511 bases upstream of the transcription start site), *IL6* (−174), and *TNF* (−308), as well as other locations (Smith and Humphries, 2009). There is preliminary evidence that inflammation-related SNPs are associated with cancer-related fatigue during and after treatment. In a sample of 185 patients undergoing radiation therapy for breast, prostate, lung, or brain cancer, polymorphisms in *TNFA* and *IL6* were associated with elevations in fatigue (Aouizerat et al., 2009; Miaskowski et al., 2010). Jim and colleagues also found an association between polymorphisms in *TNFA* and *IL6* and fatigue in a small sample of 53 prostate cancer patients undergoing androgen deprivation therapy (Jim et al., 2012). In a large sample of lung cancer survivors, Rausch and colleagues found that polymorphisms in *IL1B* and *IL1RN* were associated with fatigue (Rausch et al., 2010). Similarly, we found an association between polymorphisms in *IL1B* and *IL6* and fatigue in breast cancer survivors (Collado-Hidalgo et al., 2008), although these findings were not replicated by another group (Reinertsen et al., 2011). Of note, cytokine polymorphisms have been linked to fatigue in other patient populations (Carlo-Stella et al., 2006; Piraino et al., 2012) and to clinical outcomes in cancer patients (DeMichele et al. 2009).

4.2. HPA axis alterations

Alterations in immune regulatory systems provide another plausible mechanism for elevated inflammatory activity and associated symptoms of fatigue in cancer populations. The HPA axis is known to regulate pro-inflammatory cytokine production (McEwen et al., 1997) via alterations in glucocorticoid production and/or decreased sensitivity of the glucocorticoid receptor (GR) to hormone ligation (Raison and Miller, 2003). We have shown deficits in both pathways in breast cancer survivors with persistent fatigue. In particular, fatigued breast cancer survivors show alterations in diurnal cortisol slope, with elevated levels of evening cortisol (Bower et al., 2005b) as well as blunted cortisol responses to psychological stress (Bower et al., 2005) that are correlated with elevations in stimulated cytokine production (Bower et al., 2007). To probe alterations in glucocorticoid receptor sensitivity, we conducted genome-wide transcriptional profiling of leukocytes from an independent sample of fatigued breast cancer survivors. Results showed a marked down-regulation of genes with response elements for the glucocorticoid receptor in fatigued vs. non-fatigued survivors, suggesting a state of functional GR resistance (Bower et al., 2011a). Reduced GR sensitivity may contribute to the tonic upregulation of NF-κB observed in fatigued survivors (Bower et al., 2011a), consistent with studies linking GR desensitization

to increased NF-κB activity in non-cancer populations (Cole et al., 2007; Miller et al., 2009; Miller et al., 2008b). Research in other cancer populations has also supported a link between HPA alterations and fatigue. For example, in a sample of ovarian cancer patients, higher levels of evening cortisol and reduced cortisol variability were associated with fatigue, vegetative depression, and functional disability (Weinrib et al., 2010).

Because these findings come from cross-sectional studies, it is unclear whether alterations in the HPA axis drive, or are driven by, inflammatory processes. In a longitudinal study of patients with malignant melanoma undergoing treatment with interferon-alpha (IFN-α), exaggerated HPA axis responses to the initial IFN-α administration predicted subsequent development of depression (Capuron et al., 2003), suggesting that HPA alterations may set the stage for behavioral disturbances in cancer patients. Further, HPA axis alterations have been observed in chronic fatigue syndrome and other fatigue-related disorders, and may act as a vulnerability factor for the development of these syndromes (Nater et al., 2008; Cleare, 2003; Crofford et al., 1994; Catley et al., 2000). Additional research on the role of HPA axis alterations in cancer-related fatigue (and other cancer-related behavioral disturbances) is required.

4.3. Cellular immune system and latent herpesvirus activation

Cancer treatments can cause pronounced and prolonged alterations in the cellular immune system (Rotstein et al., 1985; Solomayer et al., 2003), which may underlie long-term alterations in inflammatory activity. In our previous research, we have shown alterations in T cell populations and myeloid dendritic cells in breast cancer survivors with persistent fatigue that are correlated with inflammatory processes (Bower et al., 2003; Collado-Hidalgo et al., 2006). Other groups have shown more global changes in the cellular immune system in relation to fatigue, including elevations in leukocyte numbers among fatigued breast cancer survivors (Landmark-Hoyvik et al., 2009; Alexander et al., 2009).

Another potential explanation for elevated inflammatory processes in cancer patients is reactivation of latent herpesviruses (Glaser et al., 2005; Nazmi et al., 2010). A recent study conducted with breast cancer patients prior to treatment found that elevated CMV antibody titers were associated with a greater likelihood of being fatigued, as well as higher levels of CRP (Fagundes et al., 2012). These findings are consistent with earlier studies showing an association between seropositivity to latent herpesviruses and depressive symptoms in cardiac patients (Miller et al., 2005; Appels et al., 2000). Cancer treatments such as chemotherapy promote viral reactivation and associated increases in inflammatory markers (Kuo et al., 2008), which may have long-term implications for immune regulation and recovery as well as behavioral symptoms.

4.4. Psychological and behavioral factors

A number of psychological and behavioral factors are correlated with fatigue in cancer patients and survivors. We focus here on factors that have been linked with inflammatory processes and may influence fatigue through this pathway. Fatigue is strongly correlated with depression in cancer populations (Jacobsen et al., 2003), and history of depression predicted post-treatment fatigue in a longitudinal study of breast cancer survivors (Andrykowski et al., 2005). Given links between depression and inflammation (Howren et al., 2009; Raison et al., 2006), including increased inflammatory response to challenge in depressed individuals (Pace et al., 2006), it is possible that prior or current depression may set the stage for elevated inflammatory processes and associated symptoms of fatigue during cancer diagnosis and treatment. Similarly, sleep disturbance is correlated with cancer-related fatigue (Bower et al., 2000) and with inflammation (Irwin, 2002) and could plausibly contribute to inflammation-related fatigue in cancer populations.

Another psychosocial factor that may increase risk for inflammation and fatigue is psychological stress, including stress in early life. Both acute and chronic stressors are associated with elevations in proinflammatory cytokines (Steptoe et al., 2007; Kiecolt-Glaser et al., 2003), though links between stress and cancer-related fatigue have not been established. Early life stress is also associated with increased inflammation (Taylor et al., 2006; Danese et al., 2007) as well as elevated fatigue symptoms (McCauley et al., 1997) and chronic fatigue syndrome (Heim et al., 2006) in non-cancer populations. There is preliminary evidence that breast cancer survivors who experienced abuse or neglect as children report higher levels of fatigue (as well as elevated distress and reduced quality of life) (Fagundes et al., 2012). Together, these findings suggest a potential role for psychological stress in the etiology of cancer-related fatigue.

Physical inactivity and elevated body mass index (BMI) are both linked with cancer-related fatigue; indeed, in a large longitudinal study of women with early-stage breast cancer, BMI emerged as one of the key predictors of fatigue at 6 months and 42 months post-treatment (Donovan et al., 2007). These factors are also correlated with increased inflammatory markers (O'Connor et al., 2009b) and might influence fatigue through inflammatory pathways.

5. Conclusions, recommendations, and implications for treatment

The evolving literature on cancer-related fatigue increasingly supports the hypothesis that inflammation is associated with fatigue symptoms in cancer populations. The evidence linking inflammation and fatigue in cancer survivors is particularly strong, with consistent findings emerging from large, well-controlled studies of breast cancer survivors. At this point, there are no well-established animal models of cancer-related fatigue, which limits our ability to probe the underlying mechanisms for this symptom. Initial evidence suggests that the tumor itself, radiation, and chemotherapy can elicit increases in inflammation as well as alterations in locomotor activity in animal models, but links between these systems are still under investigation. The development of animal models of cancer-related fatigue is an important avenue for future research.

This review also highlighted several new frontiers in research on cancer-related fatigue. There is growing interest in neural mechanisms underlying inflammation-related fatigue, with a focus on dopaminergic pathways. However, there has been minimal examination of these processes in cancer patients and survivors. This is a promising avenue for future research. Another important research direction is the identification of factors that increase risk for inflammation and fatigue. There are a number of plausible biological and psychological factors that may contribute to cancer-related fatigue through inflammatory pathways. However, longitudinal studies of fatigue are still extremely limited, and to our knowledge none have simultaneously examined biological and psychological risk factors for fatigue and associated mechanisms in a prospective study design. This type of integrated assessment is critical for determining who is at risk for fatigue and why, which will direct the development and implementation of personalized, targeted interventions for prevention and treatment. Although not highlighted here, there is also substantial interest on "symptom clusters" in cancer populations and the potential for common underlying mechanisms.

At the methodological level, we have found stronger links between cancer-related fatigue and downstream markers of

inflammatory activity (e.g., sTNF-RII, IL-1RA, CRP) as compared to noisier instantaneous plasma cytokine levels (e.g., IL-1β, IL-6). These downstream markers may provide a more robust, stable, and sensitive marker of systemic inflammation, facilitating the detection of relationships with fatigue. Thus, we recommend their incorporation in future research, particularly studies with post-treatment survivors.

Another issue of great importance in this field is the development of effective treatments for cancer-related fatigue. If fatigue is driven by activation of pro-inflammatory cytokines, cytokine antagonists may have promise for reducing this symptom in cancer populations. Indeed, clinical trials have documented beneficial effects of cytokine antagonists on fatigue in patients with inflammatory disorders (Tyring et al., 2006), and there is preliminary evidence that these therapies may also decrease fatigue in patients with advanced cancer. In a study of patients with advanced malignancies undergoing weekly docetaxel chemotherapy, those randomized to receive eterancept, a TNF decoy receptor, reported significantly lower levels of fatigue (Monk et al., 2006). Our research group (Bower, Ganz, Irwin, & Cole) conducted a small pilot study to evaluate the acute effects of infliximab, a monoclonal antibody against TNF, in breast cancer survivors with severe, persistent fatigue. The five women enrolled in this trial had completed their cancer treatments at least one year previously, showed no evidence of cancer recurrence, and were determined to be healthy after careful screening. Participants completed daily diaries for two weeks before and after receiving a single dose of infliximab to assess changes in the severity and duration of daily fatigue. All five women reported reductions in daily fatigue, including a mean 1.9 point decrease in "worst" fatigue from pre- to post-treatment (range = 0.3–2.7 point decrease). These very preliminary findings require replication in a larger, double-blind trial, but do offer initial support for a possible role of cytokine antagonists as therapeutic agents for post-treatment fatigue.

Many cancer patients and survivors will not be eligible for or interested in treatment with cytokine antagonists or other pharmacotherapies (e.g., methylphenidate; Minton et al., 2011). Fortunately, psychological and behavioral approaches also show considerable promise for treating cancer-related fatigue. In randomized controlled trials with cancer populations, exercise has been associated with reduced fatigue (Kangas et al., 2008); exercise also leads to reductions in inflammatory markers in cancer patients (Fairey et al., 2005), and may influence fatigue through effects on inflammatory processes. We recently evaluated a specialized yoga intervention for breast cancer survivors with persistent fatigue and found clinically significant improvements in fatigue and vigor in survivors randomized to yoga vs. health education (Bower et al., 2011c). Cognitive-behavioral therapy is also effective in reducing fatigue among cancer survivors with severe, persistent fatigue (Gielissen et al., 2006). Both yoga and cognitive-behavioral therapies are associated with reduced inflammatory activity (Kiecolt-Glaser et al., 2010; Pullen et al., 2008), including decreased activity of the pro-inflammatory transcription factor NF-κB (Antoni et al., 2012), suggesting one pathway for their beneficial effects. Ultimately, identifying the mechanisms underlying cancer-related fatigue and developing targeted interventions to prevent and/or ameliorate this symptom will enhance survivorship and long-term quality of life in the growing population of cancer survivors.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Review

Sleep disturbance, inflammation and depression risk in cancer survivors

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ABSTRACT

Over two-thirds of the 11.4 million cancer survivors in the United States can expect long-term survival, with many others living with cancer as a chronic disease controlled by ongoing therapy. However, behavioral co-morbidities often arise during treatment and persist long-term to complicate survival and reduce quality of life. In this review, the inter-relationships between cancer, depression, and sleep disturbance are described, with a focus on the role of sleep disturbance as a risk factor for depression. Increasing evidence also links alterations in inflammatory biology dynamics to these long-term effects of cancer diagnosis and treatment, and the hypothesis that sleep disturbance drives inflammation, which together contribute to depression, is discussed. Better understanding of the associations between inflammation and behavioral co-morbidities has the potential to refine prediction of risk and development of strategies for the prevention and treatment of sleep disturbance and depression in cancer survivors.

1. Introduction

With over 11.4 million cancer survivors, nearly 5% of the US population has been diagnosed with cancer in 2006 (Office of Cancer Survivorship, 2009). Over the next decade, the number of cancer survivors will grow dramatically due to the aging of the population and the resultant increased cancer incidence. Moreover, due to earlier detection and successful treatment for the disease, over two-thirds of individuals diagnosed with cancer today can expect long-term survival, with many others living with cancer as a chronic disease controlled by ongoing therapy.

Despite considerable advancements in diagnosis and treatment, symptoms or problems often arise during treatment and persist long-term to complicate improved survival and reduce quality of life. Indeed long-term behavioral co-morbidities such as depression and sleep disturbance are prominent and are thought to relate to the onset of cancer-related somatic symptoms such as fatigue and pain, and possibly cancer recurrence or second malignancies. In this review, the inter-relationships between cancer, depression, and sleep disturbance are described, with a focus on the role of sleep disturbance as a risk factor for depression. In addition, increasing evidence links alterations in inflammatory biology dynamics to these long-term and late effects of cancer treatment, and the hypothesis that sleep disturbance drives inflammation, which together contribute to depression, is discussed. Given the growing number of cancer survivors, effective management of the late effects of cancer and its treatment is needed. To this end, better understanding of the associations between inflammation and post-treatment symptoms has the potential to inform risk identification and the development of strategies for prevention and treatment of such behavioral co-morbidities as sleep disturbance and depression.

2. Depression

2.1. Prevalence

Major depression in patients with cancer occurs at a high rate, with a median point prevalence (15–29%) that is approximately three to five times greater than the general population (Miller et al., 2008; Raison et al., 2003; Rooney et al., 2011). However, prevalence estimates vary from 1.5% to 50%, depending on the cancer type, as well as the definition of depression and method of assessment (Fann et al., 2008; Massie, 2004). Indeed, the majority of studies find that 20–30% of women with breast cancer, for example, experience elevated depressive symptoms (Fann et al., 2008). Yet, the prevalence of major depressive disorder may be considerably lower; major depressive disorder is a clinical syndrome that lasts for at least 2 weeks and causes significant impairment in normal functioning. Among women newly diagnosed with breast

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cancer, about 9% also experience major depression as determined by a structured clinical interview (Coyne et al., 1995). In contrast, among women with recurrent breast cancer, the prevalence of depression appears to be considerably higher (Gotay et al., 2007) with rates substantially exceeding that of the general population (Massie, 2004). Mitchell et al. recently examined the meta-analytical pooled prevalence of depression defined by the Diagnostic and Statistical Manual of Mental Disorders using 24 studies with 4007 individuals across seven countries in palliative-care settings, and found rates of 16·5% (95% CI 13·1–20·3) for DSM-defined major depression, and 9·6% (3·6–18·1) for DSM-defined minor depression, although all types of depression occurred in 20·7% (12·9–29·8) of patients (Mitchell et al., 2011). However, despite the high prevalence of depression in association with cancer, there are few consistent correlates of this risk, as neither age, sex, nor clinical setting are associated with depression. Furthermore, there are inadequate data to examine the effects of cancer type and illness duration on depression occurrence (Mitchell et al., 2011).

2.2. Depression disease burden

As the population ages and the number of cancer survivors grows dramatically, depression is will increase by 2030 to a position of the greatest contributor to illness burden (Mathers and Loncar, 2006). Moreover, because adults and especially older adults with depression often do not receive diagnosis and treatment (Alexopoulos, 2005), and only about 30–35% achieve remission using current treatment approaches (Evans et al., 2005), over two-thirds of the depression disease burden persists (Andrews et al., 2004; Chisholm et al., 2004) leading to staggering health care costs (Vos et al., 2004).

Depressed patients are also less likely to adhere to recommendations for treatment including cancer therapies. Meta-analytic findings demonstrate that depression triples the risk for nonadherence to medications (Fann et al., 2008). In turn, the use of emergency and medical inpatient services is disproportionately increased in persons diagnosed with cancer. For example, among Medicare beneficiaries diagnosed with cancer, use of emergency departments and medical inpatient services is twice as likely if they have significant depressive symptoms than if they do not (Himelhoch et al., 2004). These findings are consistent with other diseases in which co-morbid depression increases health care use, functional disability, and work absence (Stein et al., 2006). Lower medical adherence which is associated with poorer understanding of treatment recommendations, which in turn heightens anxiety about treatment adverse effects in depressed cancer patients (Ell et al., 2005).

Depression also increases risk for morbidity and mortality in several chronic diseases, including AIDS (Ickovics et al., 2001) and cardiovascular disease (Barefoot et al., 2000). In addition, some evidence suggests that depressed cancer patients have increased morbidity and, possibly, mortality (Gallo et al., 2007; Spiegel and Giese-Davis, 2003), although findings among cancer survivors are less consistent (Goodwin et al., 2004; Steel et al., 2007). Nevertheless, when the association between depressive symptoms and mortality rates were compared between participants with and without cancer among 10,025 participants in the National Health and Nutrition Examination Survey (Onitilo et al., 2006), results demonstrated that participants with both cancer and depressive symptoms had a 19% increased risk of death compared to participants with cancer only after adjusting for confounders. Likewise, Mykletun et al. (2007) found a 33% increased risk of death from cancer associated with disorder-level depressive symptoms in a prospective study of 61,349 adults in Norway followed for 4.4 years after depression assessment. Moreover, when the depression is unremitting and chronic, adverse health outcomes are more prominent as compared to the effects of an acute depressive episode (e.g., Evans et al., 2005; Stommel et al., 2002). Finally, Pril et al. recently reported that the presence of major depression was associated with worse survival in patients with non-small cell lung carcinoma, although treatment of such depression did not yield survival benefit (Pirl et al., 2012).

2.3. Risk factors: role of cancer in catalyzing the occurrence of depression

In the midst of receiving a diagnosis of cancer and managing the subsequent psychological and physiological challenges, rates of depressive symptoms markedly rise with increases typically highest in the first 6 months after cancer diagnosis, followed by declines over time with adjustment to the initial shock of diagnosis and acute effects of cancer treatment (Schag et al., 1993). For example, cancer treatments alone are associated with increased risks of depressive symptoms; prior to medical treatment about 10% of patients diagnosed with various cancers show depressed mood or anhedonia, two hallmark symptoms of depression, whereas during treatment over 20% endorse one of these symptoms (Fann et al., 2009). Importantly, one large-scale prospective study suggests that cancer diagnosis and treatment provokes depression leading to a 4-fold increase in depression occurrence during the first two years after diagnosis as compared those who remain medically healthy. Polsky et al. (2005) examined depressive symptoms prior to and after disease diagnosis in five biennial waves of the Health and Retirement Study involving more than 8000 adults aged 51–61 years without depressive symptoms at study onset. Within 2 years following diagnosis, individuals with cancer had the highest risk of significant depressive symptoms relative to no incident disease (Hazard Ratio = 3.55) and other diagnosed diseases (e.g., heart disease, arthritis). Moreover, the experience of cancer can prompt an episode of depression in addition to triggering depressive symptoms. Kangas et al. (2005) found that 29% of head/neck/lung cancer patients met interview-assessed criteria for major depressive disorder within 1 month of diagnosis, of whom only 29% met criteria for prior lifetime major depressive disorder. At 6 months, 22% met criteria for major depressive disorder, and 27% of those were new diagnoses. In comparison, note that the major depressive disorder point prevalence in women in the US general population is estimated at 5–9% and lifetime prevalence at 21% (Kessler et al., 2005); men have lower rates. Thus, the prevalence of depression in cancer survivors might be nearly 4-fold greater than that in the general population. Studies also document elevated suicidal ideation/behavior in individuals with cancer, including breast cancer (for review see: Miller et al. (2008)).

3. Sleep disturbance

3.1. Prevalence

Even before treatment, patients with cancer report significant sleep impairment, and among cancer survivors, sleep problems persist well beyond primary treatment. In an unselected sample of women with non-metastatic breast cancer, Savard et al. (2001) found that 19% met diagnostic criteria for insomnia, and that 58% of the women reported that the cancer or its treatment (e.g., adjuvant tamoxifen) either caused or aggravated their sleep problems. Indeed, cancer survivors have a 2- to 3-fold increase in the prevalence of insomnia symptoms compared to healthy adults, with nearly 51% reporting difficulties sleeping during the cancer survivorship period (Savard and Morin, 2001). However, patients with different types of cancer reported different rates of sleep problems. In a survey of more than 1000 patients with different types of cancer, 31%

reported insomnia, with lung cancer patients having highest prevalence of sleep problems in general, and breast cancer patients having the highest prevalence of insomnia coupled with fatigue (Davidson et al., 2002). However, in a sample of 823 patients with diverse cancers receiving chemotherapy longitudinally, Palesh et al. found that breast cancer patients had the highest number of overall insomnia complaints, although lung cancer patients showed the highest prevalence of insomnia diagnosis or syndrome (Palesh et al., 2010). Importantly, the severity of insomnia persisted over the course of chemotherapy treatment with similar rates of insomnia complaints at cycle 1 and cycle 2. Polysomnographic data confirm reduced sleep efficiency, prolonged latency to fall asleep, and increased awake time during the night among cancer survivors (Savard et al., 2001), and lung cancer show marked reductions in sleep efficiency as compared to insomniacs or breast cancer survivors (Silberfarb et al., 1993). However in one study, total sleep time as indexed by actigraphy increased during a course of chemotherapy in breast cancer patients (Liu et al., 2012b).

3.2. Sleep disturbance and disease burden

Sleep disturbance comes at a considerable price. For example, sleep disturbance is a predictor of cancer-related fatigue. As pointed out by Fiorentino and Ancoli-Israel in a recent review of insomnia and its treatment in women with breast cancer (Fiorentino and Ancoli-Israel, 2006), sleep disturbance often co-occurs with fatigue in breast cancer patients and survivors. Disturbed sleep can be a predictor of fatigue; in turn, less physical activity as a result of fatigue may exacerbate sleep disturbance. However, among women undergoing chemotherapy for breast cancer, fatigue was related to daytime sleepiness and subjective reports of poor sleep quality, but not total sleep time as objectively assessed by actigraphy (Liu et al., 2012b). Hence, is unclear whether these two symptoms of fatigue and sleep disturbance merely co-occur in the same patient or have a causal association. Nevertheless, other data as further discussed below indicate that sleep disturbance is a potent predictor of depression relapse in persons with a history of prior depression (Cho et al., 2008), although there are no prospective data to address this question in cancer survivors.

Epidemiological data also implicate insomnia as a predictor of all-cause disease mortality over and above age, gender, and medical burden (Althuis et al., 1998; Ayas et al., 2003; Dew et al., 2003; Mallon et al., 2002; Newman et al., 2000). Indeed, EEG measures of prolonged sleep latency, sleep efficiency, and percentage of REM sleep predict all-cause mortality taking into the account of other known factors (e.g., age, gender, medical burden) (odds ratio 2.1, 95% CI: 1.25–3.66; odds ratio 1.93, 95% CI: 1.14–3.25; odds ratio, 1.7, 95% CI: 1.01–2.91) (Dew et al., 2003).

3.3. Risk factors: role of cancer

In cancer survivors, sleep impairments are primarily characterized by problems falling asleep (Andrykowski et al., 1998), with difficulties of sleep maintenance (Engstrom et al., 1999) and duration also reported (Savard and Morin, 2001). Given the varying kinds of sleep disturbance found in cancer survivors, it is thought that a number of different clinical factors can precipitate and/or perpetuate insomnia in cancer survivors. Old age, for example, increases the vulnerability for insomnia in cancer survivors (Savard and Morin, 2001). Furthermore, it is generally assumed that insomnia is secondary to psychological distress and anxiety of cancer diagnosis and treatment, although Cimprich (1999) found that sleep problems are frequent even in those patients who report low levels of anxiety. Pain is less likely to be a factor in sleep problems among cancer survivors who show no indication of residual or recurrent disease (Fortner et al., 2002).

Among cancer survivors, other cancer specific factors should also be considered. For example, Berger and Farr (1999) reported that women undergoing chemotherapy showed a progressive increase in the number of awakenings with the duration of the treatment cycle. However, Liu et al. found no change in subjective sleep quality with an increase in total sleep time, during chemotherapy for women with breast cancer (Liu et al., 2012b) similar to the findings of Palesh et al. across diverse cancer types (Palesh et al., 2010). Other studies also report that the prevalence of sleep problems was not related to time since diagnosis nor to treatment type (Savard and Morin, 2001) and that sleep disturbance was similar across groups who receive different treatment (e.g., surgery, chemotherapy, radiation) (Davidson et al., 2002), although nocturnal menopausal symptoms increases reports of insomnia. Nevertheless, high rates of sleep complaints occur after initiation of adjuvant therapy (e.g., tamoxifen) for breast cancer, which can hasten premature menopause. Together, these data suggest that insomnia develops a chronic course in a substantial proportion of cancer survivors, especially in older adults (Savard and Morin, 2001), contributing to fatigue, impairments in quality of life, and possibly depression. Despite such evidence, it also important to note that findings are mixed and adjuvant endocrine therapies (e.g., tamoxifen) have not been consistently related to either the onset or maintenance of insomnia symptoms (Berger and Higginbotham, 2000; Stein et al., 2000). Circadian rhythm disturbances might also contribute to sleep disturbance, but consideration of the associations between cancer and biological rhythms is beyond the scope of this review. In sum, multiple cancer-specific factors increase vulnerability for sleep complaints and likely contribute to the perpetuation of these sleep problems and the onset of sleep disturbance in breast cancer survivors (Savard and Morin, 2001). However, additional research is needed to uncover cancer-specific physiological, psychological, and behavioral factors that contribute to the development of insomnia during chemotherapy, similar to the conclusion offered by Palesh et al. in the largest study to date that longitudinally examined insomnia symptoms and clinical characteristics in a diverse sample of cancer patients undergoing chemotherapy.

4. Sleep disturbance and depression risk

Insomnia is the most frequent sleep disturbance in depressed patients, and such sleep impairment is viewed as a symptomatic dimension of current depression. Sleep disturbance often lingers and its persistence can represent a residual phase of a major mood disorder. Alternatively, emergence of disturbed sleep may serve as a precursor or prodrome of depression that occurs later in life. Indeed, insomnia is increasingly viewed as an independent risk factor for depression. Although there are no prospective data in cancer survivors, some limited prospective data are available in non-cancer adults and older adults. For example, as part of the Epidemiologic Catchment Area study, Ford and Kamerow (1989) found that persistent insomnia was associated with the incidence of new major depression. As such, these findings were among the first to identify the need for research to determine if early recognition and treatment of sleep disturbance, a modifiable risk factor (Cole and Dendukuri, 2003), might forestall or prevent incident depression. Even less is known about the prospective association between sleep disturbance and depression recurrence, as opposed to incident new major depression. Whereas sleep disturbance has been shown to predict depression relapse and recurrence in late life (Buysse et al., 1996; Dew et al., 2001; Dombrovski et al., 2007; Flint and Rifat, 2000), these data are limited to populations who were conveniently recruited from specialty clinics. Hence, the generalizability of such observations to community dwelling adults and/or cancer survivors is not known.

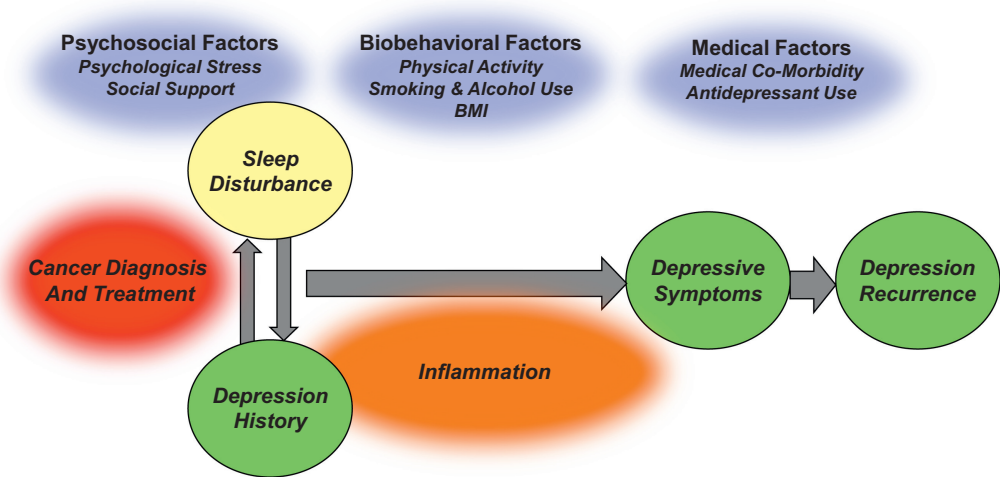

Fig. 1. Model depicting the impact of depression history and sleep disturbance on occurrence of depressive symptoms and depressive episode(s), taking into account the relationships with inflammation and psychosocial-, biobehavioral-, and medical factors in cancer survivors.

Furthermore, despite evidence that sleep disturbance and other factors (e.g., life stress, social support) are associated with persistence of depression in community dwelling adults (Beekman et al., 2001, 1995; Denihan et al., 2000), their prospective contribution to depression recurrence has not been extensively evaluated nor examined in large groups of cancer survivors. For example, in a meta-analysis of 20 prospective studies (Cole and Dendukuri, 2003), only four studies used multivariate approaches to identify sleep disturbance as a predictor of depression onset among elderly community subjects and none of these studies addressed cancer-related variable, and many of these studies included small samples. However, in community-dwelling older adults, Cho et al. recently found in a two-year longitudinal study that sleep disturbance is a predictor of incident depression among non-depressed elderly community subjects, and that the risk posed by sleep disturbance is greater – maybe specific – for depression recurrence than for depression incidence (Cho et al., 2008). For example, in this sample depression occurrence was strongly predicted by prior depression history (odds ratio 38.65, 95% CI 4.72–316.43, $P = 0.001$), with a cumulative incidence of depressive disorders of 16.9% (11.0–24.3%) at 2 years. Moreover, sleep disturbance at baseline was significantly associated with depression recurrence (odds ratio 4.84, 95% CI 1.40–16.73, $P = 0.01$), taking into account other predictors of depression recurrence including antidepressant medication use (3.25, 1.23–8.53, $P = 0.02$), and other depressive symptoms (1.15, 1.02–1.30, $P = 0.03$). Given that over one-third of individuals with prior depression history were using antidepressant medications, and such use was not protective in ameliorating the risk of sleep disturbance on depression occurrence, interventions that specifically target sleep disturbance might have a unique role in reducing subsequent depressive morbidity. Importantly, cancer diagnosis and treatment introduce many extraneous variables that might alter the association between sleep disturbance and depression risk; research that targets cancer survivors is needed to determine whether risk models that perform well in older adults generalize to cancer survivors.

5. Inflammation, sleep disturbance and depressive symptoms in cancer

5.1. Overview

As shown in Fig. 1, among the biological mechanisms contributing to onset and maintenance of sleep disturbance and/or depression, inflammation has emerged as one important pathway, which may be especially relevant in cancer survivors. In association with increased prevalence of sleep disturbance and depression, prominent increases in proinflammatory cytokine activity occur in cancer survivors. Indeed, epidemiological studies have shown that chronic inflammation predisposes individuals to various types of cancer including breast cancer, and underlying inflammatory responses are linked to 15–20% of all deaths from cancer worldwide (Mantovani et al., 2008). In addition, chronic inflammation is reported to be associated with recurrence of breast cancer (Cole, 2009). Moreover, cancer treatments are potential inducers of inflammation. For example, chemotherapy is associated with acute increases in inflammatory markers (Mills et al., 2008, 2004; Wang et al., 2010), which may persist long into survivorship, with some data suggesting that such increases may be associated with fatigue and sleep disturbance during chemotherapy treatment (Liu et al., 2012a,b). Among the panoply of molecules involved in cancer-related inflammation, key endogenous factors can be identified including transcription factors such as nuclear factor-κB (NF-κB), and major inflammatory cytokines (such as IL-1β, IL-6, and TNF-α) (Karin, 2006). As described below, wake–sleep cycles have emerged as homeostatic regulators of inflammatory biology, in which sleep loss induces activation of NF-κB to coordinate the production of inflammatory mediators and systemic inflammation. In turn, proinflammatory cytokines are thought to contribute, in part, to the onset of depressive symptoms. In addition, inflammatory processes are being investigated as explanatory mechanisms for the demonstrated links between depression and morbidity and all-cause and disease-specific mortality, including cancer.

Studies in laboratory animals and humans provide compelling evidence that administration of innate immune cytokines induces a syndrome of "sickness behavior" that has many overlapping features with the behavioral symptoms commonly experienced by cancer patients, including sleep disturbance and depression (Dantzer et al., 2008; Irwin and Cole, 2011). Such behavioral effects of inflammatory cytokines are secondary to the capacity of peripheral cytokine signals to access the brain, activate inflammatory responses within the brain, and interact with neurobiolgical pathways involved in behavioral states such as sleep disturbance and depression (Dantzer et al., 2008; Irwin and Cole, 2011; Miller et al., 2008). Within the brain, pro-inflammatory cytokines decrease the activity of key behavior-modulating neurotransmitter systems including norepinephrine, dopamine and serotonin (Miller et al., 2009) all of which play a major role in the regulation of

multiple behaviors and are the primary targets for currently available psychopharmacologic treatments of depression (Dantzer et al., 2008; Irwin and Cole, 2011; Miller et al., 2008).

5.2. Sleep disturbance and inflammation

Sleep has key consequences for proinflammatory cytokine expression. Experimental sleep restriction, with a loss of total sleep time, leads to daytime increases in production and circulating levels IL-6 levels (Meier-Ewert et al., 2004; Redwine et al., 2003; Shearer et al., 2001; Vgontzas et al., 1999). In addition, loss of sleep for only part of the night induces an upregulation of leukocyte expression of pro-inflammatory cytokine genes and increased NF-kB activity (Irwin et al., 2006b, 2008). These effects are especially pronounced in women (Irwin et al., 2010, 2008), possibly due to sex differences in sympathetic nervous system upregulation of IL-6 production (O'Connor et al., 2007), which together might contribute to sex differences in the incidence of inflammation-related behavioral and autoimmune diseases. Observational studies have also identified elevated levels of C-reactive protein (CRP) and other inflammation-related biomarkers in night-shift workers who have a disruption of sleep–wake activity, as well as insomniacs who report poor sleep quality and disruption of sleep continuity (e.g., difficulties with sleep onset or sleep maintenance with an overall decreases in total sleep time) (Motivala and Irwin, 2007). Little is know about the effects of hypersomnia on inflammatory markers, although there is evidence that long sleepers (i.e., total sleep time more than 8 h per day) have an increased risk of mortality similar to short sleeper (i.e., total sleep time less than 6 h per day) (Kripke et al., 2002). However, despite evidence of increased inflammation in cancer survivors and compelling observational and experimental data indicating a relationship between sleep disturbance and innate immune cytokines such as IL-6, the relationship between sleep and inflammatory markers in cancer survivors has not been rigorously examined.

Systemic inflammation may also have reciprocal effects and play a role in the homeostatic regulation of sleep (Imeri and Opp, 2009). Animal genetic studies and LPS administration studies in humans (Imeri and Opp, 2009; Mullington et al., 2000) have linked changes in non-rapid eye movement (NREM) sleep to elevated levels of circulating type I IFN and pro-inflammatory cytokines. In animals, cytokines such as TNF increase NREM- and decrease REM sleep (Imeri and Opp, 2009), although Hogan et al. found that IL-6 administration produces a fragmentation of sleep in rats (Hogan et al., 2003). In humans, pharmacological administration of a single dose of IL-6 or treatment with IFNα in humans induce decreases in NREM, slow-wave sleep and increases in REM sleep (Imeri and Opp, 2009; Raison and Miller, 2003). In contrast, a single dose of endotoxin, which induces physiologic, as opposed to pharmacological, elevations of proinflammatory cytokines, has been found to enhance sleep depth (Mullington et al., 2000). However, these pharmacologic and experimental approaches yield elevations of proinflammatory cytokines that are several fold greater than found in the resting, baseline condition, and a further question concerns the association between varying levels of proinflammatory cytokines at rest and sleep measures. In healthy individuals, levels of IL-6 and TNFα obtained prior to sleep onset, and without experimental or pharmacologic induction, temporally correlate with increased sleep latency and REM sleep independent of other clinical factors (Irwin et al., 2004). Moreover, TNF antagonism that blocks endogenous levels of TNFα has also been found to normalize REM sleep levels (e.g., in abstinent alcohol-dependent patients, who have elevated amounts of REM sleep) (Irwin et al., 2009).

In summary, regulation of sleep architecture by the innate immune system may play a substantial role in structuring overall inflammatory homeostasis (Imeri and Opp, 2009; Motivala and Irwin, 2007), given the substantial fraction of time we spend asleep, the general immunological activation that occurs during sleep (Motivala and Irwin, 2007) and epidemiological links between abnormally high REM sleep and mortality (Dew et al., 2003). Future research is needed to determine whether increases of proinflammatory cytokines are a key biological mechanism through which disordered sleep accelerates morbid outcomes, including depression risk, in cancer survivors.

5.3. Depression and inflammation

Proinflammatory cytokines are thought to contribute, in part, to the onset of depressive symptoms, and possibly to the onset and incidence of major depressive disorder in susceptible populations such as cancer survivors. A recent meta-analysis (Howren et al., 2009) revealed significant effect sizes for the relation of depression and C-reactive protein (CRP) (51 studies), interleukin (IL)-6 (62 studies), IL-1 (14 studies), and IL-1 receptor antagonist (IL-1ra) (9 studies), with effect sizes ranging from $d = 0.15$ to 0.35. Effect sizes remained significant when body mass index was controlled, and the effects were larger when medication use (e.g., antidepressants, statins) was controlled. Clinically depressed patient samples demonstrated the strongest relations, although relations also were reliable in community samples. Among the seven study samples with cancer, depression was related significantly to IL-6 (Musselman et al., 2001b; Seruga et al., 2008; Soygur et al., 2007). Tumor necrosis factor (TNF-α) also has been linked to depression (Seruga et al., 2008); relevant research on other inflammatory markers was not available in cancer samples. Howren et al. (2009) noted the plausibility of causation in either direction, although as described above, basic animal research has implicated proinflammatory activity in inducing a motivational state that parallels depressive symptoms (Dantzer et al., 2008). Indeed, some large scale prospective studies have found that elevated levels of systemic inflammation (e.g., C-reactive protein, CRP) are prospectively associated with depression (Gimeno et al., 2009; Matthews et al., 2007), although others have suggested that depression leads to elevated levels of inflammatory markers (Duivis et al., 2011). Nevertheless, administration of the cytokine interferon-α to cancer patients and other clinical samples induces increases of depressive symptoms that are responsive to treatment with antidepressants (Miller et al., 2008). Together, these naturalistic and experimental observations suggest that activation of inflammatory pathways may be causally linked to the onset of depressive symptoms, at least in certain vulnerable patient populations such as those with cancer.

Inflammatory cytokines can alter the metabolism of key monoamines that are involved in the pathogenesis of mood disorders (Dantzer et al., 1999). For example, cytokine-induced behavioral changes are associated with alterations in the metabolism of relevant neurotransmitters such as serotonin, norepinephrine, and dopamine, all of which play a major role in the regulation of depressive symptoms. (Miller et al., 2008, 2009). Proinflammatory cytokines through stimulation of multiple inflammatory signaling pathways (e.g., nuclear factor κB, NF-κB) activate neurotransmitter metabolism, which in turns leads to decreased availability of tryptophan, the amino acid precursor for serotonin. Furthermore, the reuptake of monoamines is influenced by cytokines and their signaling pathways, which further reduces the availability of these neurotransmitters contributing behavioral symptoms such as depression.

Additionally, recent evidence suggests that polymorphisms in several cytokine genes are potential markers for genetic susceptibility for depressive symptoms and treatment response (Miller et al., 2009). Together, these results suggest that blockade of inflammatory cytokines might diminish risk and improve treatment response of depression, although there are no studies in

cancer survivors with evidence of increases in markers of inflammation. These data also raise the possibility that circulating inflammatory biomarkers and inflammation-related genetic polymorphisms might be used to help guide targeted therapy for specific major depressive disorder subtypes that are thought to have an inflammatory component such as depression in older adults or in those with inflammation-associated disease including cancer. It is important to note further that links between inflammation and depressive symptoms have also led to the hypothesis that the increased prevalence of major depressive in modern industrialized society may stem from decreased exposure to tolerogenic microorganisms, which contributes to dysregulated inflammatory signaling (a psychiatric version of the ''hygiene hypothesis'') (Raison et al., 2010).

Given the ''main effects'' of inflammation-related gene polymorphisms on depression (i.e., mediated via genetic effects on inflammatory cytokine levels), it is also conceivable that inflammatory signaling may interact with brain neurotransmitter systems to affect the risk or severity of depression. Under such a model, polymorphisms in neurotransmitter-related genes (e.g., the serotonin transporter promoter polymorphism *5HTTLPR*, coding polymorphisms in *BDNF* and *COMT* genes; (Uher and McGuffin, 2008) might affect CNS vulnerability/resilience to the depression-promoting effects of inflammatory cytokines. With this hypothesis, inflammation could be viewed as a moderator of brain-related genetic risk factors for depression, serving as a physiologic ''stress'' to actualize a genetic ''diathesis'' as previously observed in studies of interaction between psychosocial stress and the *5HTTLPR* polymorphism (Caspi et al., 2003). Consistent with this notion, antidepressant medications (e.g., serotonin- and norepinephrine reuptake inhibitors) interact with brain neurotransmitter gene polymorphisms (serotonin transporter gene) to affect monoamine metabolism, which in turn have been shown to influence the development of cytokine-induced depressive symptoms in humans (Bull et al., 2009). Alternatively, variants neurotransmitter genes such as the serotonin transporter gene might influence inflammatory profile and risk for depression. Indeed, it is thought that variants in the serotonin transporter gene (i.e., 5-HTTLPR polymorphisms) influence susceptibility to depression in which those carrying two short alleles of the 5-HTTLPR gene have increased risk of depression. Recent evidence demonstrated that SS individuals exhibit a proinflammatory bias under resting conditions and/or during laboratory stress (Fredericks et al., 2010). Moreover, among individual who have the serotonin transporter gene variant, SLC6A4 gene, increases in both depressive symptoms and IL-6 plasma levels have been identified (Su et al., 2009).

Functional neuroimaging studies in humans have begun to map the specific neural circuits associated with cytokine-induced depressive symptoms, and have identified altered connectivity among the subgenual anterior cingulate cortex, amygdala and medial prefrontal cortex (Eisenberger et al., 2009; Harrison et al., 2009) and reduced ventral striatum response to reward cues (Eisenberger et al., 2010a). It is known, for example, that clinical depression is associated with alterations in brain circuits that are involved in emotion processing (e.g., anterior cingulate cortex). In addition to playing an important role in detecting physical and social threat, and subsequently recruiting attention and coping resources to minimize danger (Lieberman and Eisenberger, 2009), increased activity of the anterior cingulate cortex has been demonstrated in individuals at risk for mood and anxiety disorders, including those with high-trait anxiety, neuroticism, and obsessive–compulsive disorder (Eisenberger and Lieberman, 2004). Using an experimental challenge, inflammatory activation increased in regional blood flow in the dorsal part of the anterior cingulate cortex (dACC) as revealed by functional magnetic resonance imaging during a social rejection task (Eisenberger et al., 2010b, 2009). In association with such cytokine-inducted activation of the dACC, depressive symptoms were elevated, and the severity of depressive symptoms correlated with dACC in women, but not in men (Eisenberger et al., 2009). Finally, we have found that experimentally-induced systemic inflammation alters neural activity that is associated with anhedonia, a key diagnostic symptom of depression (Eisenberger et al., 2010a). Along with increases in self-reported and observer-rated depressed mood, activity in the ventral striatum was reduced in response to monetary reward cues. Hence, inflammation alters reward-related neural responding in humans and these reward-related neural responses mediate the effects of inflammation on depressed mood (Eisenberger et al., 2010a).

In summary, we and others have found that activation of proinflammatory cytokines leads to marked increases in depressed mood (Eisenberger et al., 2010b, 2009). In contrast, pharmacologic blockade of inflammation activity improves sleep (Irwin et al., 2009) and reduces depressive symptom severity (Tyring et al., 2006). Further research is needed to examine the bi-directional relationships between inflammation and depression occurrence, and the potential role of sleep disturbance in driving activation of inflammatory biology. Such work has the potential to guide development of personalized strategies for depression treatment, and possibly depression prevention in cancer survivors who are at high risk for depression occurrence and/or recurrence.

6. Development of biological treatments targeting inflammatory biology

Biological pathways such as inflammation are hypothesized to contribute to depression occurrence, which together are driven in part by biobehavioral risk factors such as sleep disturbance. Hence, it is important to utilize such pathophysiological models of depression to inform the development of interventions that have the potential to treat and/or prevent depression (Munoz et al., 2010; Reynolds, 2009). Insight into the role of these biological mechanisms has the potential to improve the efficiency of risk identification and depression prevention and possibly treatment, which may be especially true for cancer survivors who show greater abnormalities in inflammatory dynamics. As an example, the use of molecular signaling methodologies can be utilized to assist in identifying the upstream mechanisms that are differentially expressed in cancer survivors, triggered by sleep disturbance, and causal in the differential increased risk of depression in cancer survivors versus adults.

Under this hypothesis, it is logical to consider treatments that target the upstream components in the cytokine-to-CNS-behavior link. For example, candidate pharmacologic treatments would include cytokine antagonists, anti-inflammatory agents, and other drugs that disrupt cytokine signaling pathways (e.g., NF-κB) (Miller et al., 2008, 2009). Indeed, several recent trials have demonstrated that TNF-α-blockade with etanercept is safe in patients with advanced cancer (Madhusudan et al., 2004, 2005), and one study found that chemotherapy fatigue was reduced with this medication (Monk et al., 2006). Moreover, given the role of NFκB pathways in cancer etiology and progression (Karin, 2006), this work would provide a foundation for collaborative efforts between behavioral scientists and oncologists on the full spectrum of activity of relevant antagonists of inflammatory pathways on health and quality of life outcomes in cancer patients. Alternatively, at the level of the CNS, inflammation induced alterations in neurotransmitter systems might be targeted by agents for specific monoamine systems. For example, use of a serotonin reuptake inhibitor, paroxetine, prior to cancer treatment has been found to reduce depression in cancer patients undergoing chemotherapy with IFNα

(Musselman et al., 2001a). In addition, the use of paroxetine has been found to effectively reduce depressive symptom severity, but not fatigue, during chemotherapy in breast cancer patients (Roscoe et al., 2005). Coupling such clinical trials with monitoring specific inflammatory biomarkers has the potential to optimize treatment response characterization. Moreover, knowledge about variants in inflammatory response genes might identify those patient populations who are most likely to benefit from such inflammation-targeted and/or neuropsychopharmacologic treatments.

7. Development of behavioral treatments targeting inflammatory biology

Given the role of psychological stress on inflammatory dynamics, interventions that specifically target stress responses such as cognitive-behavioral psychotherapies may be especially relevant in the link between sleep disturbance, inflammation, and depression risk. Furthermore, given that cancer treatment is associated with the onset of sleep disturbance and increases in depressive symptoms, the use of such interventions prior and during cancer treatment may hold considerable promise, although to our knowledge there is an absence of such research to date.

Such behavioral therapies include components such as relaxation training, enhancement of coping and/or emotion regulation skills, and establishment of appropriate sleep–wake habits and beliefs about sleep. For example, a recent cognitive-behavioral therapy program focusing on coping, sleep, physical activity, and social support was found to lead to significant improvements in fatigue in 54% of cancer survivors compared with 4% of patients assigned to a control condition, although inflammation was not evaluated (Gielissen et al., 2006). However, among those with an underlying inflammatory disorder (i.e., rheumatoid arthritis), we determined that an intervention that specifically targets emotion regulation is more effective in improving depression outcomes than an omnibus cognitive behavioral treatment, and that this benefit is particularly robust among those who have a prior history of depression (Zautra et al., 2008). The intervention for emotion regulation was based on practices of mindfulness, whereas the cognitive behavioral treatment involved a program of specific verbal/written protocols guiding participants through exercises designed to restructure dysfunctional thoughts and ruminations that contribute to negative perceptions of self and the environment. Benefits on the cellular expression of proinflammatory cytokines were found after 16 weeks in persons who had an improvement of depression (Zautra et al., 2008).

Several other behavioral interventions have been found to modulate inflammatory signaling in the innate immune system including aerobic exercise (Nicklas et al., 2008), compassion meditation (Pace et al., 2009) and Tai Chi (Irwin and Olmstead, 2011; Lavretsky et al., 2011) in association in improvements in sleep disturbance, for example. Moderate intensity physical activities such as walking for 20–30 min per day, which provides aerobic training without activating anaerobic respiration led to benefits on inflammatory markers after 12 months (Nicklas et al., 2008), but only among those who had elevated levels at baseline. Similarly, a meditation that involved thought focusing directed toward compassion of others has been to reduce sympathetic arousal mechanisms, and alter inflammatory responses to stress with benefits on inflammatory markers after 6 weeks (Pace et al., 2009). Finally, Tai Chi Chih, a Westernized form of the Chinese martial art Tai Chi, involving a slow moving meditation involving 20 min of aerobic exercise with relaxed breathing and attentional focus and body awareness led to benefits on inflammatory markers after 25 weeks (Irwin and Olmstead, 2011) with impacts reported on biomarkers of inflammation including pro-inflammatory cytokine levels (e.g., CRP) and *ex vivo* cellular production of TNF and IL-6 in response to LPS stimulation. Moreover, the latter intervention has been found to improve sleep quality (Irwin and Olmstead, 2011), and augment the treatment efficacy of antidepressant medications concomitant with reductions in inflammation (Lavretsky et al., 2011). Importantly, the regulation of leukocytes by these behavioral treatments may contribute to their neurobiological and behavioral impact if, for example, neurally-mediated reductions in peripheral inflammation feed back to reciprocally reduce depressive symptoms.

8. Summary: toward depression prevention in cancer survivors

Multiple clinical, behavioral (e.g., sleep disturbance), and biological factors (e.g., inflammation) have been found to contribute to depression risk in non-cancer adults. However, cancer-related variables may alter the depression risk profile initially found in non-cancer persons; prospective research in cancer survivors is needed to address this critical gap.

Insight into the selective profile of risk indicators in cancer survivors has the potential to advance the design of a depression prevention trial. For example, consideration of profiles of two or more risk factors (Schoevers et al., 2006) is a necessary strategy to optimize health gains, minimize effort and concomitant costs, and lead to a substantial drop in number needed to treat (NNT) in a prevention trial. Unfortunately, the majority of prior depression prevention studies have selected broad target groups (e.g., primary care patients; those with medical disorders) rather than using disease specific and/or multivariate approaches in selection of a high risk population (Munoz et al., 2010). No depression prevention study has been conducted in cancer survivors despite the high prevalence of depression in this population.

Defining the unique and specific role of sleep disturbance in depression risk represents an important paradigm shift in depression treatment and/or prevention trials. Most prior research, conducted in non-cancer populations, have typically employed broad-based psychoeducational treatments within the cognitive behavioral "family", in order for participants to learn practical skills intended to help them cope with and overcome depressive feelings, possibly in the context of interpersonal events (e.g., conflicts, transitions) (Munoz et al., 2010). Given that cancer specific factors uniquely contribute to sleep disturbance in cancer survivors, development of a specific intervention that targets this modifiable risk factor is an efficient strategy. Moreover, using an efficacious treatment such as cognitive behavioral therapy for insomnia, which is found to be as effective as pharmacologic treatments, shows considerable promise in improving sleep disturbance (Irwin et al., 2006a; Nicassio et al., 1997).

Finally, understanding the role of inflammatory biology dynamics in sleep disturbance and depression has the potential to refine prediction of risk and treatment response. Alternatively, the efficacy of behavioral interventions might be bolstered by the use of medications or other interventions (e.g., exercise) that attenuate inflammation. As such, an increasing focus on inflammation represents a major advance for current clinical practices, by providing a plausible biologic mechanism to explain variability in risk and response to treatment in cancer populations with depression.

Conflict of Interest

The authors of this manuscript have nothing to declare.

Acknowledgments

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Biobehavioral influences on recovery following hematopoietic stem cell transplantation

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is a rigorous therapy that carries significant risk of morbidity and mortality to individuals with hematologic malignancies undergoing this treatment. While relationships between psychosocial factors, immune function, and clinical outcomes have been documented in other cancer populations, similar studies of cancer patients undergoing HSCT have not yet been conducted. The clinical significance of these relationships may be particularly salient in this population given the critical role of a timely immune recovery and optimal immune regulation in preventing infections, mitigating risk for graft-versus-host disease, and eliminating malignant cells, thereby reducing morbidity and mortality. Evidence for the potential role of biobehavioral processes following HSCT is reviewed, mechanisms by which psychosocial factors may influence immune processes relevant to post-transplant outcomes are discussed, and a framework to ground future psychoneuroimmunology (PNI) research in this area is provided. The review suggests that the recovery period following HSCT may provide a "window of opportunity" during which interventions targeting stress-related behavioral factors can influence the survival, health, and well-being of HSCT recipients.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a rigorous therapy that carries significant risk of morbidity and mortality to individuals with hematologic malignancies undergoing this treatment. While there are a growing number of psychoneuroimmunology (PNI) studies of other cancer populations, there is a dearth of research on patients undergoing HSCT. The clinical significance of PNI relationships may be particularly salient in this population given the critical role of a timely immune recovery and optimal immune regulation in reducing morbidity and complications and preventing recurrence. This review focuses on evidence suggesting the importance of biobehavioral processes in the recovery following HSCT and potential mechanisms by which psychosocial factors influence immune processes relevant to critical post-transplant outcomes. Fig. 1 outlines the biobehavioral pathways discussed.

2. Hematopoietic stem cell transplantation (HSCT)

HSCT is performed by administering intense chemotherapy with or without radiation therapy followed by infusion of autologous or allogeneic hematopoietic stem cells. See Copelan (2006) for an excellent overview. In brief, autologous transplantation involves the infusion of the patients' own hematopoietic stem cells and allows patients to receive high-dose chemotherapy that can eradicate disease at the cost of ablating the bone marrow. It is most frequently used for multiple myeloma, other plasma cell disorders, and aggressive lymphomas. In allogeneic transplantation, patients receive hematopoietic stem cells or bone marrow product from an HLA-matched donor or umbilical cord. It is used to treat acute and chronic leukemias, myelodysplastic and myeloproliferative disorders, lymphomas, and aplastic anemias and other bone marrow failure disorders. While high-dose therapy provides short-term disease control, the therapeutic potential of allogeneic transplant derives from a "graft-versus-malignancy" effect in which donor-derived immune cells detect and eliminate remaining circulating malignant cells. Consequently, reduced intensity, nonmyeloablative regimens have increasingly been used to take advantage of this effect while reducing the toxicity and morbidity.

HSCT is a risky procedure with a high rate of treatment-related morbidity and mortality. Common side effects include nausea, diarrhea, anorexia, and fatigue. Significant early complications include gastrointestinal mucosal injury (mucositis), veno-occlusive disease of the liver, pulmonary failure, and infections. Allogeneic transplantation also carries a high risk of acute graft-versus-host disease (aGVHD). Two-year allogeneic transplant-related mortality

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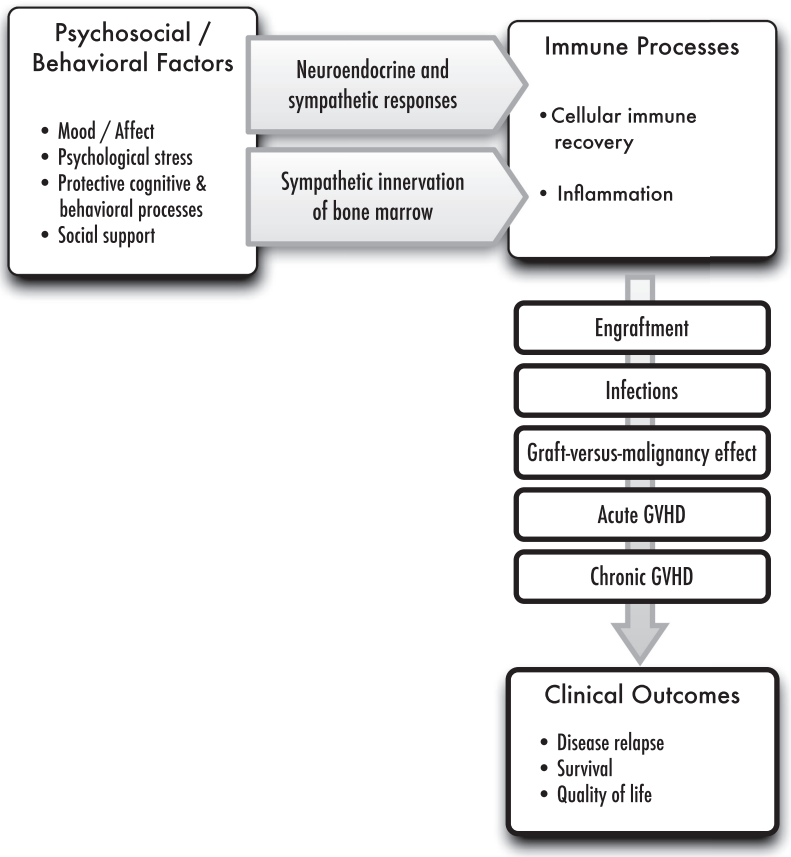

Fig. 1. This biobehavioral model of HSCT illustrates the contributions of psychosocial processes and behavioral factors to recovery following HSCT. Stress-related behavioral factors can activate the HPA and SNS axes. The secreted products of these pathways (glucocorticoids, catecholamines) and the direct sympathetic innervation of the bone marrow microenvironment can modulate cell recovery following transplant and promote (or moderate) an inflammatory environment that predisposes the HSCT recipient to moderate or severe GVHD. Immune recovery plays a critical role in several different clinical events following HSCT, including preventing infections, promoting the activity of effector cells on residual disease, and mitigating acute and chronic GVHD, thereby reducing the risk for disease recurrence, ensuring survival, and facilitating a more optimal quality of life.

ranges from 6–51%, depending on pre-transplant disease status and comorbidities (Bacigalupo et al., 2004). Later complications include increased risk for cardiovascular disease, diabetes, chronic fatigue, secondary malignancies, and chronic graft-versus-host disease (cGVHD), a cause of significant long-term disability. While autologous transplantation is not associated with GVHD or most of the long-term risks seen following allogeneic transplant, there is a high rate of relapse, approximately 40–70% (e.g., Porrata and Markovic, 2004). While many patients are discharged from the hospital within a month, functional recovery occurs slowly over 6 to 12 months and the burden of ongoing medical care remains high with the need for many medications, frequent clinical visits, and common subsequent complications often related to GVHD and infection.

It is therefore not surprising that many HSCT patients report significant emotional distress. While elevated distress typically subsides over the first year, some degree of emotional distress persists among a significant subset of patients (Andrykowski et al., 2005; Syrjala et al., 2004), and depression is one of the most commonly reported concerns following HSCT. A prospective, longitudinal study found that the psychological recovery lagged behind the physical recovery after transplant, with 33% of HSCT recipients reporting clinically significant depressive symptoms at 90 days post-transplant and 79% continuing to report significant general psychological distress at 1 year post-transplant (Syrjala et al., 2004). A study of 662 long-term HSCT survivors who were an average of 7 years post-transplant reported continued decrements in several quality of life domains, including physical and functional abilities along with social and psychological adjustment, with prevalent problems including depression, fatigue, and cognitive dysfunction (Andrykowski et al., 2005). There is growing interest in understanding the psychological sequelae after HSCT, particularly given emerging evidence that depression and other emotional concerns are associated with poorer quality of life (e.g., Syrjala et al., 2004) and may affect the post-transplant course and outcome.

3. Stress-related psychosocial factors and post-transplant outcomes

A growing literature suggests that psychosocial factors may serve as risk or protective factors for HSCT outcomes. A review by Hoodin et al. (2006) summarized considerable evidence that emotional status predicts mortality following HSCT, with 10 of 15 relevant studies included in the review documenting significant links. The adverse influence of depression was the most frequently identified risk factor and appeared to exert the most salient effects for early post-transplant mortality. Earlier studies reviewed were often limited by retrospective designs and small sample sizes and/or failed to account for medical and demographic factors known to contribute to mortality following HSCT. However, Hoodin et al. (2006) observed that more recent, methodologically rigorous studies were more likely to find significant effects of emotional states on HSCT outcomes. Following this review, addi-

tional evidence for the negative prognostic significance of psychological distress and depression has emerged. For example, a large prospective study followed 138 allogeneic transplant recipients for at least 2 years post-transplant. The authors examined a comprehensive panel of medical and sociodemographic characteristics as potential predictors of survival. Depressive symptoms assessed at the time of hospital admission for HSCT emerged as an independent predictor of mortality after accounting for other relevant factors. Moreover, depression was not correlated with other predictors, suggesting it was not simply a marker of an unfavorable health status (Grulke et al., 2008). In another large study, the presence of depression or anxiety was a significant risk factor for mortality following HSCT; in fact, these factors predicted mortality more strongly than cardiac conditions, diabetes, cerebrovascular disease, and other common comorbidities (Sorror et al., 2005). Although not all studies have found significant links between depression and HSCT outcomes (e.g., Pereira et al., 2010), taken together with the prior findings, these studies suggest depression should be considered a risk factor for a poorer outcome following HSCT.

Potentially protective psychological factors (or their absence) have been investigated as predictors of mortality, including social support, optimism, and spirituality. In a study of patients undergoing autologous transplantation, those who perceived their social support to be "problematic" showed increased risk of mortality; however, perceptions of "positive" social support were not associated with mortality (Frick et al., 2005). Another large study found that optimistic expectations about HSCT outcomes predicted better survival at 2 months post-transplant after accounting for relevant medical and demographic predictors (Lee et al., 2003). However, there were no significant effects beyond 2 months post-transplant, and the study was limited by the heterogeneous sample of both autologous and allogeneic transplant recipients. A recent study of allogeneic recipients found that those who reported "spiritual absence" (a lack of spiritual or religious coping resources) had a higher mortality rate by 1 year post-transplant (Pereira et al., 2010). The authors note that the findings are limited by a modest sample size, precluding the ability to control for a larger number of medical factors that may be associated with mortality.

In sum, while the evidence for other psychosocial factors is still preliminary and has been limited by design issues and sample size, there is stronger evidence that depression is a risk factor for greater mortality and reduced survival time following HSCT. There are several possible pathways by which depression may influence HSCT outcomes. Depressed patients may be more likely to engage in health-impairing behaviors such as tobacco and alcohol use, both of which are associated with poorer outcomes and increased mortality following HSCT (e.g., Ehlers et al., 2011; Stagno et al., 2008). Depressed patients may also be less likely to comply with the rigorous requirements of post-transplant care, including taking multiple medications properly and attending frequent clinic appointments. Depression may also adversely influence immune processes important to recovery following HSCT. While systematic research linking mood disturbance, immune function, and HSCT outcomes is still lacking, we will discuss potential biobehavioral pathways for these effects.

Although hematologic cancers have not been the focus of most biobehavioral cancer research, it has been hypothesized that immune-mediated diseases such as leukemias and lymphomas are particularly susceptible to biobehavioral influences (e.g., Andersen et al., 1994; Costanzo et al., 2011). In addition, the early recovery following successful treatment of a malignancy can be viewed as a "window of opportunity," a time period when psychosocial factors are more likely to have an influence on immune processes and disease course. These interactions may be of particular significance for HSCT patients because any modulating influence on immune processes could have a salient effect on relapse and survival. Specifically, the speed and success of immune reconstitution following transplant are directly associated with overall and progression-free survival (e.g., Porrata and Markovic, 2004) and the most prevalent and serious clinical complications of HSCT are mediated by immune processes. As such, understanding PNI relationships may be particularly fruitful for improving the outcomes of patients undergoing HSCT. Fig. 1 outlines a biobehavioral framework for the contributions of behavioral factors to immune-mediated clinical outcomes following HSCT. The specific immune processes, clinical outcomes, and potential biobehavioral mechanisms are elaborated in the next section.

4. Biobehavioral research targets: immune processes critical to HSCT outcomes

4.1. Cellular immune recovery/engraftment

Engraftment is monitored clinically by absolute neutrophil counts, white blood cell counts and platelet counts. Time-to-engraftment varies based on stem cell source and graft type, but commonly occurs around 10–20 days post-transplant. Recovery of other immune cell populations can take considerably longer. NK cell kinetic and functional recovery occurs early, beginning 2–3 weeks after transplant and returning to normal levels within 1–2 months (Auletta and Lazarus, 2005; Porrata and Markovic, 2004). An early recovery of innate immunity confers greater protection against infection and occurs sooner than the restoration of B and T cell functions, which may take many months to longer than a year to be fully reconstituted. Monocyte (Mo) and associated dendritic cell (DC) subpopulations are the next to reemerge, providing a critical bridge between innate and adaptive immunity. Mature Mo expressing CD16+ receptors are thought to promote leukocyte recovery, activate cytokines, and facilitate the immune response to minimal residual disease (MRD) following HSCT (Tanaka et al., 1999). B cell numbers begin to recover by 3 months post-transplant, but B cell function and immunoglobulin production can be impaired for several months or even years (Auletta and Lazarus, 2005; Porrata and Markovic, 2004). T cells are among the last to recover. Re-emergence of CD4+ and T regulatory (Treg) subpopulations lag substantially behind the more quickly recovered CD8+ cells, leading to an inverted CD4/CD8 ratio and a resulting dysregulation that can facilitate the development of opportunistic infections, GVHD, and other complications. In many patients, there is a long-term or even permanent reduction in the diversity of the T cell repertoire.

4.1.1. Infections

The recovery of immune competence is critical for minimizing tissue damage and providing protection against bacterial and viral pathogens. Damage to mucocutaneous barriers and delayed immune recovery contribute to the high incidence of infections in the acute post-transplant period, with 75% of patients developing infections during the initial month following transplant (Majhail and Weisdorf, 2008), particularly bacterial infections. In the months following transplantation, ongoing T cell dysfunction and hypogammaglobulinemia place patients at continued risk for infection, including reactivation of Varicella zoster virus and Cytomegalovirus (CMV).

4.1.2. Graft-versus-malignancy effect

A timely immune recovery reduces risk for disease relapse and improves survival via a "graft-versus-malignancy" effect. In the allogeneic setting, lower relapse rates are due to the activity of effector cells on MRD, with a substantial literature indicating links

between the recovery of lymphocyte subsets and reduced risk for recurrence and mortality (e.g., Kim et al., 2006; Thoma et al., 2012). While less well-characterized, a similar effect may occur after autologous transplantation. Specifically, there is evidence for the prognostic significance of absolute lymphocyte count (ALC) at 15 days post-transplant, with findings indicating that an ALC above a threshold of 500 cells/mL is associated with reduced relapse risk and better overall and progression-free survival (Porrata et al., 2008; Porrata and Markovic, 2004). Examination of lymphocyte subsets suggests that CD16+CD56+CD3− NK cells may be the primary population driving these beneficial effects (Porrata et al., 2008). As NK cell kinetic and functional recovery occurs early, NK cell subsets are likely to be important in early surveillance and containment of MRD. The role of Mo has not been as thoroughly investigated as lymphocyte populations. However, a recent study found that higher absolute Mo counts at 30 days post-transplant predicted better overall and progression-free survival following allogeneic transplantation (Thoma et al., 2012).

4.1.3. Potential biobehavioral mechanisms

Fig. 1 outlines pathways by which psychosocial factors may modulate immune recovery and related clinical complications, thereby influencing HSCT outcomes. It is now widely accepted that many of the host- or recipient-derived cells essential to the recovery of hematopoiesis and immunity also express receptors for factors that are responsive to the extensive crosstalk between psychological state and the neuroendocrine and immune systems. These effects are mediated by the sympathetic nervous system (SNS), hypothalamic–pituitary–adrenal (HPA) axis, and a number of other hormones and peptides. Effects may occur via glucocorticoid and beta-adrenergic receptors on mononuclear leukocytes and/or neuroendocrine modification of lymphocyte trafficking (Dhabhar and McEwen, 1997; Elenkov et al., 2000; Khan et al., 1986). Beta-adrenergic signaling pathways relevant to cancer control have been well-characterized (e.g., Cole and Sood, 2012), including influences on cellular immune function. While much of the prior research has focused on lymphocyte subsets, Mo may also be sensitive to these influences due to the high density of beta-adrenergic receptors (Kavelaars et al., 1997).

Also relevant to immune recovery following HSCT is the sympathetic innervation of the bone marrow hematopoietic environment. There is evidence of particularly extensive innervation in the sinusoidal and parenchymal areas that influence hematopoiesis (e.g., Elenkov et al., 2000). Mouse models of bone marrow transplantation have shown that early hematopoietic progenitors are especially sensitive to catecholamines and that adrenergic influences play a role in triggering stem cells into cycle (e.g., Byron, 1972) and their migration out of the bone marrow (e.g., Katayama et al., 2006). Another series of studies demonstrated that adrenergic antagonists can stimulate platelet and granulocyte formation but inhibit lymphocyte proliferation and NK cell activation, suggesting that adrenergic stimulation may have different effects on myelopoiesis and lymphopoiesis (Maestroni and Conti, 1994). However, social stress appeared to have the opposite effect; mice repeatedly exposed to a social stressor showed increased numbers of neutrophils and monocytes but a reduction of B and T cells in the bone marrow, suggesting that stress favors myelopoiesis at the expense of lymphopoiesis (Engler et al., 2004). Similarly, corticosteroid administration in a murine model depleted pre-B cells, did not affect erythrocytes or monocytes, and increased granulocytes in the bone marrow (Laakko and Fraker, 2002). Taken together, the findings suggest that stress-related physiological pathways can modulate stem cell proliferation and differentiation in the bone marrow, providing a plausible pathway by which stress-related behavioral factors can influence the reconstitution of innate and adaptive immunity following HSCT.

Cellular processes and secreted soluble mediators essential to the initial expansion and differentiation of the progenitor cells and then their continued proliferation may also be influenced by stress-related behavioral factors. Among the cytokines that have traditionally been studied in the field of PNI, IL-6 and the larger IL-6 family (e.g., G-CSF leptin, LIF, OSM, IL-11) are especially important. Additional cytokines to target that may also be sensitive to biobheavioral influences and can be distinguished by their involvement in the restoration of key cell subsets, including neutrophils (IL-8), NK cells (IFNalpha, IL-2, IL-7, IL-12), and dendritic cell/Mo lineages (TNFalpha, IL-4, GM-CSF). Beyond the essential functional actions of these cytokines, many chemokines have been shown to have stimulatory or inhibitory roles, affecting cell proliferation, migration, and activation. A full summary of the many other relevant biological pathways that influence the cell recovery after HCST goes beyond the scope of this paper but can be found in excellent reviews (e.g., Auletta and Lazarus, 2005).

4.2. Inflammation

Inflammation in the post-transplant period is common due to tissue damage from conditioning therapy and the high occurrence of infections and plays a key role in the pathogenesis of GVHD in the allogeneic transplant setting. GVHD occurs when donor T cells attack recipient tissues including the liver, skin, and intestinal mucosa. A "cytokine storm" of inflammatory factors ensues, with elevated levels of cytokines such as TNFα and IL-1 serving as reliable indicators of risk for the development of more severe GVHD (Ferrara and Reddy, 2006). GVHD occurs in acute and chronic varieties, each with a distinct but related underlying pathophysiology and clinical implications.

4.2.1. Acute graft-versus-host disease (aGVHD)

Acute GVHD typically occurs within the first 60 days post-transplant and is mediated by alloreactive donor T cells that have been activated by *recipient* antigen-presenting cells. Severe aGVHD is associated with significant morbidity and mortality and is the single most important factor affecting survival other than relapse (Ferrara and Reddy, 2006). Most patients receiving an allogeneic transplant (approximately 60–80%) experience some degree of aGVHD (Majhail and Weisdorf, 2008).

4.2.2. Chronic graft-versus-host disease (aGVHD)

Chronic GVHD is mediated by alloreactive donor T-cells that are activated by *donor-derived* antigen-presenting cells and usually appears later, generally 50–200 days post-transplant. Approximately 50% of patients will develop cGVHD, with less than 5% of those who develop severe forms surviving long-term (Horowitz and Sullivan, 2006). Moreover, cGVHD can substantially undermine the recovery following transplant and is a significant cause of long-term disability for HSCT survivors.

4.2.3. Potential biobehavioral mechanisms

The biobehavioral model depicted in Fig. 1 also incorporates the effects of inflammation on GVHD, and there is preliminary evidence for a relationship between psychosocial factors and GVHD. A small study found that patients who reported greater anxiety prior to HSCT were at elevated risk for developing severe aGVHD (Gregurek et al., 1996). In a study of spirituality and mortality following HSCT, those reporting greater spiritual absence were particularly likely to succumb to complications of GVHD (Pereira et al., 2010). Both studies were limited in their ability to account for other predictors of GVHD, and neither study examined potential mechanisms for these relationships.

An augmented inflammatory response may play a critical role in meditating these psychobiological relationships. Specifically, an

accepted clinical dogma about GVHD is that alloreactive donor immune cells recognize host antigens; however, it is only in the setting of other "danger" signals that the alloreactivity will lead to disease. These "danger" signals derive from higher levels of inflammatory mediators, including TNFα, IL-1, and IFNγ, released under a variety of conditions such as tissue injury from treatment and infection (Ferrara and Reddy, 2006). Links between distress or depression and inflammatory responses have been well documented in the PNI field. These relationships may be mediated by nonimmune processes, including HPA dysregulation, or more directly by the activation of lymphoid cells and proinflammatory cytokines (Black, 2002; Miller et al., 2002). It has been shown that distress or depression can sensitize the body's defense mechanisms to engage in a prolonged state of readiness, resulting in a heightened inflammatory response when challenged (Johnson et al., 2002). Consequently, distress or depression will likely tilt the balance of the internal milieu in a way that would contribute to the initiation and perpetuation of GVHD. Interactions between stress responses and the fundamental danger signals of the body, including the release of heat shock proteins into circulation, has been of interest in the larger PNI field and may be of particular relevance in the context of HSCT.

4.3. Bidirectional pathways

While this review has focused on the downstream effects of behavioral factors on immune recovery and regulation, the relationship is likely to be bidirectional. It is already known that proinflammatory cytokines can activate central nervous system circuitry associated with the withdrawal and conservation of energy, evoking adverse neurobehavioral and affective responses including depressed mood, fatigue, enhanced pain sensitivity, sleep disturbance, decreased activity, anorexia, and neurocognitive dysfunction. Such mechanisms have been of interest in understanding behavioral comorbidities and quality of life concerns of cancer patients (Miller et al., 2008; Seruga et al., 2008). HSCT recipients are at particularly high risk for inflammation-related impairments due to cytokines released in response to tissue damage associated with conditioning therapy, infection, and the "cytokine storm" associated with GVHD. A recent study of allogeneic transplant recipients tracked both inflammatory cytokines and several symptoms during the initial 30 days post-transplant (Wang et al., 2008). It found that increases in IL-6 and sTNF-R1 were associated with worsening of fatigue, poor appetite, pain, drowsiness, dry mouth, and disturbed sleep. While the study had a small sample, the frequent assessments and a sophisticated analytical approach that allowed the authors to model dynamic relationships and account for numerous potential covariates were strengths of this study. While still preliminary, findings suggest that assessment of inflammatory mediators can contribute to understanding of the factors that undermine quality of life following HSCT.

5. PNI research directions for HSCT: predictors, mediators, and clinical outcomes

5.1. Psychosocial/behavioral factors

It will be critical for biobehavioral HSCT studies to delineate the behavioral constructs that are most likely to have a salient clinical influence. Evidence from studies of psychosocial factors and HSCT outcomes clearly points to depression as a significant risk factor. However, some degree of depressed mood is a very common response following a treatment regimen as intense and difficult as HSCT. The changes in mood may have to be more severe or prolonged to affect downstream physiology. Prior research suggests that chronically depressed cancer patients are at risk for poorer outcomes, while those who experience only acute depression following diagnosis are at no greater risk (Stommel et al., 2002). Ascertaining the severity and duration of mood changes necessary to affect clinical outcomes will be an important challenge. In addition, related indicators of distress and mood disturbance, such as worry and intrusive thoughts, posttraumatic stress symptomatology, and generalized negative affect will be valuable to assess in developing a fuller clinical profile of HSCT recipients at risk for poorer outcomes.

Considering measures of cognitive and behavioral processes that promote well-being and alleviate distress will also be critical to understanding the full range of psychological experience. It is already known that protective influences such social support and optimism can mitigate the adverse effects of stress-related behavioral factors on immune function and cancer outcomes (reviewed in Costanzo et al., 2011), and we have reviewed preliminary evidence linking these contstructs to HSCT outcomes (e.g., Frick et al., 2005; Lee et al., 2003). In addition, recent studies suggest that many HSCT survivors are able to find meaning or perceive some benefit from their experience, reporting closer intimate relationships, an enhanced sense of personal strength, or an increased appreciation of life (Andrykowski et al., 2005; Widows et al., 2005). This type of psychological growth has been shown to have a beneficial influence on immune function and health in other cancer populations (Dunigan et al., 2007; McGregor et al., 2004), and therefore is likely to be a fruitful area for future inquiry alongside more traditional distress measures. It will be important to elucidate the summative influence of psychosocial risk and protective profiles that have the most salient influence on downstream physiology and HSCT outcomes in order to discern which factors should be targeted in behavioral interventions.

5.2. Immune processes

While neutrophil counts have been a primary diagnostic endpoint used in evaluating early post-transplant recovery, the recovery of other leukocyte subsets are critical in mitigating risk for infection and enhancing the graft-versus-malignancy effect, as described previously. Good candidates to consider for investigating the mechanisms linking stress-related behavioral factors to HSCT outcomes include NK cells, which appear be primary players in eradicating MRD and reducing risk for recurrence (Porrata et al., 2008) and CD16+ Mo, which may also play a critical role in promoting the overall leukocyte recovery and an appropriate immune response to MRD (Tanaka et al., 1999). Both NK cells and Mo are also known to be extremely responsive to soluble neuroendocrine mediators and have been shown to be sensitive to psychological factors. The protracted recovery of T cell profiles is an important problem in the HSCT setting. A quicker T cell recovery and restoration of healthier ratios of T cell subsets can reduce morbidity and mortality related to infections, and regulatory T cells (Tregs) can mitigate the undesirable aspects of GVHD. Within the PNI literature, there has been considerable research on the influence of stress-related factors on T cell differentiation and proliferation, and this could be applied to the HSCT setting. Assessments will require sophisticated immunophenotyping and the use of state-of-the-art multicolor flow cytometry approaches to discern the diversity and profiles of the returning leukocytes.

Cytokines are another obvious biomarker to employ in assessing soluble mediators in systemic circulation due to the central role of inflammatory cytokines in promoting and maintaining GVHD as well as the involvement of cytokines in cell differentiation and proliferation. Attention to the less commonly studied soluble mediators and cell growth promoters is also warranted, such as GM-CSF and the cytokines that promote NK cell differentiation

and function such as IL-12 and IL-17. A pragmatic challenge for this line of research, however, is that assessing the levels circulating in plasma may not be sufficient. Studies of cell propensity to produce and secrete cytokines (e.g., intracellular RNA), the cytokine response following in vitro stimulation in culture, and the more sophisticated use of advanced flow cytometry methods will be needed to better examine the production of these ligands and expression of their receptors.

5.3. Clinical complications and outcomes

Addressing issues of clinical significance of PNI relationships in the HSCT setting is critical in translating findings to meaningful interventions. While following patients for extended periods of time to determine relapse and survival rates is ideal, the time commitment can pose challenges. More proximal HSCT outcomes that can be examined in a shorter-term study include the development of infections, the occurrence and resolution of aGVHD, and the initial emergence of cGVHD. As previously discussed, these complications are the primary causes of morbidity and early mortality and have a clinically meaningful impact on quality of life. Additional indices of recovery such as days of hospitalization, number of rehospitalizations, time to return to work/school, and performance status can also be assessed as milestones with a medical relevance and can serve as a functional metric of the patient's quality of life.

5.4. Confounds and challenges

There are a number of logistical and conceptual challenges when conducting clinical PNI research in the context of HSCT. There is significant heterogeneity with respect to diagnosis, graft type, stem cell source, conditioning regimen, and supportive care. The pre-transplant treatment and disease course can differ significantly based on diagnosis, responses to initial treatment approaches, and other risk characteristics, with some patients undergoing HSCT as part of first-line therapy, whereas others receive transplants in second or later remissions. This disease and treatment history heterogeneity needs to be carefully considered when choosing an appropriate and sufficiently homogeneous target population and/or otherwise addressed in the study design and data analysis strategy.

In addition, many aspects of post-transplant care can affect the immunological measures and mediators of interest. For example, routine GVHD prophylaxis during the first few months post-transplant includes calcineurin-inhibitors, corticosteroids, and other immunosuppressive medications. Antivirals, antibiotics, and antifungals are also commonly administered, sometimes in a pre-emptive manner and also upon early signs of infection. Cytokines may be used as biological response modifiers to stimulate a faster and stronger recovery, including the common use of GM-CSF following HCST. In addition, monoclonal antibodies against certain immunomodulatory proteins are employed in experimental protocols to reduce the inflammatory components of GVHD as well as to selectively activate or inactivate certain effector cells. Because some of these interventions are routine and standardized, selection of a homogeneous target population receiving the same supportive care protocol is a useful approach and can even be creatively incorporated into the study design and aims as one means to evaluate the influence of psychosocial variables on patients' responsiveness to immunomodulatory agents. Where selective inclusion/exclusion criteria are not practical, sophisticated statistical modeling strategies that can account for differences in medications and treatment approaches are critical.

Another challenge is the pace of change and evolution in the field of HSCT medicine. In the allogeneic setting, a combination of factors including the increased use of reduced-intensity conditioning regimens, better GVHD prophylaxis, and early treatment of viral and fungal infections, has decreased treatment-related mortality for patients in first or second remission from 34–37% prior to 1990 to 6–25% (Bacigalupo et al., 2004). Given the rapid pace of change, research findings on psychosocial influences and clinical outcomes can quickly become outdated. In addition, the researcher must endeavor to keep up with the technical innovations taking place in fields such as multicolor flow cytometry and the multiplexing strategies for cytokines and other analytics, along with the advances in molecular biology and genomics. The ability to examine physiological pathways in more exquisite detail will facilitate discoveries about the mechanisms that mediate better clinical outcomes of HSCT and hopefully allow us to tailor interventions to stimulate these pathways.

6. Conclusions and translational directions: improving health, well-being, and survival

Although there are many conceptual and practical challenges, the clinical significance of a timely immune recovery and immune-induced complications, along with the psychological challenges of a risky and difficult treatment regimen, make HSCT an especially important context for understanding biobehavioral influences on immunity, cancer progression, and survival. We have described the critical role of immune function for successful recovery following HSCT, focusing on the importance of a timely recovery of leukocyte subsets and optimal regulation of inflammatory processes in order to prevent infections, mitigate risk for GVHD, and eliminate malignant cells, thereby reducing risk for disease relapse and ensuring survival (see Fig. 1). Many of the traditional neuroendocrine mechanisms known to be of importance in other PNI contexts are likely to be highly relevant in HSCT, including both HPA and SNS function. Sympathetic innervation of the bone marrow and adrenergic modulation of hematopoiesis are likely to have a particularly critical role in this setting. The high potential for translational relevance makes this an especially appealing area to apply the PNI perspective.

Well-designed studies with carefully selected target populations, appropriate behavioral and immune targets and assessment strategies, and attention to clinical outcomes are needed to identify characteristics of patients who are most likely to be benefit from interventions that target stress-related behavioral factors, help to determine the optimal timing for these interventions, and provide guidance regarding the types of treatments that may be most effective. We have already outlined the psychosocial and immune processes that appear most promising and have suggested that the early recovery period may be a promising window in which to intervene. Because HSCT requires substantial preparative therapies and procedures (e.g., stem cell mobilization and collection for autologous transplantation, induction therapy and procurement of donor product in the allogeneic setting), there is a unique opportunity to intervene during the weeks or even months prior to HSCT. There is also ample opportunity to implement interventions during the typically long hospital stay and/or frequent follow-up clinic visits during the acute recovery. Psychological interventions utilizing cognitive-behavioral approaches, stress management strategies, and newer approaches that incorporate acceptance and mindfulness-based modalities have shown promise in other cancer populations (see Costanzo et al., 2011). Interventions targeting health practices relevant to stress-related processes, such as treatment of sleep and circadian disturbances, may also be beneficial. Pharmacological approaches, including emerging research on beta blockers, and more effective use of psychotropic medications such as SSRIs and SNRIs may have additional therapeutic value in the HSCT setting.

The advent of HSCT has extended the lifespan of patients and in many cases permits the chance for full remission and long-term survival. However, this treatment does not come without costs, including a protracted immune and clinical recovery and the related physical and psychological sequelae that can undermine quality of life for many months or even years. The challenge for behavioral scientists is to understand the biobehavioral processes that may deter immune restoration or engender a better recovery, thereby influencing the clinical course and outcome. This is a critical step in developing novel therapeutic strategies tailored to the unique needs of this population with the potential to contribute to improved health, quality of life, and longer survival following HSCT.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Review

Effects and potential mechanisms of exercise training on cancer progression: A translational perspective

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ABSTRACT

Over the past decade there has been increasing research and clinical interest in the role of exercise therapy/rehabilitation as an adjunct therapy to improve symptom control and management following a cancer diagnosis. More recently, the field of 'exercise – oncology' has broadened in scope to investigate whether the benefits extend beyond symptom control to modulate cancer-specific outcomes (i.e., cancer progression and metastasis). Here we review the extant epidemiological evidence examining the association between exercise behavior, functional capacity/exercise capacity, and cancer-specific recurrence and mortality as well as all-cause mortality individuals following a cancer diagnosis. We also evaluate evidence from clinical studies investigating the effects of structured exercise on blood-based biomarkers associated with cancer progression/metastasis as well findings from preclinical investigations examining the effects and molecular mechanisms of exercise in mouse models of cancer. Current gaps in knowledge are also discussed.

1. Introduction

The health benefits of regular exercise have been recognized for centuries. Indeed, structured exercise training is established as the cornerstone of primary and secondary disease prevention in multiple clinical settings. In contrast, it has not been until the past decade or so that exercise has gained acceptance as a potential adjunct therapy following a cancer diagnosis (Jones and Demark-Wahnefried, 2006; Jones et al., 2010a). To date, approximately, 80 studies have been conducted investigating the effects of structured exercise training in patients following a diagnosis of cancer. Meta-analyses and systematic reviews report that structured exercise training is a safe and well-tolerated therapeutic strategy associated with significant improvements in a broad range of cancer-related toxicities including fatigue, exercise capacity, and physical quality of life (Jones et al., 2011; McNeely et al., 2006; Speck et al., 2010). Based on the extant literature, several national and international organizations have published exercise guidelines for cancer patients both during and following the completion of adjuvant therapy (Hayes et al., 2009; Schmitz et al., 2010).

While the importance of exercise therapy to control and/or mitigate the adverse consequences of cancer therapy are undisputed, there is growing interest in determining whether the benefits extend beyond symptom control to modulate cancer-specific outcomes (i.e., cancer progression and metastasis). Elucidation of the effects and underlying mechanisms will be critical to inform hypothesis-driven clinical trials and ensure the optimal safety and efficacy of exercise in cancer control. Accordingly, over the past several years, exercise–oncology researchers from a wide variety of disciplines have started to investigate the association between exercise behavior, objective measures of exercise capacity/functional capacity, and prognosis following a cancer diagnosis as well as the cellular and molecular mechanisms underlying these associations. The purpose of this paper is to review: (1) the extant epidemiological evidence examining the association between exercise behavior, functional capacity/exercise capacity, and cancer-specific recurrence and mortality as well as all-cause mortality, and (2) studies elucidating the host and cellular mechanisms underlying the exercise–prognosis relationship. In terms of the latter, we evaluate evidence from clinical studies investigating the effects of structured exercise training ('structured' exercise is defined as studies testing a specific plan of physical activity designed to improve exercise capacity as opposed to studies designed to increase physical activity) on changes in blood-based biomarkers proposed to mediate the association between exercise behavior/exercise capacity and cancer-specific prognosis.

Further, we also review the evidence from preclinical investigations examining the effects of exercise on tumor progression and metastasis in mouse models of cancer.

2. Search strategy

A comprehensive literature review using PubMed, MEDLINE, Sport Discus, and Cochrane Controlled Trials Register (1966

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through January, 2012) was conducted using the following MESH terms and text words: exercise, cardiorespiratory fitness, exercise capacity, cardiopulmonary fitness, functional capacity, oncology, and cancer. Relevant reference lists were also hand-searched. Studies in pediatric patients (<18 years) and adult patients with hematological malignancies were excluded.

3. Relationship between exercise and prognosis: epidemiological evidence

As summarized in Table 1 and 20 epidemiological studies to date have examined the association between self-reported exercise behavior and prognosis following a diagnosis of cancer. (Bertram et al., 2011; Borugian et al., 2004; Chen et al., 2011; Dal Maso et al., 2008; Holick et al., 2008; Holmes et al., 2005; Irwin et al., 2011, 2008b; Jones et al., 2011; Kenfield et al., 2011; Meyerhardt et al., 2006a, 2009a,b, 2006b; Moorman et al., 2011; Morikawa et al., 2011; Pierce et al., 2007; Richman et al., 2011; Ruden et al., 2011; Sternfeld et al., 2009). In brief, the majority of studies were conducted in women with early breast cancer (50%), with fewer conducted in patients with gastrointestinal malignancies (25%) and prostate cancer (10%). One study each was performed in patients with ovarian, non-small cell lung cancer, and primary malignant glioma. Overall, 15 studies (75%) found a significant inverse relationship between exercise and prognosis (cancer-specific or all-cause mortality) with a range of risk reduction between 15% to 67% and 18% to 67% for cancer-specific mortality and all-cause mortality, respectively. A dose–response was reported in 11 (55%); five studies found no significant association between exercise and cancer-specific mortality. Finally, the dose of exercise required for mortality reduction was not uniform either within or between cancer populations ranging from $\geqslant$9 metabolic equivalent time (MET)-hours per week of exercise (MET-h wk^{-1}) of moderate intensity exercise (equivalent to approximately 180 min wk^{-1} of moderate intensity exercise) to $\geqslant$27 MET-h wk^{-1} (equivalent to $\geqslant$500 min wk^{-1} of moderate intensity exercise).

Of importance, the association between exercise and cancer-specific mortality is not uniform and appears to vary according to volume of physical activity and even cancer type. For example, the first reported study in women with early breast cancer found that that women reporting $\geqslant$9 MET-h wk^{-1} (equivalent to walking briskly for 30 min, 5 days/week), had a 6% reduction in unadjusted absolute mortality risk at 10 years compared with women who reported less than 3 MET-h wk^{-1} (equivalent to walking 2–2.9 mph for 1 h) (Holmes et al., 2005). In contrast, Holick et al. that found to $\geqslant$21 MET-h wk^{-1} (brisk walking for 75 min, 5 d wk^{-1}) was required for significant reductions in cancer specific and all-cause mortality in women with invasive breast cancer.

The majority of studies in women with early breast cancer have examined the association between exercise levels in the period following the completion of primary adjuvant therapy (i.e., surgery, radiotherapy, chemotherapy) and prognosis. Of interest, Borugian et al. found that self-reported exercise before adjuvant therapy was not associated with breast cancer mortality at 10 years. The reasons for the discrepant findings are not known but may relate to significant reductions in exercise behavior during primary adjuvant therapy (Irwin et al., 2003) or the lack of additional protective effect of exercise in combination with adjuvant therapy. To this end, exercise may be more effective as a 'secondary' adjuvant therapy. In other words, from a disease recurrence perspective, exercise may be more effective as a chronic maintenance adjunct therapy following the completion of primary adjuvant therapy akin to long-term endocrine therapy, although evidence to support this notion is currently lacking. The lack of uniformity of the association between exercise and prognosis following a breast cancer diagnosis also appears to translate to other solid malignancies. Specifically, in a series of studies by Meyerhardt and colleagues, they found that $\geqslant$18 MET-h wk^{-1} (equivalent to walking briskly for 60 min, 5 days/week) was associated with 36% to 40% reductions in colorectal-specific mortality and all-cause mortality in one study whereas $\geqslant$ 27 MET-h wk^{-1} (equivalent to walking briskly for 90 min, 5 days/week) was required to confer the equivalent reduction in colorectal-specific mortality and all-cause mortality in 668 patients with colorectal cancer.

The lack of consistent effects of exercise on cancer-specific outcomes may be partially explained by differences in tumor biology (phenotype) or expression of certain molecular markers. For example, Holmes et al. (2005) found that $\geqslant$9 MET-h wk^{-1} was associated with a relative risk reduction in mortality of only 9% in women with estrogen receptor (ER) negative tumors relative to a mortality reduction of 50% in women with ER positive tumors. This finding was corroborated by Irwin et al. (2008) In a detailed analysis of tumor samples obtained at the time of surgical resection, Meyerhardt et al. (2009c) found that patients with tumors exhibiting loss of p27, a protein that regulates cell cycle, the hazard ratio (HR) for colon cancer-specific mortality was 1.40 (95% CI, 0.41–4.72) for those reporting $\geqslant$18 MET-h wk^{-1} compared with <18 MET-h wk^{-1}. The corresponding HR for patients with tumors exhibiting expression of p27, was 0.33 (95% CI, 0.12–0.85) for those reporting $\geqslant$18 MET-h wk^{-1} compared with <18 MET-h wk^{-1}. In subsequent analyses Morikawa et al. found that patients reporting $\geqslant$18 MET-h wk^{-1} whose tumors did not express CTNNB1 (β-catenin), a key mediator of the WNT signaling pathway that plays an important role in colorectal carcinogenesis, had an adjusted HR for colorectal cancer-specific survival of 0.33 (95% CI: 0.13–0.81) compared with patients reporting <18 MET-h wk^{-1} (Morikawa et al., 2011). Conversely, there was no significant relationship between exercise and prognosis in patients with tumors that were positive for nuclear CTNNB1 (adjusted HR: 1.07; 95% CI: 0.50–2.30). Although more studies are clearly required, the putative evidence provides promising preliminary evidence that regular exercise may be protective in select types (i.e., histological or molecular sub-types) of solid malignancies.

4. Relationship between fitness and prognosis: epidemiological evidence

The vast majority of studies to date in the field of exercise–oncology have examined the relationship between self-reported exercise behavior (via surveys) and prognosis in cancer patients. While there are several advantages to self-report methodology, particularly in large observational epidemiological studies, these tools are subjective, lack sensitivity and have poor reliability and validity. In contrast, exercise tolerance tests provide an objective measure of exercise capacity or functional capacity. There are several methods available that provide an objective determination of exercise capacity. For a more detailed review of exercise testing methods in clinical oncology, the reader is referred to a prior review by our group (Jones et al., 2008).

The strong, independent prognostic importance of exercise capacity and functional capacity in numerous clinical populations is well-established (Warburton et al., 2006). However, a paucity of studies have examined the prognostic importance of these measures in patients with cancer. As summarized in Table 2, five studies to date have examined this question. In the first study, our group examined the prognostic value of peak oxygen consumption (VO_{2peak}), the gold standard assessment of exercise capacity. $VO_{2\text{-}peak}$ was a strong independent predictor of death in 398 surgical candidates with NSCLC after adjustment for KPS, age, gender, and pulmonary function (Jones et al., 2010c). In further work, we found

Table 1
Association between self-reported exercise and cancer-specific mortality and all-cause mortality.

Tumor type (study)	*N*	Cohort/setting	Cancer-specific mortality			All-cause mortality		
			Risk reduction	Physical activity dose	Dose response	Risk reduction	Physical activity dose	Dose response
Breast adenocarcinoma (Borugian et al., 2004)	603	Breast cancer patients after surgery before the start of adjuvant treatment	1.0 (multi-variate adjusted relative risk). Exercise was not associated with breast cancer mortality	>1 time/wk	No	n/a	n/a	n/a
(Holmes et al., 2005)	2987	Stage I–III breast cancer; Nurses' Health Study	0.5 (multi-variate adjusted relative risk)	9–14.9 MET-h/wk	No	0.56 (multi-variate adjusted relative risk)	15–23.9 MET-h/wk ($p < 0.05$)	No
(Pierce et al., 2007)	1490	Stage I–IIIa breast cancer; Women's Healthy Eating and Living Study	0.58	⩾1320–6420 MET-min/wk	Yes	n/a	n/a	n/a
(Holick et al., 2008)	4482	Invasive breast cancer free of recurrence >2 years since diagnosis	0.51 (multi-variate adjusted relative risk)	⩾21 MET-h/wk ($p < 0.05$)	Yes	0.44 (multi-variate adjusted relative risk)	⩾21 MET-h/wk ($p < 0.05$)	Yes
(Irwin et al., 2008)	933	Breast cancer survivors; Health, Eating, Activity and Lifestyle Study	0.65 (multi-variate adjusted relative risk)	⩾9 MET-h/wk	Yes	0.33 (multi-variate adjusted relative risk)	⩾9 MET-h/wk ($p < 0.05$)	Yes
(Dal Maso et al., 2008)	1453	Invasive breast cancer survivors	0.85 (multi-variate adjusted relative risk)	⩾2 h/wk	n/a	0.82 (multi-variate adjusted relative risk)	⩾2 h/wk NS	n/a
(Sternfeld et al., 2009)	1970	Stage I–IIIa breast cancer; Life After Cancer Epidemiology	0.69 (multi-variate adjusted relative risk)	3 to <6 h/wk moderate activity	No	0.66 (multi-variate adjusted relative risk)	3 to <6 h/wk moderate activity ($p < 0.05$)	No
(Bertram et al., 2011)	2361	Stage I–III breast cancer survivors within 4 years, no current chemotherapy; WHEL study	n/a	n/a	n/a	0.47 (multi-variate adjusted relative risk)	⩾24.7 MET-h/wk NS	Yes
(Chen et al., 2011)	4826	Stage I–III breast cancer, 6 mo after diagnosis; Shanghai Breast Cancer Survival Study	0.60 (multi-variate adjusted relative risk)	⩾2 times/wk ($p < 0.05$)	Yes	0.70 (multi-variate adjusted relative risk)	⩾2 times/wk ($p < 0.05$)	Yes
(Irwin et al., 2011)	4643	Invasive breast cancer survivors; Women's Health Initiative	0.60 (multi-variate adjusted relative risk)	⩾9 MET-h/wk ($p < 0.05$)	Yes	0.58 (multi-variate adjusted relative risk)	⩾9 MET-h/wk ($p < 0.05$)	Yes
Colon/colorecta adenocarcinoma (Meyerhardt et al., 2006)	832	Patients with stage III colon cancer	0.51 (multi-variate adjusted relative risk), cancer recurrence or death from any cause (disease-free survival)	⩾18 MET-h/wk ($p < 0.05$)	No	0.37 (multi-variate adjusted relative risk)	⩾27 MET-h/wk ($p < 0.05$)	Yes
(Meyerhardt et al., 2006)	573	Women with stage I–III colorectal cancer; Nurses' Health Study	0.39 (multi-variate adjusted relative risk)	⩾18 MET-h/wk ($p < 0.05$)	Yes	0.43 (multi-variate adjusted relative risk)	⩾18 MET-h/wk ($p < 0.05$)	Yes
(Meyerhardt et al. 2009)	484	Men and women with colon cancer; Nurses' Health Study and Health Professional Follow-up Study	0.64 (multi-variate adjusted relative risk)	⩾18 MET-h/wk ($p < 0.05$)	n/a	0.60 (multi-variate adjusted relative risk)	⩾18 MET-h/wk ($p < 0.05$)	n/a
(Meyerhardt et al. 2009)	668	Men with stage I–III colorectal cancer; Nurses' Health Study	0.47 (multi-variate adjusted relative risk)	⩾27 MET-h/wk ($p < 0.05$)	No	0.59 (multi-variate adjusted relative risk)	⩾27 MET-h/wk ($p < 0.05$)	No
(Morikawa et al., 2011)	995	Men and women with stage I–IV colorectal cancer; Nurses' Health Study and Health Professionals Follow-up Study	In patients with negative CTNNB1 status and stages I–III cancer: 0.33 (multi-variate adjusted relative risk). No difference in survival for CTNNB1 + patients	⩾18 MET-h/wk ($p = 0.05$)	Yes	n/a	n/a	n/a
Prostate adenocarcinoma (Kenfield et al., 2011)	2705	Men with prostate cancer from Health Professionals Follow-up Study	0.65 (multi-variate adjusted relative risk)	⩾9 MET-h/wk ($p < 0.05$)	Yes	0.67 (multi-variate adjusted relative risk)	⩾9 MET-h/wk ($p < 0.05$)	Yes
(Richman et al., 2011)	1455	Men with prostate cancer from CaPSURE Study	0.43 (for progression)	Walking ⩾3 h/wk at ⩾3 mph ($p < 0.05$)	No	n/a	n/a	n/a
Lung adenocarcinoma (Jones	118	Stage IIIb and IV NSCLC	n/a	n/a	n/a	0.67 (multi-	⩾9 MET-h/wk	n/a

(*continued on next page*)

Table 1 (*continued*)

Tumor type (study)	*N*	Cohort/setting	Cancer-specific mortality			All-cause mortality		
			Risk reduction	Physical activity dose	Dose response	Risk reduction	Physical activity dose	Dose response
et al., 2012b,2011)						variate adjusted relative risk)	($p < 0.05$)	
Ovarian adenocarcinoma (Moorman et al., 2011)	638	Invasive disease; NC Ovarian Cancer Study	n/a	n/a	n/a	0.69 (multi-variate adjusted relative risk)	$\geqslant$2 h/wk ($p < 0.05$)	No
Primary glioma (Ruden et al., 2011)	243	WHO grade III/IV recurrent glioma	n/a	n/a	n/a	0.64 (multi-variate adjusted relative risk)	$\geqslant$9 MET-h/wk ($p < 0.05$)	Yes

Abbreviations: MET, metabolic equivalent task; NSCLC, non-small cell lung cancer; WHO, World Health Organization; NS, not-significant.

that VO_{2peak} was also a strong predictor of all-cause mortality in 52 women with metastatic breast cancer. Again, VO_{2peak} added incremental value to the prediction of mortality beyond traditional markers of prognosis in this population (Jones et al.).

Three studies have also investigated the prognostic importance of measures of functional capacity, specifically 6-min walk distance (6MWD), in patients with cancer. Kasymjanova et al. investigated the association between 6MWD and survival in 64 patients with inoperable NSCLC (Kasymjanova et al., 2009). Relative to <400 m, a 6MWD $\geqslant$ 400 m was associated with a 56% reduction in the risk of death after adjustment for important covariates. In two subsequent studies by our group, we found that 6MWD was a strong, independent predictor of prognosis that provided incremental mortality risk prediction beyond traditional risk factors in patients with metastatic NSCLC, but was not associated with survival in patients with primary malignant recurrent glioma (Jones et al., 2011; Ruden et al., 2011). Of interest, in our metastatic NSCLC study, 6MWD remained an independent predictor of mortality after adjustment for self-reported exercise behavior, suggesting that an objective measure of exercise exposure (e.g., functional capacity) may provide a more sensitive prediction of mortality in patients with advanced cancer.

5. Mechanisms underlying the exercise-prognosis relationship

While the emerging literature base suggests that both self-reported exercise behavior and objective measures of exercise capacity/functional capacity are associated with survival in select cancer populations, the underlying biological mechanisms remain to be elucidated. Postulated mechanisms underlying the potential effects of exercise and/or fitness on cancer progression include modulation of metabolic (e.g., markers of glucose–insulin homeostasis) and sex-steroid (e.g., estrogens) hormone levels, improvements in immune surveillance, and reduced systemic inflammation and oxidative damage (McTiernan, 2008). However, to date, there is a paucity of correlative or direct evidence on these postulated host pathways in the clinical setting, or whether modulation of these pathways modulates the hallmarks of cancer in preclinical (animal) studies. Of course, there is a significant genetic contributor to exercise capacity therefore it is currently not clear how genetic predictors of exercise capacity relate to those contributing to cancer progression/metastasis or how the contribution of lifestyle factors (to exercise capacity) influence the fitness–prognosis relationship beyond genetic contributions. These factors need to be considered when elucidating the mechanisms by which exercise and/or fitness may influence tumorgenesis. In the following sections, we review the available evidence from clinical studies investigating the effects of structured exercise on changes in blood-based biomarkers as well as data from preclinical investigations investigating tumor progression and metastasis in mouse models of cancer. Of note, unless otherwise stated, we assume that all blood-based measurements were performed in the resting state (and not immediately following an acute exercise bout).

5.1. Clinical data

As summarized in Table 3 and 24 studies to date have investigated the effects of structured exercise training on changes in circulating concentrations of host-related factors in persons with cancer (Allgayer et al., 2004, 2008a; Evans et al., 2009; Fairey et al., 2003, 2005a,b; Galvao et al., 2010; George et al., 2010; Irwin et al., 2006, 2007, 2005, 2009; Janelsins et al., 2011; Jones et al., 2012b; Ligibel et al., 2008; Na et al., 2000; Payne et al., 2008; Pierce et al., 2009; Schmitz et al., 2005; Segal et al., 2003, 2009; Tosti et al., 2011; Yuasa et al., 2009; Zeng et al., 2011).

Table 2
Association between objective measures of cardiorespiratory fitness, functional capacity and all-cause mortality.

Tumor type (study)	*N*	Cohort/setting	Objective measure	Prognostic findings
Non-small cell lung cancer (Kasymjanova et al., 2009)	64	Newly diagnosed patients with stage IIIA, IIIB, or IV NSCLC with a life expectancy of at least 4 months	6MWT conducted three times: at time of enrollment, pre-chemotherapy, and after two cycles of chemotherapy	Patients with an initial 6 MWT $\geqslant$ 400 m had significantly longer survival than patients with initial 6 MWT < 400 m (multivariate adjusted HR: 0.44; 95% CI: 0.23–0.83; $p = 0.001$)
(Jones et al., 2010a,b,c)	398	Patients with suspected stage I–IIIA NSCLC who were candidates for primary surgery with an estimated life expectancy of at least 2 years; CALGB protocol 9238 Study	CPET on cycle ergometer to determine $VO_{2\ peak}$ measured once before surgery	Patients achieving $VO_{2\ peak}$ > 1.29 L/min had significantly better survival than patients with a $VO_{2\ peak}$ < 0.96 L/min (multivariate adjusted HR: 0.56; 95% CI: 0.39–0.80; $p = 0.0037$)
(Jones et al., 2010a,b,c)	118	Patients with histologically confirmed stage IIIB, IV or recurrent metastatic NSCLC	6MWT assessed one time	Patients achieving 6MWT $\geqslant$ 450 m had significantly better survival than patients with 6MWT <358.5 m (multivariate adjusted HR: 0.48; 95% CI: 0.24–0.93; $p = 0.025$). Every 50 m improvement in distance was associated with a 13% reduction in risk of death
Glioma (Ruden et al., 2011)	243	Patients with histologically confirmed WHO grades 3 and 4 malignant glioma who had received or were receiving salvage therapy	6MWT assessed one time	6MWT was not an independent prognostic factor. There was no significant difference in all-cause mortality between patients achieving 6MWT > 489 m and 6MWT <390 m (multivariate adjusted HR: 0.97; 95% CI: 0.63–1.48)
Breast adenocarcinoma (Jones et al., in press-a,2012a)	52	Women with histologically confirmed IV breast cancer receiving cytotoxic chemotherapy	CPET on cycle ergometer to determine $VO_{2\ peak}$ measured once	CPET performance was not predictive of overall survival. Patients achieving $VO_{2\ peak} \geqslant$ 15.4 mL/kg-min did not have significantly different survival rates from those with a $VO_{2\ peak} \leqslant$ 15.4 mL/kg-min (multivariate adjusted HR: 0.59; 95% CI: 0.29–1.19; $p = 0.141$)

Abbreviations: 6MWT, 6 min walk test; CPET, cardiopulmonary exercise testing; NSCLC, non-small cell lung cancer; WHO, World Health Organization.

The most widely investigated pathway is the effect of exercise on circulating metabolic factors, especially the insulin–glucose axis. Of the nine studies that evaluated these factors, most suggest that exercise is associated with changes in insulin-like growth factor-1 (IGF-1) concentration, insulin-like growth factor binding protein-3 (IGFBP-3) concentration, and IGF-1:IGFBP-3 M ratio, but there were no significant changes in insulin and glucose levels. Fairey et al. were the first to report that supervised exercise training following traditional exercise prescription guidelines (i.e., moderate intensity aerobic training at 60–75% of baseline VO_{2peak}, 3×/wk, 30–45 min/session for 15 weeks) was associated with significant alterations in IGF-1 concentrations (−7.4 ng/mL), IGFBP-3 concentrations (+180.5 ng/mL), and IGF-1:IGFBP-3 M ratio (−0.006) in 53 early-stage postmenopausal breast cancer patients compared to a sedentary control group (Fairey et al., 2003). There were no differences in fasting measures of glucose, insulin, or insulin resistance. Similar results were observed in larger studies of both aerobic and resistance exercise in breast cancer patients (Irwin et al., 2005; Janelsins et al., 2011; Schmitz et al., 2005). Ligibel et al. randomly assigned 101 overweight, sedentary early stage breast cancer patients to a 16-week home aerobic and resistance exercise program or sedentary control (Ligibel et al., 2008). Interestingly, in this population, patients experienced a borderline significant decrease in fasting insulin (28%, $p = 0.07$), a result that was corroborated by Irwin and colleagues (Irwin et al., 2009). Beyond breast cancer, Galvão et al. investigated the effects of a combined aerobic and resistance exercise training intervention metabolic factors in 57 prostate cancer patients undergoing androgen deprivation therapy. No significant differences were observed in any metabolic outcomes.

Exercise-induced effects on immune surveillance and inflammatory pathways have received some attention. Nine studies evaluated immunity and inflammation, but the results were quite variable. Na et al. reported that physical activity was associated with significant changes in *in vitro* function of natural killer cells isolated from gastric cancer patients (Na et al., 2000). Fairey et al. observed similar results as a result of 15-weeks of aerobic training in early stage breast cancer patients (Fairey et al., 2003). In this study, the authors also used ELISA to examine mononuclear cell cytokine production, but they did not see significant differences in either pro-inflammatory (e.g., IL-1, TNF-α, IL-6) or anti-inflammatory cytokines (e.g., IL-4, IL-10, transforming growth factor-1). A 2008 study by Payne et al. also reported no significant effects of exercise on serum IL-6 and cortisol levels in 20 postmenopausal breast cancer patients receiving hormonal therapy (Payne et al., 2008). In contrast, Allgayer et al. conducted a study of 23 stage II–III colorectal cancer patients after they completed primary therapy and found that short-term moderate intensity exercise was associated with decreases in IL-1 receptor agonist response to stimulation with LPS in heparinized whole blood and a more pro-inflammatory state, which the authors defined as decreased ratios of IL-1ra/IL-6 and IL-1ra/IL-1β (Allgayer et al., 2004). One common measure of systemic inflammation is C-reactive protein (CRP), an acute-phase biomarker that has been widely shown to rise in the blood in accordance with systemic inflammation. Four studies, three in breast cancer and one in prostate cancer, have shown that exercise is associated with decreases in CRP levels (Fairey et al., 2005b; Galvao et al., 2010; George et al., 2010; Pierce et al., 2009).

Fewer studies have examined the effects of exercise training on markers of oxidative balance/status. Allgayer et al. found that short-term moderate intensity exercise significantly reduced urinary excretion of 8-oxo-dG, a marker of oxidative damage to DNA, in colorectal cancer patients after the completion of primary adjuvant therapy (Allgayer et al., 2008). In contrast, Jones et al. found that moderate to high-intensity cycle ergometry was associated with significant increases in F2-isoprostanes, eicosanoid markers of oxidative stress, in 16 patients with stage I–IIIB NSCLC (Jones et al., 2012b). Finally and somewhat surprisingly, a limited number of studies have examined the effects of exercise on circulating concentrations on sex-hormone factors – factors that are clearly of most relevance breast and prostate

Table 3
Effects of exercise training on blood-based biomarkers from clinical studies.

Tumor type (study)	*N*	Design/cohort/setting	Biological mechanisms
Breast adenocarcinoma (Fairey et al., 2003)	53	RCT. Postmenopausal breast cancer patients randomized to a supervised 15-week aerobic training intervention or sedentary control	Exercise was associated with decreases in IGF-1 (10.9%, $p = 0.045$) and IGF-1:IGFBP-3 M ratio (18.2%, $p = 0.017$) and increase in IGFBP-3 ($p = 0.021$). No significant effect on fasting insulin, glucose, or insulin resistance
(Irwin et al., 2005)	710	Observational. Stage 0–IIIA breast cancer survivors; Health, Eating, Activity, and Lifestyle Study	Exercise was associated with significantly lower C-peptide ($p = 0.001$) and leptin levels ($p = 0.001$) and higher IGF-1 ($p = 0.0037$). There was a trend toward increased IGFBP-3 levels ($p = 0.055$)
(Fairey et al., 2005a,b)	53	RCT of exercise training in postmenopausal survivors of stage I–IIIB breast cancer who had completed surgery, RT, and/or chemo with or without tamoxifen or arimidex; Rehabilitation Exercise for Health after Breast Cancer Trial	Exercise was associated with a trend toward decreased C-reactive protein levels ($p = 0.066$)
(Fairey et al., 2005a,b)	53	RCT of exercise training in postmenopausal survivors of stage I–IIIB breast cancer after surgery, RT, and/or chemo with or without tamoxifen or arimidex; Rehabilitation Exercise for Health after Breast Cancer Trial	Exercise was associated with increases in natural killer cell cytotoxic activity ($p = 0.035$) and unstimulated mononuclear cell function ($p = 0.007$). No significant differences were observed in blood mononuclear cell production of pro-inflammatory (IL-1, TNF-α, IL-6) and anti-inflammatory cytokines (IL-4, IL-10, transforming growth factor-1)
(Schmitz et al., 2005)	85	RCT. Posttreated breast cancer patients 4–36 months after adjuvant therapy.	Six months of weight training significantly decreased IGF-II ($p = 0.02$) and IGFBP-3 levels ($p = 0.03$). There were no significant changes in glucose, insulin, IGF-I, IGFBP-1, and IGFBP-2
(Irwin et al., 2006)	474	Observational. Stage 0–IIIA breast cancer survivors; Health, Eating, Activity, and Lifestyle Study	Exercise was associated with a significant decrease in mammographic dense area ($p = 0.046$) and percent density ($p = 0.026$) in postmenopausal women with BMI $\geqslant$ 30 kg/m^2. Exercise was associated with a significant increase in percent density in premenopausal women with BMI < 30 kg/m^2 ($p = 0.037$)
(Irwin et al., 2007)	522	Observational. Stage 0–IIIA breast cancer survivors; Health, Eating, Activity, and Lifestyle Study	Exercise was associated with a significant decrease in mammographic dense area in postmenopausal women with BMI $\geqslant$ 30 kg/m^2 ($p = 0.036$). Exercise was associated with an increase in dense area in women with BMI < 25 kg/m^2, but this difference was not statistically significant
(Payne et al., 2008)	20	RCT. Postmenopausal breast cancer patients receiving hormonal therapy	No significant differences were observed between groups with respect to cortisol or IL-6 levels. Serotonin levels ($p = 0.009$) were significantly affected by exercise
(Ligibel et al., 2008)	101	RCT. Sedentary, overweight early stage breast cancer patients to home-based aerobic and resistance training program or sedentary control for 16 weeks	Exercise was associated with a 28% reduction in fasting insulin ($p = 0.03$) and a nonsignificant improvement in insulin sensitivity ($p = 0.09$)
(Pierce et al., 2009)	741	Observational. Stage 0–IIIA breast cancer survivors; Health, Eating, Activity, and Lifestyle Study	Exercise was associated with significantly lower concentrations of C-reactive protein ($p = 0.005$) and non-significant decreases in serum amyloid A concentrations ($p = 0.06$)
(Evans et al., 2009)	14	Pre-post. Posttreated breast cancer patients within 6 months of completion of all major cancer therapy and healthy controls matched for age and physical activity level; Get REAL & HEEL Breast Cancer Program	Breast cancer patients had significantly lower post-exercise blood lactate levels following high-intensity exercise (70% of VO_{2max}, $p < 0.0005$). There was no significant difference between patients and sedentary controls at low (40%) and moderate (60%) intensity
(Irwin et al., 2009)	75	RCT. Sedentary postmenopausal breast cancer survivors diagnosed 1–10 years prior with stage 0–IIIA breast cancer after adjuvant treatment at least 6 months before enrollment	Exercise was associated with a decrease in insulin ($p = 0.089$), IGF-I ($p = 0.026$), and IGFBP-3 ($p = 0.0006$) levels
(George et al., 2010)	746	Observational. Stage 0–IIIA breast cancer survivors; Health, Eating, Activity, and Lifestyle Study	Physical activity offset the negative effects of poor diet quality on C-reactive protein levels ($p = 0.03$) in breast cancer survivors
(Tosti et al., 2011)	14	Pre-post. Stage I–III invasive breast cancer within 6 months of completion of all major cancer treatments and no evidence of metastatic disease, matched to healthy controls	Patients with breast cancer had significantly lower blood lactate response to exercise ($p \leqslant 0.05$) across a variety of intensities. There was a trend toward more elevated glucose responses in women with cancer before and after exercise ($p < 0.08$)
(Janelsins et al., 2011)	21	RCT. Sedentary patients with stage 0–IIIb breast cancer after primary treatment 1–30 months prior to enrollment	Insulin levels did not rise in exercising patients compared to an increase in sedentary controls that received psychosocial therapy (significance not reported). IGF-1 decreased more in exercising patients than controls and IGFBP-3 increased in the exercising group (compared with a decrease in the control group), but neither of these changes was significant
(Zheng et al., 2011)	75	Pre-post/observational. Physically inactive postmenopausal women diagnosed with stage 0–IIIA breast cancer after completion of adjuvant treatment at least 6 months prior to enrollment.	Exercise was associated with a significant reduction in methylation of the L3MBTL1 tumor suppressor gene ($p = 2.9 \times 10^{-5}$)
Colon/colorectal adenocarcinoma (Allgayer et al., 2004)	23	RCT. Stage II or III colorectal cancer patients at least 4 weeks after completion of primary therapy (surgery and radiation and/or chemotherapy)	Short-term (2 weeks) of moderate intensity exercise was associated with decreased IL-1 receptor agonist response to LPS ($p < 0.05$) and a more pro-inflammatory state (decreased LPS antagonist/IL-1β, TNF-α, and IL-6 cytokine ratio; $p < 0.05$ for IL-6, all others non-significant)

(*continued on next page*)

Table 3 (*continued*)

Tumor type (study)	*N*	Design/cohort/setting	Biological mechanisms
(Allgayer et al., 2008)	48	RCT. Colorectal cancer patients after completion of primary therapy (surgery and radiation and/or chemotherapy)	Short-term (2 weeks) of moderate intensity exercise significantly decreased urinary 8-oxo-dG excretion levels ($p = 0.02$). High intensity exercise resulted in a non-significant increase in 8-oxo-dG levels ($p = 0.18$)
Prostate adenocarcinoma (Segal et al., 2003)	155	RCT. Men with a confirmed prostate cancer diagnosis scheduled to receive at least 3 months of androgen deprivation therapy after enrollment	Exercise did not significantly affect testosterone or PSA levels
(Segal et al., 2009)	121	RCT. Men with a confirmed prostate cancer diagnosis scheduled to receive radiation therapy with or without androgen deprivation therapy after enrollment	Neither resistance nor aerobic exercise significantly affected hemoglobin, testosterone, or PSA levels
(Galvao et al., 2010)	57	RCT. Men with confirmed prostate cancer at least 2 months of androgen deprivation therapy and were expected to undergo at least 6 more months of ADT with no evidence of disease	Exercise was associated with a significant decrease in C-reactive protein ($p = 0.008$). No significant differences were observed in testosterone, PSA, cholesterol, triglyceride, insulin, glucose, or homocysteine levels
Gastric adenocarcinoma (Na et al., 2000)	35	RCT. Stomach cancer patients after surgery	Exercise was associated with a significant sequential change in function *in vitro* of natural killer cells isolated from stomach cancer patients ($p < 0.05$)
(Yuasa et al., 2009)	106	Observational. Patients with primary gastric carcinoma	There was an association between physical activity and decreased methylation of CACNA2D3 (commonly methylated, poor prognostic factor in gastric cancer) ($p = 0.03$)
Lung adenocarcinoma (Jones et al., 2011,2012b)	16	Pre-post. Stage I–IIIB non-small cell lung cancer	Exercise was associated with an increase in urinary F2-isoprostanes (markers of oxidative stress) ($p = 0.08$)

Abbreviations: RCT, randomized controlled trial; 8-oxo-dG-8-Oxo-2′-deoxyguanosine; BMI, body mass index; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein-3; RT, radiation therapy; LPS, lipopolysaccharide; PSA, prostate-specific antigen.

cancer. In terms of the latter, three studies to date have examined the effects of exercise on levels of testosterone in men with both early and locally-advanced prostate cancer; all studies to date have reported no change in testosterone or prostate-specific antigen between men randomized to exercise compared with sedentary control (Galvao et al., 2010; Segal et al., 2003, 2009).

Based on the current evidence, there are no clear conclusions regarding the efficacy of exercise training to modulate the postulated host-related mechanisms underlying the exercise–prognosis relationship. However, the postulated pathways appear reasonable targets since there is considerable evidence in healthy populations that exercise training can modulate circulating levels of metabolic hormones (Umpierre et al., 2011), immune/inflammatory factors (Walsh et al., 2011), and sex-steroid hormones (Friedenreich et al., 2010; McTiernan et al., 2004). Modulating effects of exercise on pro-oxidative and anti-oxidative products is less conclusive at present. Clearly, the pathways postulated to underpin the exercise–cancer prognosis relationship encompasses essentially all host-related factors in humans. As a consequence, there are literally hundreds of potential candidate biomarkers that could, in theory, modulate exercise efficacy. To tackle this issue, the integration of high-throughput discovery approaches in preclinical and/or clinical investigations will be critical to identify a parsimonious panel of exercise-responsive factors – the mechanistic action of which can then be further interrogated using an integrated approach involving *in vivo* and *in vitro* experiments. Following the identification of the major players within and across the various host-related pathways, the clinical importance of these factors can be examined in existing epidemiological datasets.

Whether exercise training can directly alter tumor biology in cancer patients has not been investigated. However, Yuasa et al. found an inverse association between self-reported exercise levels and methylation of CACNA2D3 in tumor tissue from 106 patients with gastric cancer (Yuasa et al., 2009). Similarly, Zeng et al. found a significant association between self-reported exercise levels and decreased methylation of the L3MBTL1 tumor suppressor gene in 75 postmenopausal women with stage 0–IIIA breast cancer (Zeng et al., 2011).

5.2. Preclinical data

The utilization of animal models is a critical scientific method to dissect the effects and underlying host-related and molecular mechanisms of exercise on tumor biology to complement clinical investigations, to facilitate a translational approach to optimize the safety and efficacy of exercise in the oncology setting. Surprisingly, while numerous research groups have utilized mouse models to examine the effects of exercise on the initiation and incidence of several different cancer types (i.e., exercise exposure followed by tumor initiation), far fewer have adopted this experimental system to investigate the effects of exercise on progression and metastasis (i.e., initiation of exercise following tumor establishment, which mimics the clinical scenario of using exercise as an adjunct cancer therapy). As summarized in Table 4, we found a total of 14 studies investigating the effects of endurance exercise on a variety of different tumor types and endpoints, in several different model systems (Baracos, 1989; Cohen et al., 1991; Foley et al., 2004; Hoffman et al., 1962; Hoffman-Goetz et al., 1994b; Japel et al., 1992; Jones et al., 2005, 2010b; MacNeil and Hoffman-Goetz, 1993; Roebuck et al., 1990; Saez Mdel et al., 2007; Uhlenbruck and Order, 1991; Zheng et al., 2011; Zielinski et al., 2004). In summary, 11 (79%) studies reported primary tumor growth as an endpoint; of these, four found that exercise resulted in inhibition of primary tumor growth (Baracos, 1989; Cohen et al., 1991; Hoffman et al., 1962; Zheng et al., 2011). For example, Hoffman et al. implanted Walker 256 sarcoma cells subcutaneously into Wistar rats that were subsequently randomly allocated to voluntary wheel running in combination with daily swimming for 21 days relative to a sedentary control group. Tumor weight was significantly lower in the exercise group (percent of control: −97%) (Hoffman et al., 1962).

In contrast, three studies reported mixed results, meaning that tumor growth was inhibited in some exercise conditions and not in others (Roebuck et al., 1990; Uhlenbruck and Order, 1991; Zielinski et al., 2004).

Roebuck et al. examined the effects of up to 18 weeks of voluntary wheel running in a pancreatic adenocarcinoma model induced by injection of azaserine (Roebuck et al., 1990). They found that F344 rats that exercised had significantly fewer tumors and

Table 4
Effects of exercise on tumor progression and metastasis in mouse models.

Tumor type (study)	Rodent model	Animal strain	Method of tumor initiation	Effect of exercise on primary tumor growth (% of control)	Description of primary tumor growth	Description of mechanistic findings	
						Intratumoral	Systemic
Breast adenocarcinoma (Cohen et al., 1991)	Rat	F344	Tail vein injection of 37.5 mg/kg NMU[b]	Inhibition (NE)	Exercise significantly delayed tumor appearance and increased tumor latency. Tumor incidence was significantly lower in exercised animals compared with controls. There was no significant difference in tumor burden or mean tumor volume	Not evaluated	Exercise did not significantly affect prolactin levels
(Hoffman-Goetz et al., 1994a,b)	Mouse	BALB/c	Lateral tail vein injection of 1×10^4 MMT 66 cells	Not evaluated	This study evaluated pulmonary metastases from a tail vein injection of tumor cells. Exercise beginning after tumor injection did not significantly affect multiplicity of lung metastases	Not evaluated	Mice that began running on a voluntary wheel only after tumor injection had significantly higher LAK cell activity than those that had access to a voluntary running wheel before and after tumor injection
(Jones et al., 2005)	Mouse	Athymic nu/nu	Subcutaneous injection of 5×10^6 MDA-MB-231 cells	No change (NE)	There was no significant difference in tumor growth delay	Not evaluated	Not evaluated
(Saez Mdel et al., 2007)	Rat	Sprague–Dawley	Gastric intubation of DMBA.[c] 5 mg weekly for 4 w.	Augmentation (+200%)	The exercise group had a significantly higher tumor growth rate. There were no significant differences in survival time or tumor multiplicity	Not evaluated	Not evaluated
(Jones et al., 2010a,b,c)	Mouse	Athymic	Orthotopic (dorsal mammary fat pad) injection of 1×10^6 MDA-MB-231 cells	No change (+21%)	There was no significant difference in survival time based on tumor growth to 1500 mm^3	The exercised group had significantly more perfused vessels. HIF-1 protein levels were significantly higher in the viable tumor the exercised group. There were no significant differences in the levels of CD31, VEGF, ATP, PGC-1α, or AMPK	Not evaluated
Sarcoma (Hoffman et al., 1962)	Rat	Wistar	Subcutaneous injection of 2 cc of Walker 256 cell suspension (concentration not specified)	Inhibition (-97%)	Tumor weight was significantly lower in the exercise group	Not evaluated	Not evaluated
(Uhlenbruck and Order, 1991)	Mouse	BALB/c	Subcutaneous injection of 2.5×10^4 L^{-1} cells	Inconclusive (−44% to +86%)	The group that ran 200 m daily had significantly lower tumor weights, but the other running distances did not significantly affect tumor weight	Not evaluated	Not evaluated
(Foley et al., 2004)	Rat	F344	Subcutaneous injection of 1×10^7 C10 cells	No change (-31%)	There was no significant difference in tumor weight as a result of exercise	Not evaluated	Exercise significantly increased insulin-stimulated glucose transport. No significant differences in blood glucose levels or lipid peroxidation in skeletal muscle based on exercise treatment
Hepatoma (Baracos, 1989)	Rat	Sprague–Dawley	Subcutaneous injection of 20 μL Morris hepatoma 777 finely chopped tumors	Inhibition (-25%)	Tumor weight was significantly lower in the exercise groups. No significant differences in tumor weight between groups exercised for different durations	Not evaluated	Not evaluated
Pancreatic adenocarcinoma (Roebuck et al., 1990)	Rat	F344, Lewis	F344 rats received 3 doses of 30 mg/kg azaserine 4–5 d apart. Lewis rats received one dose of 30 mg/kg azaserine[a]	Inconclusive (−1% to −11%)	F344 rats that were exercised had significantly fewer foci and smaller volume percentage of foci, but there was no difference in focal diameter. No significant differences in Lewis rats	No significant difference in the amount of DNA synthesis based on treatment condition	Not evaluated

Transformed salivary gland cells (Japel et al., 1992)	Mouse	NMRI	Subcutaneous injection of 1.5×10^6 S-180 cells	Not evaluated	Not evaluated	Not evaluated		Moderate intensity physical exercise begun after injection of tumor cells did not significantly change macrophage phagocytosis
Transformed fibroblasts (MacNeil and Hoffman-Goetz, 1993)	Mouse	C3H/He	Lateral tail vein injection of 3×10^5 CIRAS1 cells	Not evaluated	This study evaluated pulmonary metastases from a tail vein injection of tumor cells. Exercise beginning after tumor injection did not alter lung tumor density relative to sedentary controls	Not evaluated		Not evaluated
Neoplastic lymphoid cells (Zielinski et al., 2004)	Mouse	BALB/c	Subcutaneous injection of 2×10^7 EL-4 cells	Inconclusive (+5%)	Exercise significantly delayed tumor appearance. There was no significant difference in peak tumor volume. Non-syngeneic tumors were rejected by the immune system of exercised mice	No significant difference in the fluid content of tumors. Significant reduction in vessel density in exercised animals on days 6, 8, 10, and 14 compared to controls		Not evaluated
Prostate adenocarcinoma (Zheng et al., 2011)	Mouse	SCID	Subcutaneous injection of 2.5×10^6 LNCaP cells. Tumors grew for 4–6 wk then animals were castrated to mimic androgen deprivation	Inhibition	Voluntary wheel running moderately inhibited androgen-independent tumor growth	Not evaluated		Not evaluated

[a] Azaserine is a glutamine analog carcinogen used to induce pancreatic cancer in preclinical models.
[b] *N*-Nitroso-*N*-methylurea (NMU) is an alkylating agent used to induce breast cancer.
[c] 7,12-Dimethylbenzanthracene (DMBA) is an aromatic hydrocarbon used to induce breast cancer.

smaller volume percentage, but Lewis rats subjected to the same treatment did not exhibit significant differences between groups. In a different model, Uhlenbruck and Order injected L-1 sarcoma cells subcutaneously into BALB/c mice that were forced to run 200 m, 400 m, or 800 m daily on a treadmill (Uhlenbruck and Order, 1991). Tumor weight was significantly lower in the group that ran 200 m daily compared with sedentary animals, but there were no significant differences in tumor weight for the other distances. Additionally, Zielinski et al. injected EL-4 neoplastic lymphoid cells subcutaneously in BALB/c mice and found that forced treadmill running delayed tumor appearance, but there was no significant difference between groups with respect to maximum tumor volume (Roebuck et al., 1990; Zielinski et al., 2004).

Finally, in three studies tumor growth was comparable between exercise and sedentary control groups (Foley et al., 2004; Jones et al., 2005, 2010b). Foley et al. found no effect of 17 days of voluntary wheel running following subcutaneous injection of C10 sarcoma cells in F344 rats (Foley et al., 2004). Jones et al. observed similar results in two independent experiments in a model of human breast cancer. In the first, MDA-MB-231 breast cancer cells were subcutaneously into athymic nude mice and then randomized to 8 weeks of treadmill running or sedentary control (Jones et al., 2005). In the second experiment, MDA-MB-231 breast cancer cells were orthotopically implanted into the dorsal mammary fat pad and then animals were randomized to 41–48 days of voluntary wheel running or sedentary control (Jones et al., 2010b). In both experiments, exercise did not significantly inhibit or augment tumor growth. It is important to note that in contrast to the aforementioned studies, one has reported augmentation of primary tumor growth with exercise training. In this experiment, Sprague–Dawley rats were administered 7,12-dimethylbenz[α]anthracene to induce breast adenocarcinoma, and following tumor establishment, all animals were randomized to forced swimming (30 min/day for 38–65 days) or sedentary control (Saez Mdel et al., 2007). Tumor growth rate increased 200% compared to control, although there were no significant differences in overall survival time.

The vast majority of cancer deaths result from metastasis, as such studies investigating the effects of exercise on metastatic dissemination and progression are of clear relevance. Only three studies to date have examined this question. In the first study, MacNeil and Hoffman-Goetz injected CIRAS1 transformed fibroblasts into the lateral tail vein of C3H/He mice and then randomized animals to voluntary wheel running or sedentary controls for three weeks (MacNeil and Hoffman-Goetz, 1993). Hoffman-Goetz et al. followed up on that work by injecting MMT 66 breast cancer cells intravenously into BALB/c mice and then randomizing animals to three weeks of treadmill running, voluntary wheel running, or sedentary control (MacNeil and Hoffman-Goetz, 1993). Exercise following tumor cell injection did not impact the development of lung metastases in either of these experiments. However, it is important to note that both of these experiments involved intravenous injection of cancer cells into otherwise healthy recipients, a model that fails to evaluate the ability of tumor cells to break through the tissue's basement membrane and invade into the capillary.

In an effort to address the limitations of prior research, our group examined the effects voluntary wheel exercise, compared with sedentary control, in male mice orthotopically implanted (into the prostate) with murine prostate cancer cells (TRAMP C-1). Results indicated that primary tumor growth rate was comparable between groups but expression of prometastatic genes was significantly modulated in exercising animals with a shift towards reduced metastasis (Jones et al., in press-a,2012a). Paradoxically, exercise was associated with significant increases in tumor vascularization, as measured by magnetic resonance blood perfusion

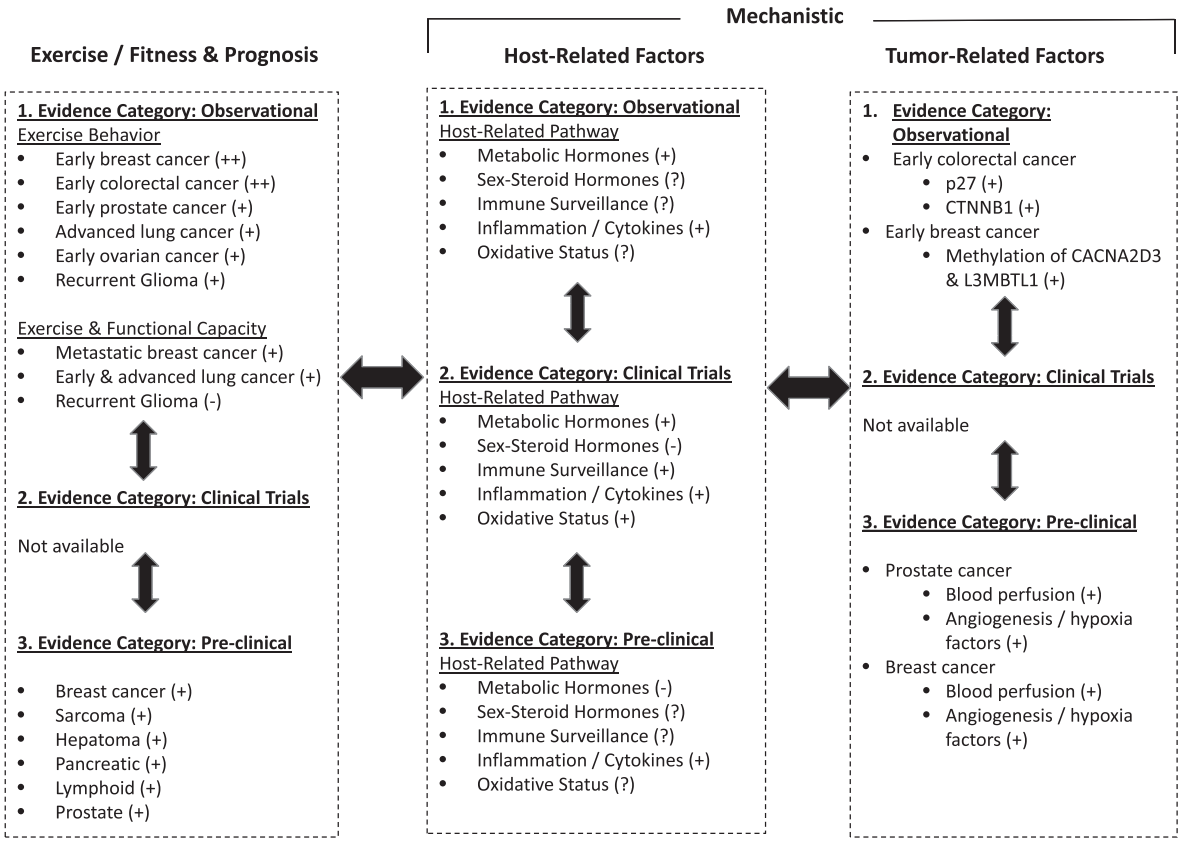

Fig. 1. Evidence-based representation of the known effects and mechanisms of exercise on tumor progression adopting a bi-directional translational research or scientific discovery (T0) paradigm. Exercise/fitness and prognosis, evidence supporting association between self-reported exercise behavior, objective measures of exercise capacity or functional capacity, and cancer prognosis; Host-Related Factors, postulated systemic (host-related) pathways mediating the association between exercise behavior and exercise/functional capacity and cancer prognosis; Tumor-Related Factors, intratumoral factors shown to mediate the association between exercise and prognosis or factors shown to be modulated in response to exercise. +++, strong evidence; ++, moderate evidence; +, weak evidence; —, null; ?, unknown at present.

imaging, while multiplex ELISAs revealed distinct reductions in plasma concentrations of interleukin-6 and CXCL1 in the exercise group. Based on the present data, we conclude that the effects of exercise on tumor progression and metastasis are equivocal. Reasons for the variable results to date likely pertain to differences in study methods, tumor cell line or carcinogen, location of tumor growth, and differences in the volume and type of exercise.

Researchers have also started to investigate the effects of exercise on host-related circulating factors as well as changes in the tumor phenotype that may mediate the exercise–tumor progression relationship. Specifically, three studies (21%) evaluated changes in intratumoral markers of neoplastic phenotype (Table 4). For example, in a model of azaserine-induced pancreatic cancer, Roebuck et al. reported that voluntary wheel running did not significantly affect the rate of DNA synthesis assessed by ^{3}H-thymidine incorporation (Roebuck et al., 1990). The other two experiments assessed markers of tumor angiogenesis. Zielinski et al. found that treadmill running decreased intratumoral blood vessel density in BALB/c mice subcutaneously with EL-4 neoplastic lymphoid cells (Zielinski et al., 2004). More recently, we found that voluntary wheel running increased hypoxia-inducible factor-1 protein levels and number of perfused blood vessels in athymic mice bearing human breast cancer xenografts, although there were no differences in protein levels of 5′ adenosine monophosphate-activated protein kinase, which regulates cellular energy homeostasis, or the angiogenic markers CD31 (a marker of endothelial cells) and vascular endothelial growth factor (Jones et al., 2010b). Two (14%) studies have examined changes in circulating concentrations of various growth factors with exercise, with both finding no changes in systemic glucose or prolactin levels (Cohen et al., 1991; Foley et al., 2004). Additionally, two studies investigated immune effects of exercise. Hoffman-Goetz et al. reported that lymphokine-activated killer cell activity was higher in exercising animals, whereas Japel et al. did not observe a difference in macrophage phagocytosis (Hoffman-Goetz et al., 1994a; Japel et al., 1992). Overall, the effects of exercise on tumor growth and metastasis are inconclusive at present due to the low number and considerable heterogeneity between studies.

The existing literature base suggests that exercise may modulate select circulating concentrations of several different growth factors across multiple host pathways in both mouse and clinical studies. In conjunction, data from animal studies suggesting that exercise may impact tumor progression. Together, these data create a novel hypothesis that changes in these circulating factors in the host may, in turn, influence release of secondary factors from other organ sites such as the bone marrow, skeletal muscle, or liver with the end result of altering ligand availability in the primary tumor microenvironment and/or distant ectopic sites to influence tumorigenesis and metastasis, respectively. In other words, exercise may be a critical strategy to modulate the host–tumor

Table 5
Future directions for exercise–oncology research on cancer progression.

Epidemiological studies
- A greater number of large-scale studies assessing both self-reported and/or objective measures of exercise exposure with long-term follow-up and adequate event rates.
- Delineate the association no how changes in exercise behavior, functional capacity/cardiorespiratory fitness measures are associated with clinical outcome across all solid tumors.
- More studies determining the differential association between exercise and prognosis as a function of tumor phenotype/gene expression.
- More studies determining the differential association between exercise and prognosis as a function of host-related circulating factors postulated to mediate the exercise–prognosis relationship.

Clinical biomarker intervention studies
- Delineate the differential effects of differences in exercise prescription dose (e.g., frequency, intensity, duration, modality) on changes in salient biomarkers in randomized trials.
- Determine effects of exercise across different tumor types across the cancer continuum (i.e., from diagnosis to palliation) to expand current efforts as well as extend to other solid tumors where exercise has not been rigorously evaluated.
- Elucidate the most salient biomarkers of interest that mediate the exercise–cancer prognosis relationship to develop a standardized 'exercise–oncology' biomarker panel that is reproducible and can be evaluated/compared across studies.
- Determine the effects of exercise on circulating biomarkers in conjunction with procurement of tumor tissue and/or imaging biomarkers whenever possible.

Preclinical studies
- Orthotopic implantation of syngeneic tumor cell lines or induction of orthotopic tumors via transgenic or chemical methods in immune competent animals to enable investigation of effects on primary tumor growth and metastasis.
- Elucidate the optimal exercise frequency, intensity, duration, and progression, as appropriate. Confirmation of 'training' effect via muscle fiber or mitochondrial function analysis.
- Determine effects on systemic mechanisms (metabolic and sex hormones, inflammation, immunity, and products of oxidation) in conjunction with examination of intratumoral/tumor microenvironmental molecular mechanisms (e.g., cell signaling pathways, angiogenesis, metabolism, migration).

Potential translational (cross-cutting/transdisciplinary) studies
- Elucidation of the optimal dose of exercise to inhibit tumor progression/metastasis in mouse models of solid tumors to guide the dose of exercise to be tested in phase II randomized trials.
- Elucidation of the effects of exercise on both circulating and intratumoral mechanisms associated with tumor growth in mouse models to guide systemic (plasma) biomarker testing in completed and ongoing clinical exercise trials in cancer patients. For further mechanistic investigations, plasma/serum from patients exposed to exercise vs. control conditions can be applied to human cancer cells in vitro to investigate effects on markers of the neoplastic phenotype.
- In epidemiological studies, identify genes or histological sub-types that may mediate the association between exercise and prognosis. Next, in preclinical studies, confirm mechanism of action by examining the effects of exercise in clinically relevant mouse models where the identified gene/pathway/histological sub-type is over-expressed or ablated. For clinical translational, plasma/serum from patients (with the identified histological sub-type or over expression of a specific pathway) exposed to exercise vs. control conditions can be applied to human cancer cells in vitro for further mechanistic studies.

interaction (Goodwin, 2008). The known effects and mechanisms of exercise on tumor progression adopting a bi-directional translational research approach is presented in Fig. 1.

Investigations that adopt a scientific discovery (also known as T0 or 'bench-to-bedside') translational approach are now required to elucidate to further understand the role and mechanisms of exercise to modulate host milieu–tumor–distant organ cross-talk. Such research will dramatically increase understanding of the mechanistic properties and application of exercise in the oncology setting.

6. Gaps in knowledge

Our recommendations for future research in this field are shown in Table 5.

7. Conclusion

There is growing recognition and acceptance of the beneficial role of exercise training/rehabilitation following a cancer diagnosis to prevent and/or mitigate disease and/or treatment-related toxicities to optimize symptom control and recovery. The results from an increasing number of epidemiological studies, however, suggest a previously unexpected role of exercise – as a therapeutic strategy to potentially delay cancer recurrence and mortality. It is important to stress that the evidence base is emergent with a small number of studies in comparison with the scientific literature base investigating the exercise–cancer prevention/incidence relationship. In addition, the strong association between exercise and prognosis following a cancer diagnosis are yet to be confirmed in randomized trials, although at least one trial is currently ongoing. For example, the Colon Health and Life-Long Exercise Change (CHALLENGE) trial is a phase III trial investigating the effects of regular exercise on recurrence and cancer-specific mortality in 962 colorectal cancer patients (Courneya et al., 2008). In conjunction with epidemiological studies and ongoing RCTs, a more thorough and structured understanding of the systemic and molecular mechanisms underlying the purported effects of exercise on tumor progression is critical. Such an understanding can be effectively achieved through the development of carefully designed correlative science (as part of forthcoming and/or ongoing randomized trials) or biomarker-driven studies together with a broad base of basic and clinical investigations encompassing a scientific discovery (T0 or 'bench-to-bedside') translational approach. This work will inform the design of adequately powered, mechanistically driven phase II–III trials to ensure that the appropriate exercise dose is prescribed to the right patients, at the right time, to facilitate the shift towards personalized, medicine to optimize therapeutic outcomes.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Invited Minireview

Psychosocial intervention effects on adaptation, disease course and biobehavioral processes in cancer

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Biobehavioral processes

ABSTRACT

A diagnosis of cancer and subsequent treatments place demands on psychological adaptation. Behavioral research suggests the importance of cognitive, behavioral, and social factors in facilitating adaptation during active treatment and throughout cancer survivorship, which forms the rationale for the use of many psychosocial interventions in cancer patients. This cancer experience may also affect physiological adaptation systems (e.g., neuroendocrine) in parallel with psychological adaptation changes (negative affect). Changes in adaptation may alter tumor growth-promoting processes (increased angiogenesis, migration and invasion, and inflammation) and tumor defense processes (decreased cellular immunity) relevant for cancer progression and the quality of life of cancer patients. Some evidence suggests that psychosocial intervention can improve psychological and physiological adaptation indicators in cancer patients. However, less is known about whether these interventions can influence tumor activity and tumor growth-promoting processes and whether changes in these processes could explain the psychosocial intervention effects on recurrence and survival documented to date. Documenting that psychosocial interventions can modulate molecular activities (e.g., transcriptional indicators of cell signaling) that govern tumor promoting and tumor defense processes on the one hand, and clinical disease course on the other is a key challenge for biobehavioral oncology research. This mini-review will summarize current knowledge on psychological and physiological adaptation processes affected throughout the stress of the cancer experience, and the effects of psychosocial interventions on psychological adaptation, cancer disease progression, and changes in stress-related biobehavioral processes that may mediate intervention effects on clinical cancer outcomes. Very recent intervention work in breast cancer will be used to illuminate emerging trends in molecular probes of interest in the hope of highlighting future paths that could move the field of biobehavioral oncology intervention research forward.

1. Psychological adaptation during the cancer experience

1.1. Psychological challenges in cancer

A diagnosis of cancer and its treatments are stressful. Chief concerns of patients once diagnosed with cancer involve fears of recurrence, being damaged by adjuvant therapy, not seeing children grow, premature death, and loss of social ties and activities (Stanton, 2006; Spencer et al., 1999). Emotional distress and negative affect states are common after a cancer diagnosis and treatment and contribute to poorer psychological well being especially if persisting after treatment (Cordova et al., 1995). Frequently reported psychosocial phenomena during the cancer experience include increased anxiety, depressed mood, social disruption, and sleep and fatigue-associated disruption (Ganz et al., 2002; Stanton, 2006). Although quality of life (QoL) generally improves markedly after cancer treatment and into the survivorship period (Bloom et al., 2007), this is not always the case (Stein et al., 2008). Poorer psychological adaptation after diagnosis and during treatment predicts diminished QoL many years later (Carver et al., 2005; Steginga et al., 2009; Wenzel et al., 2005) and may be associated with physiological processes (e.g., neuroendocrine and immune system regulation) relevant for their health status and QoL (Antoni et al., 2006a,b).

1.2. Cognitive, behavioral and social factors and adaptation to cancer

Individuals vary considerably in their psychological responses to and recovery from the stress of diagnosis and treatment of cancer. Work examining individual difference factors in psychological adaptation suggests the importance of cognitive, behavioral and social factors, which forms the rationale for many of the psychosocial interventions developed for cancer patients (see Antoni, 2003 for review). A small sample of these is now listed. Cognitive factors influencing how the experience of cancer is appraised (optimistic

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rational appraisals) may enhance adaptation and QoL during treatment, during the year after surgery, and several years later (Brothers and Andersen, 2009; Carver et al., 2005). Behavioral factors (e.g., having relaxation skills during cancer treatment) are associated with less distress and better adaptation after cancer treatment (Andersen et al., 2007a,b; Luebbert et al., 2001). Many other behavioral factors are relevant for adaptation and health outcomes including physical exercise, diet, and medication adherence as reviewed elsewhere (Andersen et al., 1994; McGregor and Antoni, 2009). Social support may be associated with both adaptation to cancer (Talley et al., 2010) and to longer-term health outcomes (Nausheen et al., 2009; Pinquart and Duberstein, 2010), The support that cancer patients receive, often coming from spouse or family members, is both the most helpful to patients in managing distress and may also be the most harmful if mismanaged (Figueiredo et al., 2004; Friedman et al., 2005; Wimberly et al., 2005). Thus, interpersonal skills may be particularly important for these patients as they communicate their needs to their support network. Taken together, this work suggests that cancer patients may psychologically adapt better to the cancer experience if they possess the cognitive, behavioral and social skills necessary to meet the challenges of treatment. It is plausible that cancer patient's physiological adaptation to stressors may also mirror their psychological adaptation.

2. Physiological adaptation processes relevant during the cancer experience

Cancer diagnosis and treatment induce acute and chronic stress and reduced QoL, which may affect neuroimmune regulation promoting inflammatory processes that could contribute to both symptom exacerbation and metastasis (Antoni et al., 2006a,b; Andersen et al., 1994). As noted elsewhere in this Special Issue of *Brain, Behavior and Immunity*, chronic stress, negative affect and social adversity have also been associated with biobehavioral alterations (increased sympathetic nervous system [SNS] signaling, hypothalamic pituitary adrenal [HPA] axis dysregulation, inflammation and decreased cellular immunity), which could interact with the tumor microenvironment to promote factors favoring tumor growth (e.g., angiogenesis), invasion (e.g., tissue remodeling and epithelial-mesenchymal transition), and metastatic signaling (e.g., anoikis), during and after cancer treatment (Lutgendorf and Sood, 2011).

Animals with tumors and treated cancer patients show HPA axis dysregulation – including elevated total and nocturnal cortisol output and decreased diurnal variation – which may be aggravated by stress and negative affect and could promote inflammatory processes (Sephton and Spiegel, 2003). It is not clear to what extent HPA dysregulation derives from stress and depression-induced leukocyte glucocorticoid receptor resistance, or if it is secondary to tumor- or treatment-produced inflammatory products, or both. Chronic stress and negative affect may support inflammatory processes, both by stimulating pro-inflammatory cytokine secretion and disrupting HPA axis-related inflammatory control (Miller et al., 2002). Importantly, altered diurnal cortisol dysregulation also relates to poorer survival in women with metastatic breast cancer (BCa) (Sephton et al., 2000).

Longitudinal studies show that while distress decreases after adjuvant therapy begins and quality of life improves after treatment for many cancer patients (Bloom et al., 2007), lingering physical challenges such as fatigue can peak during treatment and persist thereafter in breast cancer patients (Schmidt et al., 2012) and other cancer survivors (Stein et al., 2008). In some breast cancer patients, distress levels may remain elevated vs. matched healthy controls up to 15 months after diagnosis (Hinnen et al., 2008). In fact, there is a growing awareness of the need to screen for psychological distress in all cancer patients (Jacobsen, 2007). Some cancer patients' ability to carry out daily activities decreases during and after treatment, distress may increase, which can trigger interpersonal strain, a cascade that may further deplete energy resulting in negative mood, disrupted sleep and fatigue. Because distress reactions appear to be a possible common denominator contributing to multiple abnormalities (decreased psychological adaptation, HPA axis, and cytokine dysregulation) characterizing cancer treatment, then one component of effective treatment might focus upon improving psychological adaptation via psychosocial intervention. Cancer patients with less social support also experience more anxiety, greater cortisol levels, and molecular evidence consistent with impaired transcription of glucocorticoid response genes, and increased activity of pro-inflammatory transcription control pathways (Lutgendorf et al., 2010b). This suggests that psychosocial interventions teaching stress management skills (relaxation and coping strategies) to decrease distress and interpersonal skills to build social support may be particularly relevant for cancer patients (Antoni, 2003).

It is plausible that poorer psychological adaptation to cancer could exacerbate stress-associated alterations in neuroendocrines, cellular immune function, and pro- inflammatory signaling which could promote cancer progression. To date there are very few studies that have experimentally demonstrated a stress-induced change in either immune system indicators or tumor growth factors that predicts survival and cancer recurrence in humans. Conducting such a test essentially involves using a psychosocial intervention to modulate psychological adaptation (decrease distress and adversity states and improve positive states) in cancer patients, monitoring changes in stress-associated biobehavioral processes (SNS activation, HPA axis regulation, inflammation and cellular immune functioning), processes that promote tumor growth (tissue modeling and invasion, angiogenesis, apoptosis, anoikis) and then following these cohorts of patients for evidence of effects on disease course (recurrence, mortality). This involves recruiting a cohort of diagnosed cancer patients into a trial of a psychosocial intervention at a critical juncture in the cancer continuum (e.g., at the time of treatment for primary disease or at the point of disease recurrence), monitoring for initial improvements in psychological adaptation (reduced distress and depression and increased positive states); and following them over months for intermediate changes in SNS and HPA activity, inflammation, cellular immunity in plasma, circulating immune cells, and tumor cells if possible; and then following them for 5–10 years for clinical outcomes. There is now exciting preliminary evidence that several of these biobehavioral changes may indeed follow from psychological interventions designed to help cancer patients adapt. Before detailing these studies we summarize the research demonstrating the efficacy of psychosocial interventions designed to help cancer patients adapt to treatment.

3. Psychosocial intervention effects on psychological adaptation, stress-related biobehavioral processes, and cancer progression

Because cognitive, behavioral and social factors can affect how cancer patients adapt to diagnosis and treatment for cancer, many investigators have evaluated the effects of psychosocial interventions on psychological adaptation during cancer treatment. These interventions were designed to *cognitively* modify outlook, stress appraisals and coping via cognitive behavioral therapy (CBT); *behaviorally* reduce tension, anxiety and distress through relaxation training, mindfulness, hypnosis, yoga, and other techniques; and *interpersonally* build skills like assertiveness and anger management, in a group format to improve perceived social support and communication. In sum, psychosocial interventions have been developed to allow cancer patients to learn relaxation and other

anxiety reduction strategies, modify cognitive appraisals, enact positive reframing and acceptance coping strategies to decrease distress, and use interpersonal skills to build and maintain social support. As these interventions work to improve psychological adaptation to the stressors of cancer diagnosis and treatment they may directly (or indirectly through stress management) improve a myriad of health behaviors such as physical exercise, diet, sleep, and medication adherence, which can each in their own right, impact health outcomes in cancer patients (Andersen et al., 1994). For a recent review of these pathways in the context of breast cancer see McGregor and Antoni (2009). The remainder of this review will focus on summarizing the empirical evidence accrued over the past 10 years for the effects of psychosocial interventions that modify cognitive, behavioral and social/interpersonal factors on psychological adaptation and how these changes in positive and negative psychological adaptation parallel alterations in stress-related biobehavioral processes, and cancer progression. Because the largest number of psychosocial intervention studies have involved women with breast cancer (BCa) (Newell et al., 2002) we will emphasize this work, though where relevant, we also highlight intervention studies conducted in patients with other cancers.

3.1. Interventions targeting stress processes to facilitate psychological adaptation

Over 300 trials of psychological interventions have been conducted in cancer patients over the past 50 years, and most have been conducted in women with BCa. Reviews covering the evidence published through the early 2000s (e.g., Newell et al., 2002) concluded that psychosocial interventions that teach relaxation and stress management, help patients ventilate feelings, improve coping strategies, and provide social support are able to improve QoL and help them manage pain and other physical symptoms. The sample sizes of these trials were generally small and efficacy varied as a function of intervention content and format (group vs. individual delivery) (Newell et al., 2002). More recent studies with larger samples have generally supported positive effects for psychosocial interventions on QoL indicators in BCa patients (McGregor and Antoni, 2009). Among studies targeting non-metastatic BCa patients published in the past 10 years, group-based cognitive behavioral and coping skills training interventions (8–24 sessions) have been shown to decrease anxiety, depressed mood and improve QoL. Many effects were still apparent over a 12-month follow-up supporting the clinical utility of these approaches. Some of these interventions blended CBT and health education (Andersen et al., 2004), CBT and sleep improvement techniques (Savard et al., 2005), or CBT and interpersonal skills training (e.g., cognitive behavioral stress management, CBSM, Antoni et al., 2006a,b). For instance, BCa patients assigned to CBSM (vs. a psychoeducational group) in the weeks after surgery but prior to the onset of adjuvant therapy showed medium to large effect size decreases in negative affect ($d = 0.33$) , thought intrusions ($d = 1.22$), rated anxiety ($d = 0.74$), and interpersonal disruption ($d = 0.53$), and increases in positive affect ($d = 0.31$), benefit finding ($d = 0.82$), and positive states of mind ($d = 1.16$) for up to one year (Antoni et al., 2006c,d). These studies collectively provide strong evidence that group-based psychosocial interventions that target stress management during active treatment can reliably modulate indicators of stress, affect and adversity and support positive experiences for extended periods of time in cancer patients.

3.2. Psychosocial intervention effects on disease progression, recurrence and survival

One of the most controversial areas of psycho-oncology research has concerned the question of whether psychosocial interventions can affect the clinical course of cancer (Spiegel, 2011). A landmark study by Spiegel et al. (1989) indicated that women with metastatic BCa who received a 12–month group-based supportive expression therapy (SET) intervention (focused on emotional expression, social support provision, and encouraging acceptance of mortality and decreasing the anxiety surrounding death lived twice as long (approximately 1.5 years longer) as women assigned to standard cancer treatment. Over the subsequent two decades attempts to replicate these effects have been mixed. Three particular clinical trials have been published over the past 10 years, each evaluating the effects of 12-month group-based psychosocial interventions on disease recurrence and survival in women with metastatic disease (Goodwin et al., 2001; Kissane et al., 2007; Spiegel et al., 2007). In each of these trials women with metastatic BCa were assigned to a 12-month course of weekly group-based SET or standard care and two of them (Goodwin et al., 2001; Kissane et al., 2007) showed no survival advantage for women assigned to SET. However, in the Goodwin et al. (2001) study intervention participants had higher levels of depression than controls at baseline, and depression has been shown elsewhere to be a negative prognostic indicator in this population (Giese-Davis et al., 2011). Although the Spiegel et al. (2007) did not show an overall survival effect for SET, secondary analyses found that the subset of women with estrogen receptor (ER) negative tumors assigned to SET had greater survival (Spiegel et al., 2007). This suggested that while women with ER+ tumors may have had more effective medical treatment options available, women with ER− tumors (including those who are triple negative: ER−, PR−, Her2Neu−) could be the major beneficiaries of psychosocial interventions going forward. A subgroup analysis of the Kissane et al. (2007) trial did not reveal a survival advantage for ER− women assigned to SET. In view of the post hoc nature of the Spiegel et al. analysis, and the lack of a similar finding in the Kissane et al. trial, the role of ER status (and possibly other clinicopathologic characteristics) in moderating the effects of psychosocial interventions in women with metastatic breast cancer deserves further study. It is noteworthy that investigating the link between psychosocial adaptation and disease course in patients with advanced cancers such as metastatic BCa may be complicated by advanced disease, and having different treatment options available for different types of breast cancer. What do we know about the effects of psychosocial interventions on disease outcomes in patients who are recruited earlier in the disease process?

Two studies to date have used psychosocial interventions to modulate psychological adaptation in patients treated for primary disease, observed increases in biobehavioral (cellular immune) processes, and then followed patients for evidence of intervention effects on disease course (recurrence, mortality) for at least 10 years (Andersen et al., 2004. 2008; Fawzy et al., 1990a,b, 1993, 2003). In the first of these (Fawzy et al., 1990a,b), patients with malignant melanoma were randomized to 6 weeks of structured group-based psychosocial intervention vs. usual care. Intervention participants revealed increased active coping and decreased negative mood at 6 weeks (Fawzy et al., 1990a), increased interferon-stimulated natural killer cell cytotoxicity (NKCC) at 6 months (Fawzy et al., 1990b), and decreased mortality and recurrence at 6 year (Fawzy et al., 1993) and 10 year follow-up (Fawzy et al., 2003). While the changes in biobehavioral processes (NKCC) at 6-month follow-up did not predict the 6-yr clinical outcomes, intervention-associated increases in active coping did predict clinical outcomes. This suggested the possibility that other biobehavioral changes that may have occurred in tandem with increases in active coping (pro-angiogenic or pro-inflammatory processes) may have mediated the effects of this intervention on disease outcomes.

Andersen et al. (2008) tested the effects of a group-based psychosocial intervention on survival and recurrence in 227 women

with non-metastatic BCa who received the intervention just after surgery. Women were randomized to standard care vs. 4 months of weekly group-based intervention and 8 months of monthly sessions. The intervention included relaxation and stress reduction exercises, coping skills training and health behavior change strategies related to diet and exercise. Intervention participants showed a significant reduction in overall and BCa specific mortality rates as well as 45% reduced risk of cancer recurrence at a median of 11 years follow-up (Andersen et al., 2008). Those who did recur were cancer free for an average of 6 months longer, after controlling for age, accrual site, disease stage and other clinicopathological factors, cancer treatment. There were no group differences in psychiatric medications and outside counseling received. Similarly those in the intervention revealed 56% less risk of death from BCa and 49% lower all-cause mortality risk at follow-up. Among those who died from BCa median survival time in the intervention group was 1.3 years longer ($M = 6.1$ yrs) than those assigned to standard care ($M = 4.8$ yrs). In addition to demonstrating effects of psychosocial intervention on clinical outcomes Andersen et al's group have also provided some evidence for intervention effects on biobehavioral mechanisms that may explain these effects.

The Andersen et al. psychosocial intervention produced alterations in some stress-related immune processes that could contribute to improved general health and possibly altered disease course. These included increases in cellular immunity measures (lymphocyte proliferative responses (LPR) to mitogens) (Andersen et al., 2004) over the 4-month pre-post intervention period. Women in the intervention did report decreased distress but also more healthy eating habits, reduced smoking rates, and differed in the range of chemotherapy doses received (Andersen et al., 2004). At the 12-month follow up health and toxicity items rated by oncology nurses revealed that intervention participants evidenced better health status based on staff ratings. Within the intervention condition, reductions in distress at the 4-month time point predicted better health at 12 months (Andersen et al., 2007a). This team also conducted analyses of blood samples collected at longer-term follow-up during which women were monitored for health status and disease recurrence. Specifically, in a subgroup of depressed women monitored over the survival follow-up period, those assigned to the intervention showed decreases in immunologic markers consistent with active infection or chronic inflammatory conditions (total white blood cells (WBC) and neutrophils) compared to controls (Thornton et al., 2009). Though speculative, these changes were posited by the authors to explain, in part, the effects of this intervention on better overall physical health status over the year after surgery (Andersen et al., 2007b), and disease outcomes (recurrence and survival) observed over the subsequent decade (Andersen et al., 2008).

Women whose cancer ultimately recurred revealed greater serum cortisol and greater levels of WBC and neutrophils 17 months prior to their recurrence compared to those who remained disease free suggesting that these immunological changes in the intervention group may have been relevant in explaining differences in clinical outcomes between groups (Thornton et al., 2008). Interestingly those women who experienced a distal recurrence at this point had weaker cellular immune responses (NKCC, LPR to mitogens) and greater elevations in WBC compared to those who experienced a local recurrence. Thus one possible explanation for the positive effects of this intervention on recurrence may be the normalization of stress- and treatment-associated neuroendocrine and immunologic regulation during a critical period following treatment and preceding recurrence. Previously it has been suggested that optimizing neuroendocrine and immunologic status may mitigate the "seeding" of micro-metastatic cells after primary treatment (Ben-Eliyahu, 2003) thereby optimizing the environment for residual disease to thrive in. Thus normalizing these processes may be critical following initial surgery and during recovery from adjuvant chemotherapy and radiation. Finally, this team followed women after the point of disease recurrence and observed a reduced risk of death over an 80-month follow-up among those who had been assigned to the intervention arm (Andersen et al., 2010). During the 12-month period following recurrence the intervention group also showed improvements in psychological adaptation (decreased negative mood and increased social support) and greater lymphocyte proliferative responses to mitogens, and greater NKCC. This trial provides the best evidence to date that a psychosocial intervention that improves psychological adaptation (decreased distress) may increase cellular immune function (lymphocyte proliferation) early in treatment, and decrease the odds of mortality and recurrence at 7–11 years. Moreover this study is the first to demonstrate that in a subgroup of the most depressed women, intervention participants showed reductions in indicators of inflammation at 17 months prior to recurrence which were, in fact, predictive of recurrence. Finally this is the first study to show that having been in a psychosocial intervention may promote persisting positive changes in psychological adaptation, immune functioning and clinical outcomes after disease recurs. This trial provides preliminary support for the value of using psychosocial interventions to modulate psychological adaptation, monitoring changes in biobehavioral processes reflecting cellular immune functioning, inflammation and other tumor-promoting processes, and following cohorts systematically for effects on clinical outcomes.

3.3. Psychosocial intervention effects on stress-related biobehavioral processes during breast cancer treatment

Over the past 10 years, other trials have evaluated the effects of stress reduction techniques such as cognitive behavioral stress management [CBSM] and meditation-based stress reduction (MBSR). These interventions have shown salutary effects on psychological adaptation, neuroendocrine and immunologic indicators in patients recruited during their medical treatment (see McGregor and Antoni, 2009 for review of BCa studies). As noted previously CBSM is a group based approach that blends cognitive, behavioral and interpersonal skills training through in-session didactic and role playing activities and homework and daily practice of stress management techniques (Antoni, 2003). MBSR applies mindfulness meditation training as a stress management technique and is often delivered in a group format over weekly training sessions and a full-day or longer retreat. Briefly, the effects of CBSM and MBSR have included decreases in late afternoon serum cortisol levels and increases in lymphocyte proliferative response and Th1 cytokine production and Th1/Th2 production ratio (McGregor and Antoni, 2009; Antoni et al., 2009; Carlson et al., 2007; Witek-Janusek et al., 2008). Since the MBSR trials were not randomized clinical trials caution is in order when interpreting the validity of these findings. We now summarize the specific neuroendocrine and immune findings from studies in our lab using CBSM.

We studied women who had completed surgery for non-metastatic BCa and were preparing for the start of adjuvant therapy. They were recruited in the weeks after surgery, competed baseline assessments, and were assigned to either 10 weeks of group-based CBSM or a one-day psychoeducational control group. Women completed questionnaires and provided blood samples at 6 and 12 month follow-up. Women in CBSM reported improvements in negative and positive mood and a wide variety of quality of life indicators as well as decreases in late afternoon serum cortisol, and increases in IL-2 and IFN-γ production from anti-CD3 stimulated peripheral blood mononuclear cells (PBMCs) (Phillips et al., 2008; Antoni et al., 2009). Thus this form of intervention, which was previously associated with decreases in distress states, and in-

creases in positive states, was also related to reductions in cortisol and increases in cellular immune function that are consistent with a hastened recovery from cancer treatment. Showing reductions in PM cortisol is important because flatter di-urnal cortisol slopes (due partly to higher PM levels) have been associated previously with decreased survival among women with metastatic BCa (Sephton et al., 2000). Intervention effects on Th1 cytokine production may be important for supporting cellular immune processes that are involved in tumor eradication, such as antigen presenting cells, cytotoxic- T-cells, and T regulatory cells (Disis and Lyerly, 2005).

In comparing the work in CBSM with the intervention of Andersen et al. (2007b), which combined relaxation with CBT and health behavior change strategies, it is noteworthy that in each trial, distress and/or cortisol decreases were paralleled by either increased frequency of relaxation practice or increased confidence in using relaxation to manage stress (Antoni et al., 2006a,b; Phillips et al., 2011). This provides some insight into one of the possible "active ingredients" of these interventions (i.e., anxiety-tension reduction) and corresponding neuroendocrine changes that may explain their effects on immunologic indicators. Since these are correlational findings, however, it is necessary to conduct experiments to separate the effects of relaxation training from other aspects of multimodal interventions such as CBSM. Across most of these psychosocial intervention studies using patients with non-metastatic BCa it is important to note that only trials showing psychological effects demonstrated physiological effects, and in some the magnitude of the changes in neuroendocrine and immune indicators paralleled the size of the psychological effects (McGregor and Antoni, 2009). It is yet to be determined whether the patients enrolled in these trials of psychosocial interventions that showed neuroendocrine and immune effects of stress management in women with primary breast cancer in the past 10 years (e.g., Antoni et al., 2009; Savard et al., 2005; Witek-Janusek et al., 2008) will demonstrate less disease recurrence and greater survival as reported by Andersen et al. (2008). However, it seems worthwhile to invest in following these extant cohorts who have already completed the intervention phase.

It is important to consider that psychosocial intervention effects on disease outcomes may be mediated by other stress-related behavioral and/or biologic processes. Some behavioral processes include improvements in health behaviors (more exercise, better nutrition, less alcohol consumption, better cancer treatment adherence) and whether psychosocial intervention participants actually receive more effective medical treatment (e.g., co-intervention effects). For instance it is worth noting that women assigned to the assessment arm of the Andersen et al., (2008) trial revealed greater individual variability in chemotherapy dose intensity than those in the intervention arm. Some biological processes that remain to be studied to explain the effects of psychosocial interventions on clinical outcomes in cancer patients include inflammation and those processes that may play a role in directly supporting tumor growth and metastasis, such as angiogenesis, tumor cell migration, tissue remodeling, and anoikis and resistance to apoptosis (Lutgendorf and Sood, 2011; Lutgendorf et al., 2010b). There is growing evidence that neuroendocrine hormones such as glucocorticoids and adrenergic hormones can influence communications between tumor, endothelial, and stromal cells which appears to be critical in modulating downstream signaling pathways important for disease progression (Lutgendorf et al., 2010a).

One paradigm for examining the effects of psychosocial interventions on cell signaling pathways that are relevant to human cancer progression is to examine whether psychosocial interventions with cancer patients are associated with transcriptional changes in circulating leukocytes reflecting molecular pathways that affect inflammation and cellular immune signaling as well as those that promote invasion and metastasis. For instance, given that chronic stress and negative affect are related to other biological processes such as inflammation, which may have relevance for cancer progression (Cole, 2009a,b; Pierce et al., 2009), it is important to examine whether stress reduction interventions (e.g., CBSM) can affect inflammatory indicators and stress-sensitive neuroendocrine processes (e.g., glucocorticoid receptor sensitivity) that control inflammation within circulating leukocytes. It would also be intriguing to show that these interventions are associated with transcriptional changes in genes controlling cellular immunity (e.g., interferon activation pathways) within these cells. Molecular changes in signaling profiles related to tissue modeling and invasion would also provide evidence that these interventions are capable of working directly on stress-related tumor promoting activities in circulating leukocytes as a model for stress-induced effects in potential stromal cells interacting in the tumor microenvironment (Lutgendorf and Sood, 2011). Showing that these molecular changes happen in harmony with changes in psychological adaptation during and after psychosocial intervention would help identify some of the biobehavioral mechanisms underlying the link between stress modulation and the course of cancer. How close are we to demonstrating such phenomena?

3.4. Intervention effects on stress-related leukocyte transcriptional changes in cancer patients undergoing treatment

In an example of this emerging line of work we now summarize a recent study that examined (a) the association of psychological adaptation and leukocyte transcriptional dynamics in women who had recently completed surgery for primary BCa; and (b) the parallel effects of group-based CBSM on psychological adaptation and transcriptional changes as these women moved through their treatment and into the recovery period (Antoni et al., 2012). Using frozen cells from women who had previously participated in an RCT testing the effects of a 10-week CBSM intervention vs. an active control (Antoni et al., 2009) we conducted genome-wide transcriptional profiling and bioinformatic analysis (Cole, 2009a,b) at study entry, and 6- and 12-month follow-up. We found that greater negative affect and less positive affect was associated with greater than 50% differential expression of 201 genes, including upregulated expression of pro-inflammatory cytokines (*IL1A*, *IL1B*, *IL6*, *TNF*) and metastasis-promoting genes (e.g., those involved in tissue remodeling and epithelial-mesenchymal transition, *LMNA*, *MMP9*) (Antoni et al., 2012). Gene Ontology analyses confirmed that these transcripts were disproportionately involved in pro-inflammatory cytokine function and wound healing. This is the first evidence that individual differences in psychological adaptation status early in BCa treatment (2–10 weeks after surgery) are significantly associated with a leukocyte transcriptional profile reflecting an up-regulation of signaling pathways associated with inflammation, invasion and metastasis. These effects were independent of disease stage, time since surgery, sociodemographic factors and anxiolytics, pain and sleep medications, and anti-depressants.

We next found that women assigned to CBSM showed decreases in negative affect and increases in positive affect, and also showed altered expression of 91 genes by >50% at 6–12 month follow-up (Antoni et al., 2012) (see Table 1 for description of genes, and magnitude of CBSM effects). These changes included downregulation of 62 genes encoding pro-inflammatory cytokines, the prostaglandin-synthesis enzyme COX2, inflammatory chemokines and their receptors, and mediators of tissue remodeling and epithelial-mesenchymal transition. Women in CBSM also revealed 29 upregulated genes relevant for cellular immune responding including Type I interferon response, Type II interferon signaling, and interferon signal transduction. Real-time Polymerase Chain Reaction (RT-PCR) analysis confirmed microarray-indicated group

Table 1
Valence and magnitude of cognitive behavioral stress management (CBSM) intervention effects on genomic indicators representing different biological pathways relevant to carcinogenesis over a 6–12 month period in women with breast cancer.

Biological pathway[a]	Genomic indicator	Cell type origin[b]	Valence	Magnitude (CBSM: control fold diff)
Inflammation				
Pro-inflammatory cytokine	IL1A, IL1B, IL6	Mo, pDC	Down-regulation	0.35–0.59
Pro-inflammatory chemokines and their receptors	CCL2, CCL3, CCL3L1, CCL3L3, CCL4L1, CCL4L2, CCL7, CXCL1, CXCL2, CXCR7	Mo, pDC	Down-regulation	0.41–0.61
Prostaglandin-synthesis enzyme	PTGS2 (a COX2 marker)	Mo, pDC	Down-regulation	0.46
Metastasis promotion				
Tissue remodeling/epithelial- mesenchymal transition	G0S2, LMNA, MMP9, OSM	Mo, pDC	Down-regulation	0.55–0.63
Cellular immunity				
Type I interferon response	IFIT1, IFIT2, IFIT3, IFIT44, IFIT44L, ISG15, MX2, OAS2, OAS3	Mo	Up-regulation	1.68–2.08
Type II interferon signaling	IFNG	Mo	Up-regulation	1.54
Interferon signal transduction	STAT1, STAT2	Mo	Up-regulation	1.51–1.58

Mo: Monocyte.
pDC: plasmacytoid dendritic cell.
[a] Inferred from TeLis bioinformatics program (Cole, 2009a,b).
[b] Inferred from Transcript Origin Analysis (Cole et al., 2011).

differences in the between-group relative expression of a sample of transcripts audited.

We then used promoter-based bio-informatic analyses to infer the families of genes contributing to these alterations using TELiS bioinformatic analysis of transcription factor-binding motif (TFBM) distributions in promoters of differentially expressed genes (Cole, 2009a,b). Our results appeared mediated by decreased activity of the transcription factors (TFs) for nuclear factor-kappa B (NFkB/Rel) and the Globin Transcription Factor (GATA) family and increased activity of interferon response factors. We also found that the women in CBSM showed increased expression of genes controlling the glucocorticoid receptor (GR) relative to controls. Parallel analyses of gene transcription controlling for concurrent serum cortisol levels showed an over-representation of GR response elements in the promoters of CBSM-up-regulated genes. Differential transcription of genes bearing GR response elements was not attributable to differential expression of genes encoding the GR. These findings are provocative and suggest that the effect of CBSM on gene profiles reflecting a down-regulation of inflammatory signaling co-occur in tandem with an upregulation of GR. Since chronic stress has been proposed to down-regulate GR and up-regulate inflammatory (NFkB) signaling in other populations (Miller et al., 2008) it is plausible that the transcriptional changes in neuroendocrine and inflammatory factors observed after CBSM are mediated by the decreases in chronic stress and negative affect as noted above. Transcript Origin Analyses (Cole et al., 2011) implicated monocytes and plasmacytoid dendritic cells (pDCs) as the most likely cells involved in CBSM-induced transcriptional changes with up-regulated genes deriving predominately from monocytes and down-regulated transcripts associated with both monocytes and pDCs. The effects of CBSM on gene expression profiles persisted when controlling for clinicopathological, cancer treatment-related, and psychiatric medications, as well as potential sociodemographic and behavioral confounders (Antoni et al., 2012).

Importantly, the genes down-regulated by CBSM included many of the transcripts that were also up-regulated in women with greater negative and less positive affect at baseline. This suggests a specificity of CBSM impact on psychological adaptation-associated genes, specifically those involved in inflammation and tissue remodeling. The immune cell types most likely mediating CBSM transcriptional alterations–antigen presenting myeloid cells – have previously been linked to distress states (Cole et al., 2011). Bioinformatic inferences of TF activity (GATA- and NF-kB/Rel-family TFs) associated with CBSM-induced transcriptional alterations have been linked to stress and SNS signaling in prior work as well (Miller et al., 2009a; Lutgendorf et al., 2009). Findings also suggest that CBSM induced GR activation, possibly representing a reversal of the distress-related GR transcriptional down-regulation shown in other work on chronic stress (Miller et al., 2008). Because these effects persisted after controlling for individual differences in circulating cortisol levels (which CBSM has shown to decrease, Phillips et al., 2008), they suggest that CBSM affects GR target gene expression primarily by enhancing GR functional sensitivity (i.e., reversing stress-induced GR desensitization) (Stark et al., 2001). If this is true it opens the possibility that CBSM and other stress reduction interventions may modulate inflammatory signaling by mitigating stress-induced GR down-regulation in the context of cancer treatment, and possibly in other chronic medical and psychiatric conditions.

Although these findings are provocative, it must be kept in mind that they are not definitive since only a partial sample of cases from a prior RCT were available for analyses. If in fact CBSM was capable of causing the changes in gene expression observed in this study it is unclear whether the accompanying changes in CNS-mediated mood changes precede or follow from changes in leukocyte signaling. It is plausible that pro-inflammatory cytokines derived from activated monocytes may signal to the brain to causally affect neural function and mood (Harrison et al., 2009; Eisenberger et al., 2010), thus inducing a bi-directional regulatory circuit that could explain associations between inflammation and CNS-mediated distress processes. These results justify future randomized trials of similar psychosocial interventions in cancer patients utilizing transcriptional analyses of specific leukocyte subsets, possibly along with functional assays designed to probe the communication between neuroendocrines (e.g., glucocorticoids) and specific leukocyte subpopulations (e.g., monocytes) and their association with longer-term clinical outcomes in order to explore whether the effects of psychosocial interventions on these signaling pathways are relevant to cancer disease progression.

Beginning this line of work with BCa patients makes sense because (a) this is a high prevalence disease and provides the availability of larges samples for clinical trials and mechanism analysis, (b) because most of the evidence showing psychosocial intervention effects on clinical outcomes in cancer populations were in BCa (e.g., Andersen et al., 2008; Spiegel et al., 1989), (c) the first work showing intervention effects on transcriptional changes has been done in BCa (Antoni et al., 2012), and (d) the growing understanding of the role of processes such as inflammation in promoting BCa disease progression (Cole, 2009a,b). Reduc-

tions in pro-inflammatory cytokines (e.g., *IL1A*) and NF-kB activity are notable in the context of BCa because chronic inflammation is believed to contribute to BCa progression and recurrence after treatment. (Pierce et al., 2009). CBSM also downregulated the expression of specific genes known to play a role in cancer progression and metastasis (e.g., *MMP9*), a pattern of change which is associated with reduced progression of cancer (Hanahan and Weinberg, 2011). It also appears that the women receiving CBSM show contemporaneous increases in gene expression associated with a recovery of interferon-mediated cellular immunity, which may be relevant for immunosurveillance of cancer micro-metastases (Benish and Ben-Eliyahu, 2010) or opportunistic infections during and after adjuvant treatment. Together these data suggest that in addition to its beneficial effects on psychological adaptation, CBSM can also alter immune cell gene expression in a manner that may both reverse the biological impact of experienced stress during treatment and could potentially influence disease progression in BCa patients (Lutgendorf et al., 2010b).

3.5. Psychosocial intervention effects in other cancer populations

There is no reason to believe that these psychosocial intervention effects are restricted to BCa. Since other cancer models have shown strong evidence for the influence of stress physiology and neuroendocrines on angiogenesis, invasion, and inflammation in tumor and stromal cells (e.g., Ovarian cancer, Lutgendorf and Sood, 2011) it is reasonable to examine the effects of psychosocial interventions on other types of cancer. The effects of psychosocial interventions on biobehavioral processes in populations other than BCa patients have only rarely been studied. One hallmark study noted previously, showed that a 6-week group-based psychosocial intervention focused on coping skills and interpersonal support was associated with improved mood, increased NKCC, and greater survival and disease-free interval in patients with malignant melanoma followed up to 10 years (Fawzy et al., 2003). More recently, immunological effects of some novel psychosocial interventions have been reported in conjunction with other populations including men with prostate cancer and women with gynecological cancers. One study showed a 2-session stress management intervention (deep breathing, guided imagery and adaptive coping skills) offered to men prior to surgery for prostate cancer related to decreases in mood disturbance and increases in NKCC one week pre- to 48 h post-surgery (Cohen et al., 2011). This is an interesting finding in view of prior work showing that a psychosocial intervention initiated prior to surgery was associated with improved 10-year survival in patients treated for gastrointestinal cancer (Kuchler et al., 2007). Another study showed that telephone-delivered psychosocial counseling intervention is associated with improved QoL and a shift toward a more Th1/Th2 cytokine bias in women with cervical cancer (Nelson et al., 2008). Finally, an integrative medicine approach – Healing Touch – was associated with a greater preservation of NKCC in women with cervical cancer as they went through chemoradiation treatments as compared to controls (Lutgendorf and Sood, 2011). There is also evidence that palliative care intervention may decrease pain and depression on the one hand and increase survival in metastatic lung cancer patients (Temel et al., 2010). All of this work deserves replication in larger samples and longer follow-ups to determine whether these interesting biobehavioral effects during treatment predict longer-term clinical outcomes.

3.6. Exploring other clinical health outcomes in cancer patients

When designing studies of psychosocial interventions in cancer patients it is reasonable to consider other stress-related clinical health outcomes beyond survival and disease recurrence such as the incidence of moderate to severe opportunistic infections (OI) during and after the completion of surgical and adjuvant therapy as well as the effects of cancer treatment on "late effects" including insulin resistance-related cardiovascular disease and diabetes, conditions, which in turn, may also influence cancer treatment efficacy and disease progression (Pall and Hurria, 2010). Surgery and other cancer-related treatment may compromise the immune system sufficiently to place treated cancer patients at risk for opportunistic disease during or after the treatment period (Antoni et al., 2006a). Stress-related changes in upper respiratory infections, reactivation of latent herpesvirus infections, and the progression of virally-associated neoplastic processes are well established. Stress reduction interventions have been shown to modify neuroendocrine (decreased urinary cortisol and NE) and immune (increased naïve T cell reconstitution, decreased antibody titers to herpesviruses, and decreased viral load) parameters as well as clinical disease (cervical neoplasia, Antoni et al., 2008) in persons with infectious disease such as Human Immunodeficiency Virus (HIV) (for review see Carrico and Antoni, 2008).

Cancer survivors might also be at elevated risk for the development of co-morbidities that are associated with alterations in insulin metabolism (abdominal obesity, dyslipidemia, cardiovascular disease and diabetes mellitus) as delayed or "late effects" of successful cancer treatment (Basaria et al., Thomson et al., 2009). For instance, adult survivors of childhood cancer show increased risk for metabolic syndrome indicators (hypertension, hypertriglyceridemia, abdominal obesity) over a 17-yr follow-up period (Hoffman et al., 2008). It has also been established that comorbidities before cancer treatment can affect clinical outcomes (decreased survival, increased disease recurrence) (Patnaik et al., 2011). For instance, conditions such as diabetes mellitus, may increase the risk for disease recurrence in colon cancer (Pall and Hurria, 2010), while obesity, another sign of altered insulin metabolism, has been associated with reduced efficacy of aromatase inhibitors in ER+ breast cancer patients (Sestak et al., 2010). Interestingly metformin, an insulin metabolism modulator, has been shown to have potential as a cancer therapeutic, especially in breast and colon cancers, which are associated with hyperinsulinemia (Dowling et al., 2011). To the extent that psychosocial factors and stress physiology can affect the pathogenesis of comorbidities related to insulin metabolism (Chrousos, 2000; Vitaliano et al., 2002; Schneiderman et al., 2010) then psychological interventions, including stress management, may mitigate the risk of and effects of these conditions in cancer survivorship. It seems reasonable that psychosocial intervention may be able to affect the risk of opportunistic disease and some of the co-morbidities listed here in treated cancer patients and may yield definitive results in much shorter follow-up periods than are required for documenting effects on disease recurrence and survival.

3.7. Caveats and methodological considerations

The cancer diagnosis and treatment may induce acute and chronic stress and reduced quality of life. These changes may be accompanied or followed by poor compliance to medical regimens, increases in negative health behaviors and decreases in positive health behaviors, which may combine with physiologic responses to stress to encourage local and metastatic disease progression. A major challenge in psychosocial intervention research in oncology is finding ways to minimize and account for the confounding effects of different disease characteristics and cancer treatments on biobehavioral indicators and clinical outcomes (Bovjberg, 1991; van der Pompe et al., 1998). Using the example of breast cancer, differences in clinicopathological characteristics can have prognostic significance (stage, HER2-neu+/−, ER/PR+/−) (Biganzoli and Boracchi, 2004; Woodward et al., 2003), and in at least one trial, were found to moderate the effects of a psychological intervention on clinical outcomes (Spiegel et al., 2007).

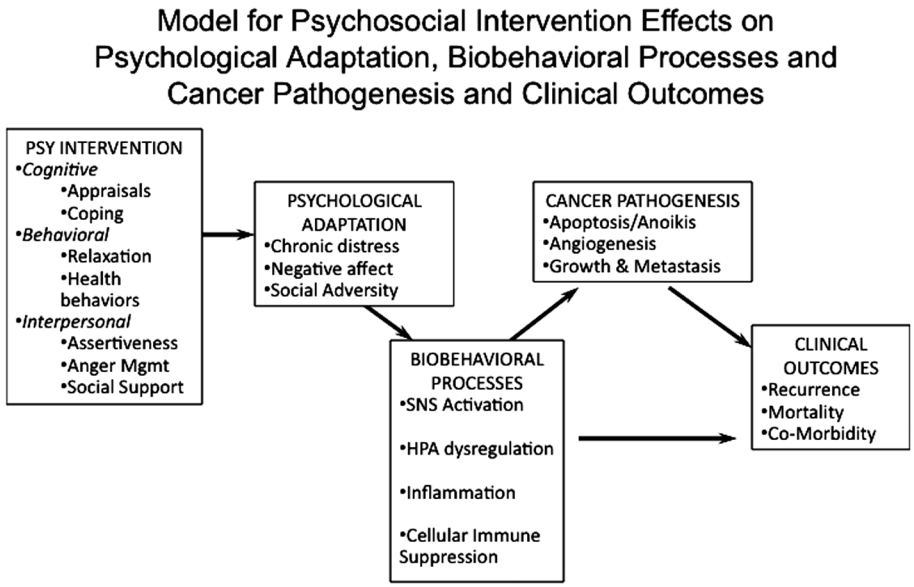

Fig. 1. This model suggests an approach for tracking changes in psychological adaptation, biobehavioral processes and cancer pathogenesis and clinical outcomes following a psychosocial intervention designed to address cognitive, behavioral and interpersonal processes as intervention targets to modulate cancer patients' stress responses during cancer treatment. Psychosocial interventions are hypothesized to decrease chronic stress, negative affect and social adversity. Improvements in psychological adaptation are hypothesized to facilitate decreases in SNS activation, HPA axis dysregulation, inflammation and cellular immune deficits. These alterations in stress-related biobehavioral processes may decrease the likelihood of cancer pathogenic processes associated with tumor cell survival, growth, invasion and metastasis, which could precede clinical outcomes such as disease recurrence, co-morbidities and mortality. Alterations in stress-related biobehavioral processes may also influence clinical outcomes (e.g., co-morbidities) independent of the cancer pathogenic processes listed.

Effects of adjuvant therapy can vary over short periods and regimens are tailored from patient to patient. There is likely inter-individual variation in neuroimmunological responses attributable to surgery, chemotherapy, radiation, immunomodulators, anti-emetics, and hormonal treatments (e.g., Tamoxifen), to name a few of the major classes of treatment. This can affect the decisions investigators make about the timing of their measurements. Decisions about the timing of an intervention also depend on what level of prevention the investigator is targeting (Miller et al., 2009a,b). Determining the timing of psychosocial intervention onset and measurement intervals involves minimizing confounders of biobehavioral readouts by working around known treatment regimens and monitoring the medical complications that occur during and after treatment is completed. This involves balancing the potential clinical yield of peri-treatment timing against the rendering of questionable findings. In cancer patients receiving adjuvant treatments, can we deliver a psychosocial intervention during a patient's peak point of stress and anxiety and still get interpretable neuroendocrine and immune results? Is it better to collect pre-intervention baseline data before the surgery or adjuvant therapy begins, intervene during active treatment, and wait for patients to complete the regimen before collecting follow-up data to observe the shape of their recovery curve, and continue to follow them over clinically meaningful periods? This latter approach may minimize some confounders of medical treatments but many powerful medical treatments have unclear post-treatment side effect kinetics. Because some have hypothesized that the critical period for stress-mediated immunosuppression increasing the risk of breast cancer metastatic spread is in the weeks after surgery (Ben-Eliyahu, 2003), it seems as though some intervention research in breast cancer patients may benefit by contending with these timing issues head on as in the case of the Andersen et al., (2008) trial. Going forward, potential confounds might be minimized in psychosocial intervention trials by controlling for elapsed days since diagnosis and surgery for the pre-intervention measures, elapsed days since most recent adjuvant treatment for intercurrent follow-ups, as well as careful monitoring of frequency/dosage of regimen, treatment actually received, corticosteroids, anti-emetics, non-steroidal anti-inflammatory agents, and other medications patients are receiving at each time point.

Although research on the efficacy of psychosocial interventions on disease progression in diagnosed cancer patients is promising, identifying vulnerable populations of patients who will most benefit from these interventions is an important direction for future research. Such high-risk populations might be identified through psychosocial criteria (e.g., depressed, highly distressed, socially isolated, a socially disadvantaged minority group member) or because of a biological factor (e.g., hereditary risk, stage of disease, tumor receptor status, or having a specific co-morbid condition like HIV/AIDS). Understanding how physical and psychosocial challenges and responses manifest differently across the cancer continuum from initial diagnosis, to curative and adjuvant treatment, to early survivorship, to disease recurrence, and to the emergence of late effects and the need for palliative care (Miller et al., 2009b) will be key in tailoring the content of psychosocial interventions developed for different cancer populations.

Examining new ways to deliver these interventions so that they have greater reach may utilize technological advances in telecommunications (web-based and mobile-phone-based delivery, Heckman et al., 2006) in combination with community-based participatory research methods to reach those populations that are unable or hesitant to receive psychosocial intervention in a medical facility. Using remote collection of biological samples and psychosocial data in home-delivered intervention trials may have real limits but could play a role in expanding the reach of intervention research in cancer populations in real-world settings. However, before these applications are developed it is important to identify the optimal format (group, couples, family, or individual-based) and dosage (number, frequency and duration of training sessions and maintenance sessions) of face-to-face psychosocial interventions needed to influence the psychological adaptation

and biobehavioral processes necessary to produce a clinical outcome. It is important to note that some of the most impressive effects of psychosocial interventions on clinical cancer outcomes in breast cancer have been achieved with very high intensity interventions spanning 12 months of group-based therapeutic contact (Andersen et al., 2008; Spiegel et al., 1989), though work in malignant melanoma has documented effects on clinical outcomes with a much shorter intervention of 6 weeks (Fawzy et al., 1993). It would be critical to see if other brief psychosocial interventions (as few as 2 sessions) that have demonstrated effects on psychological adaptation and biobehavioral indicators (e.g., Cohen et al., 2011) are also associated with positive clinical outcomes over time.

Finally, intervention research designed to elucidate biologic pathways linking psychological adaptation processes to cancer health outcomes has focused mostly on a limited number of neuroendocrine and immune indicators (for review see McGregor and Antoni, 2009). It is important to expand our observations to explore the cellular, biochemical, and molecular activities underlying tumor development and progression that have been related to stress factors and other psychosocial phenomena in the past 10 years, and which may operate in concert with stress-related immunologic changes in influencing disease outcomes (Lutgendorf and Sood, 2011). As attention in cancer research has shifted to a greater emphasis on the tumor microenvironment, it is now reasonable to examine how psychosocial processes and interventions affect the stromal cells (e.g., circulating and tumor-associated myeloid cells) that could interact in the tumor microenvironment and how such changes relate to the clinical course of disease.

4. Conclusion

The evidence available to date demonstrates the ability of many different psychosocial interventions to improve responses to the stress and adversity of the cancer experience in order to improve psychological adaptation. Improvements in psychological adaptation (decreased negative affect and social disruption and increased positive affect and quality of life) have been linked to an improved physiological profile during and after treatment, which may increase the odds for disease-free survival in some cancers. It is crucial to both establish the reliable effects of these interventions on clinical outcomes (recurrence and survival) in more cancer populations and a wider representation of the biobehavioral processes that might explain these effects. Emerging technologies now allow us to expand biobehavioral research in oncology by applying microarray and bioinformatics analyses of immune and tumor cell transcriptional activity. This could illuminate the juncture of neuroimmune communications underlying inflammatory and tumor promoting cell signaling. Several leukocyte gene expression profiles that have been consistently tied to stress and adversity on the one hand, and inflammation and tumor metastasis on the other, are accessible for researchers conducting clinical trials of psychosocial interventions in cancer patients. By co-examining intervention-associated changes in psychological adaptation and leukocyte transcriptional changes, and longer term follow-ups of clinical disease, one could amass systematic biobehavioral evidence for how these interventions work in patients undergoing cancer treatment.

Although it is unclear when the optimal time to initiate these interventions would be within cancer treatment it seems plausible to begin by randomizing patients in the early post-diagnostic, post-surgical or post-recurrence period, and establish a cohort of patients who can be followed systematically for changes in psychological adaptation, biobehavioral indicators and heath status over time. One can then correlate intervention-associated "early and later changes" in biobehavioral processes with clinical course of disease over the subsequent period of years. Using a few of the intervention studies conducted in breast cancer we have suggested some of the ways one might proceed along these lines.

Fig. 1 presents a model for guiding this line of thinking by listing some of the cognitive, behavioral and interpersonal intervention components, and indicators of psychological adaptation, biobehavioral processes, cancer pathogenesis, and health outcomes that might be considered by those designing psychosocial intervention studies in the future. Implicit in this model is a consideration of the many potential confounders that may be operating on each of these sets of variables, a discussion of which is beyond the scope of this review. It is reasonable to propose that psychosocial interventions that address cancer patient's focal concerns in the period before and after surgery for primary disease may reduce stress-associated exacerbations of biobehavioral processes that could promote disease progression. Emerging work suggests the possibility that providing psychosocial interventions shortly after the diagnosis of recurrent disease may also facilitate psychological adaptation and affect biobehavioral processes and/or clinical outcomes (Andersen et al., 2010).

Basic research and preliminary intervention studies suggest that stress factors and psychological interventions may modulate biobehavioral processes in patients diagnosed with ovarian, cervical and prostate cancers. The next step is to test whether these changes predict the clinical course of these diseases. It is important to understand that though these conditions are collectively referred to as cancers, they are different diseases with different causes, promoters, treatments, and prognostic course after initial treatment and recurrence. Creating a rationale for examining psychosocial intervention effects on health outcomes in each of these conditions will require linking the most well-validated intervention approaches to improved psychological adaptation in parallel with changes in biobehavioral processes that are known to be related to the pathophysiology of each of these separate diseases. The trajectory toward optimal health outcomes in the long term may begin with differential recovery from medical treatment for primary disease or newly diagnosed recurrent disease as measured in psychological and physiological adaptation indicators that can be targeted through pre-emptive psychosocial intervention.

Conflict of Interest

Dr. Antoni reports receiving publication royalties from books and related training materials that he has authored on CBSM treatments in health psychology.

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Does tumor necrosis factor-alpha (TNF-α) play a role in post-chemotherapy cerebral dysfunction?

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ABSTRACT

Post-chemotherapy treated cancer patients frequently report cognitive difficulties. The biology of this phenomenon is poorly understood, with uncertainty about possible direct toxic effects on the brain, secondary effects from systemic inflammation, host factors/genetic predisposition to cognitive complaints, or hormonal changes influencing cognitive function. To elucidate possible mechanisms associated with post-treatment cognitive dysfunction among breast cancer survivors, in 2007 we established a prospective, longitudinal, observational cohort study of early stage breast cancer patients, recruited at the end of initial treatments (primary treatment exposure included surgery, ±radiation, ±chemotherapy), and prior to the initiation of adjuvant endocrine therapy. We assessed cognitive complaints, neuropsychological (NP) test performance, markers of inflammation, and brain imaging at baseline, 6 months and 12 months after enrollment. In this analysis of data from the first 93 patients enrolled in the cohort study, we focus on the relationship of circulating levels of proinflammatory cytokines to cerebral functioning and chemotherapy exposure. Among the proinflammatory cytokines tested (IL-1ra, sTNF-RII, CRP, and IL-6) at baseline, only sTNF-RII was increased among chemotherapy exposed patients, with a significant decline in the year after treatment ($p = 0.003$). Higher baseline sTNF-RII in chemotherapy patients was significantly associated with increased memory complaints. In chemotherapy exposed patients, the longitudinal decline in sTNF-RII was significantly correlated with fewer memory complaints over 12 months ($r = -0.34$, $p = 0.04$). Higher baseline sTNF-RII was also associated with relatively diminished brain metabolism in the inferior frontal cortex ($r = -0.55$, $p = 0.02$), as well as relatively increased inferior frontal metabolism after 1 year, in chemotherapy-exposed subjects. These preliminary findings suggest that post-chemotherapy increases in TNF-α may be playing an important role in the manifestations of cognitive complaints in breast cancer survivors.

1. Introduction

With the growing number of cancer survivors, there has been increased interest in the risk factors for and mechanisms by which certain post-treatment symptoms occur and persist (Castellon et al., 2004; Ahles and Saykin, 2007; Donovan et al., 2004; Stein et al., 2000; Bower et al., 2000; Bower, 2007, 2008; Collado-Hidalgo et al., 2008; Miller et al., 2008). Cognitive dysfunction after cancer treatments has been among the most feared post-treatment concern in cancer survivors, and this problem has been particularly associated with cranial radiation, intrathecal chemotherapy, as well as systemic chemotherapy and biotherapy (e.g. interleukin-2, interferon-α) (Meyers, 2000, 2008; Butler et al., 1994; Wefel et al., 2008; Capuron et al., 2004). Breast cancer patients and survivors have been among the most extensively studied with regard to cognitive dysfunction among adult cancer survivors. Early studies were primarily cross-sectional and conducted in long term survivors (Schagen et al., 1999; van Dam et al., 1998; Ahles et al.,

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2002; Castellon et al., 2004), and suggested that chemotherapy exposure had an adverse effect on neuropsychological (NP) test performance. Neuroimaging studies also identified abnormalities in brain functioning, with MRI and PET scanning suggesting that chemotherapy exposure alters functional and metabolic activity in the frontal and cerebellar portions of the brain (Ferguson et al., 2007; McDonald et al., 2010; Silverman et al., 2007). A series of prospective longitudinal studies have been conducted to examine cognitive changes before chemotherapy administration and then after treatment (Wefel et al., 2004; Jenkins et al., 2006; Ahles et al., 2010). These studies have had mixed results, and have not consistently found NP impairment after chemotherapy exposure, with some studies showing NP test performance abnormalities prior to the administration of chemotherapy (Wefel et al., 2004; Ahles et al., 2008).

In 2007 we began a prospective, longitudinal, observational cohort study of early stage, newly-diagnosed, breast cancer patients, who were recruited immediately after the completion of primary treatment (surgery, adjuvant chemotherapy, radiation therapy), and prior to the initiation of adjuvant endocrine therapy if indicated (the UCLA Mind Body Study-[MBS]). By design, not all patients entering the MBS cohort had received chemotherapy, and the planned analyses focused on differences between these two groups (chemotherapy vs. no chemotherapy), before and after the initiation of adjuvant endocrine therapy, as well as an examination of how reproductive factors might modify the initial treatment exposures. At the time our study was designed, many longitudinal prospective studies were underway to examine *pre-chemotherapy treatment cognitive function* in the setting of breast cancer adjuvant therapy (Jenkins et al., 2006; Ahles et al., 2008, 2010; Wefel et al., 2010; Schagen et al., 2006), with post-treatment follow-up that was often short term, as well as small samples that were often confounded by concomitant use of endocrine therapy (tamoxifen or aromatase inhibitors) in the comparison groups and in patients exposed to chemotherapy. As clinical observations suggested that changes in menopause status associated with chemotherapy could be contributing to post-treatment cognitive complaints, our cohort study focused on recovery post-primary adjuvant therapy, and the interaction with menstrual status and targeted endocrine therapies in the follow-up year.

The MBS also evaluated self-reported cognitive complaints, as there is an emerging literature supporting the ability of individuals to subjectively detect changes in cognitive function long before they are documented with more objective tests (Saykin et al., 2006). In breast cancer patients, this is supported by two recent studies demonstrating that patients who have received chemotherapy report cognitive complaints (memory and executive function) that align with relevant NP test domains and abnormalities in related anatomic regions on brain imaging (Deprez et al., 2012; Kesler et al., 2011).

Little is known about the potential mechanisms that might contribute to changes in cognitive function associated with cancer treatments (Ahles and Saykin, 2007; Wefel et al., 2008). In line with recommendations from two international workshops on this topic (Tannock et al., 2004; Vardy et al., 2008), our research program's conceptual model included robust assessments of potential mechanisms by which cancer treatments (chemotherapy, radiation, hormonal treatments) could affect cerebral functioning, including the role of behavioral symptoms (fatigue, depressive symptoms, insomnia), and immune alterations (proinflammatory cytokines), in addition to endogenous endocrine exposures (estrogen, cortisol) (see Fig. 1). In this context, for cerebral functioning, we included measures of self-reported cognitive function, results of formal NP testing, and brain metabolism as assessed by positron emission tomography (PET) scanning (the latter in a substudy population). Although all of the factors in our conceptual model may affect cerebral functioning, they often interact among each other, e.g., chemotherapy leads to premature menopause (change in estradiol level), as well as having a potentially independent effect on cerebral functioning.

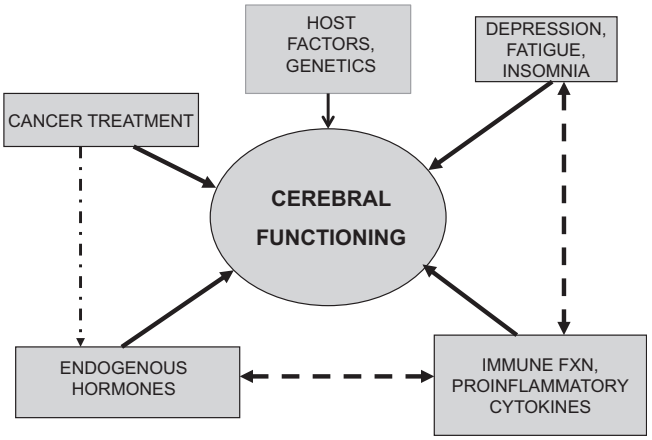

Fig. 1. Conceptual model for the study, which focuses on the multiple factors influencing cerebral functioning (as measured by self-report, neuropsychological testing, brain imaging). Cancer treatment includes surgery, radiation therapy, chemotherapy and targeted endocrine therapy (tamoxifen, aromatase inhibitors) and endogenous hormones include estrogen and cortisol. All of these factors may influence cerebral functioning, but can also interact with each other, for example, inflammatory cytokines may have direct effects on the brain, but can also influence cerebral functioning via their causal role in fatigue and other behavioral symptoms.

The main purpose of this examination of the MBS cohort study was to determine whether or not there was a significant relationship between recent chemotherapy exposure in women with early stage breast cancer and proinflammatory cytokines, and how these markers of inflammation might interact with various aspects of cerebral function and other behavioral symptoms. Based on our prior work examining persistent fatigue in breast cancer survivors (Bower et al., 2002, 2007, 2009; Collado-Hidalgo et al., 2006), we anticipated finding increases in proinflammatory cytokines in these post-treatment patients, but did not know whether this would affect cognitive complaints or neuropsychological performance, as we had not assessed these outcomes previously in this patient population. Therefore, to assess whether or not the laboratory studies should be continued across the entire longitudinal cohort study, we conducted this analysis that focuses on inflammation and cognitive dysfunction related to chemotherapy treatment exposure as the main effect.

In this report, we present data on the relationship between chemotherapy treatment exposure and cognitive complaints, behavioral symptoms, markers of inflammation, and NP performance at baseline prior to the start of endocrine therapy (*cross-sectional evaluation*). In longitudinal analyses, we focus on the trajectory of inflammatory markers over time, according to chemotherapy exposure, and their relationship to cognitive complaints as well as cerebral metabolism in the initial group of participants in the PET scan substudy (*longitudinal evaluation*). While successful recovery after breast cancer treatments has been described by our group previously in an earlier cohort study (Ganz et al., 2011b), the longitudinal relationship of chemotherapy exposure to post-treatment inflammation and recovery of symptoms has not been explored previously, to the best of our knowledge.

2. Methods

2.1. Study participants and recruitment

This was an observational cohort study that recruited women with early stage breast cancer from the Los Angeles community,

with the goal of studying the effects of endocrine therapy for breast cancer on cognitive function. To that end, women entering the cohort may have either had chemotherapy or not prior to study enrollment. Eligibility for this study included: (1) women aged 21–65 years; (2) newly diagnosed with Stage 0, I, II, IIIA breast cancer; (3) completion of primary treatment (surgery, radiation, and/or chemotherapy) within the past 3 months; (4) prior to start of endocrine targeted therapy if planned; (5) geographically accessible for follow-up in 1 year; (6) English language proficient; (7) able to provide informed consent. Exclusions and ineligibility were (1) evidence of current or past disorder/disease of the central nervous system or any medical condition that might be expected to impact cognitive functioning (e.g. multiple sclerosis, thyroid dysfunction); (2) history of head trauma with loss of consciousness greater than 30 min; (3) epilepsy, dementia, or severe learning disability; (4) current psychotic-spectrum disorder (e.g. schizophrenia, bipolar disorder, major depressive disorder) or current substance abuse or dependence; (5) history of whole brain irradiation or surgery; (6) history of past cancer treatment with chemotherapy; (7) active diagnosis of autoimmune and/or inflammatory disorder (e.g., systemic lupus erythematosis, rheumatoid arthritis, vasculitis) or disorders that may influence inflammatory processes (e.g., insulin-dependent diabetes; uncontrolled allergic condition or asthma); (8) chronic use of oral steroid medication; (9) hormone therapy (estrogen, progestin compounds) other than vaginal estrogen. An upper age-limit was used in this study due to the known age-related changes in cognitive function and brain volume beyond age 65, as well as the desire to limit confounding with treatment exposure (i.e. chemotherapy, which is less frequently given to older women based on tumor characteristics and lack of benefit in this age group). Medical conditions that were excluded were chosen for their known potential impact on cognitive function or inflammation.

Initial study recruitment occurred through practices of physicians treating breast cancer patients. When this yielded few participants, we used the Los Angeles County SEER registry for rapid case ascertainment of stage eligible patients from selected hospitals where the collaborating physicians practiced. After mailed notification to physicians of our intent to contact the patient (with physician advice if contact was contraindicated), we mailed the patient a study invitation letter and brochure. This was the major source of study participants. Patients contacted us for more information, and were screened by telephone to determine eligibility. Interested and eligible women were scheduled for an in-person appointment where they had anthropometric assessments (weight, height, waist circumference), and underwent neuropsychological (NP) testing, collection of biological specimens, and completion of psychosocial questionnaires. Assessments were conducted in the morning (before 11 am) after a two hour fast. Women rested for 20 min before the blood draw. (*Note:* a CONSORT recruitment figure will be provided when the full cohort is reported.) Fig. 2 shows the overall study design, including PET scan brain imaging that was done in a sub-study. Some preliminary results from the baseline sample of patients examining the relationship between markers of inflammation and common treatment associated symptoms (e.g., fatigue, sleep disturbance, depressive symptoms) have been reported elsewhere (Bower et al., 2011). The research was approved by the UCLA institutional review board and all participants provided informed consent.

2.2. Demographic, clinical, and behavioral measures

Demographic and clinical information was obtained from self-report and medical record abstraction. Fatigue was assessed using the Fatigue Symptom Inventory (FSI), a valid and reliable 14-item measure specifically designed to assess fatigue in cancer populations (Hann et al., 1998; Donovan et al., 2008; Donovan and Jacobsen, 2010). The Beck Depression Inventory-II (BDI-II), a 21-item measure with excellent reliability and validity, was used to assess symptoms of depression during the past two weeks (Beck et al., 1996). Subjective sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI), a 19-item questionnaire with high internal consistency, test–retest reliability, and diagnostic validity with insomnia diagnosis (Buysse et al., 1991). For the FSI, BDI-II, and PSQI, higher scores indicate worse symptoms. The Squire Memory Questionnaire (SMQ) (Squire et al., 1979), a validated 18-item self-report measure, was used to provide an assessment of memory complaints, where lower scores indicate greater severity of memory complaints.

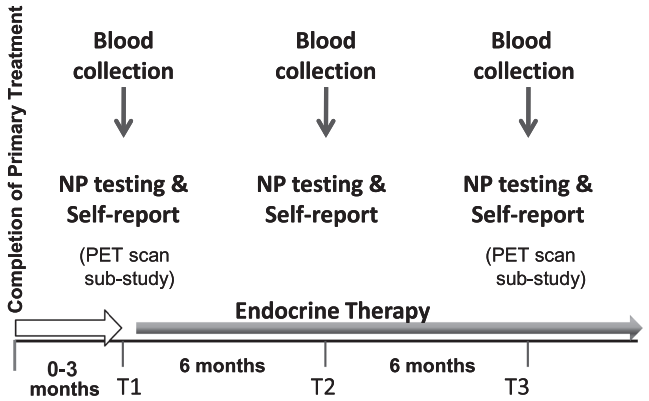

Fig. 2. Study design. Patients entered the study within 3 months of completing primary breast cancer treatment but before initiating breast cancer targeted endocrine therapy. A comprehensive assessment occurred at study entry (baseline-T1), 6 months later (T2) and 12 months later (T3). A substudy focused on brain imaging with FDG PET scans at T1 and T3 only.

2.3. Neuropsychological (NP) assessments

NP testing was conducted by a trained technician, closely supervised by a licensed clinical neuropsychologist. Review of NP testing data occurred throughout the study via case presentations and problem-focused discussions. The test battery used validated, reliable, and commonly used NP tests (see Appendix A), and took approximately 120 min to administer. All of the NP test scores were standardized to *z*-scores where positive scores indicated outcomes better than the age-matched *normative* means and negative scores worse than the normative means (normative references found in Appendix A). These scores were then used to create NP test domains based upon prior factor analytic studies of larger NP data sets and groupings used in other studies with this population (Tulsky and Price, 2003). We identified *a priori* variables most salient for the cognitive domain being studied. An estimate of full-scale IQ (the Wechsler Test of Adult Reading (WTAR), see Appendix A) was administered at baseline (T1) and provided a covariate in all NP related analyses.

2.4. Biological specimens

Blood samples for circulating inflammatory markers were collected by venipuncture into EDTA tubes, placed on ice, centrifuged for acquisition of plasma, and stored at −80 °C for subsequent batch testing. We evaluated four inflammatory markers that have been examined in association with cancer-related fatigue in previous research: IL-1 receptor antagonist (IL-1ra) and soluble TNF receptor type II (sTNF-RII), surrogate markers for IL-1 and TNF-α activity, respectively, as well as IL-6 and C reactive protein (CRP)(Bower et al., 2002, 2007, 2009; Collado-Hidalgo et al.,

Table 1
Patient demographic and medical characteristics by chemotherapy status.

	Total (n = 93)		Chemo (n = 49)		No chemo (n = 44)		p-Value
	%	n	%	n	%	n	
Age at baseline, mean (SD)	51.3 (7.8)		49.9 (8.5)		52.8 (6.7)		0.0685
Months from diagnosis to T1, mean (SD)	7.0 (2.8)		8.7 (2.4)		5.0 (1.6)		<0.0001
Race							
White	85%	79	84%	41	86%	38	0.7172
Marital status							
Married	71%	65	77%	37	64%	28	0.1571
Education							
Post college	49%	46	49%	24	50%	22	0.9471
College	33%	31	35%	17	32%	14	
Post HS	17%	16	16%	8	18%	8	
WTAR, mean (SD)	113.8 (9.2)		113.8 (9.0)		113.9 (9.5)		0.9710
Employment							
Full or part-time	61%	56	54%	26	68%	30	0.1688
Household income							
>$100K	70%	62	60%	28	81%	34	0.0285
BMI, mean (SD)	24.7 (4.5)		24.3 (4.6)		25.1 (4.4)		0.3922
Stage at diagnosis							
0	17%	16	0%	0	37%	16	<0.0001
1	45%	41	35%	17	56%	24	
2	34%	31	57%	28	7%	3	
3	4%	4	8%	4	0%	0	
Surgery							
Mastectomy	28%	26	33%	16	23%	10	0.2869
Lumpectomy	72%	67	67%	33	77%	34	
Radiation							
Yes	76%	71	80%	39	73%	32	0.4367
Hormone replacement therapy							
Yes	27%	24	21%	10	33%	14	0.1810
Change in period							
Post-menopausal	48%	45	45%	22	52%	23	<0.0001
No change	18%	17	4%	2	34%	15	
Became irregular	5%	5	0%	0	11%	5	
Stop but resumed	3%	3	4%	2	2%	1	
Amenorrhea	25%	23	47%	23	0%	0	
Chemotherapy regimen: Anthracycline							
Yes	–	–	29%	14	–	–	–
Targeted endocrine therapy at 6-months							
Yes	72%	67	78%	38	66%	29	0.2117
If Yes, type:							
Tamoxifen	54%	36	45%	17	66%	19	0.0910
Aromatase inhibitor	46%	31	55%	21	34%	10	

WTAR = Wechsler Test of Adult Reading; BMI = Body Mass Index.

2006; Orre et al., 2009; Alexander et al., 2009; Wang et al., 2010, 2012). Plasma levels of IL-1ra and sTNF-RII were determined by regular sensitivity ELISA (lower limit of detection of 31 and 234 pg/ml, respectively), and IL-6 by high sensitivity ELISA (lower limit 0.2 pg/ml) (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. CRP levels were determined by a high sensitivity ELISA (Immundiagnostik, ALPCO Immunoassays, Salem, NH) according to the manufacturer's protocol, but with an extended standard curve to a lower limit of detection of 0.2 mg/L. All samples were run in duplicate, and assays were repeated on two separate assay days for sTNF-RII and IL-1ra; the intra- and inter-assay precision of all tests was less than or equal to 10%.

2.5. PET scan imaging

Acquisition and analysis of PET scans were performed as we have detailed previously (Silverman et al., 2007), with modifications as described here. We present the entire PET scan protocol, although only data from the resting FDG studies were examined in this interim analysis. The PET scan protocol is as follows: First, the radiotracer [O-15]water was used to assess acute changes in cerebral blood flow associated with performance of control and memory tasks. In a standard word-pair association cognitive tasking protocol, cue words were projected from a computer onto each subject's bilateral visual fields, through the use of electronic goggles worn by subjects during scanning. These words were presented 10 min (short-term recall task) or 1 day (long-term recall task) after having been presented paired with other words. Tasks were presented twice each, in counterbalanced fashion, i.e. a control read-repeat task, short-term read-recall task, long-term read-recall task, long-term read-recall task, short-term read-recall task and control read-repeat task. All subjects doing the [O-15] water activation studies performed both short-term and long-term recall tasks. PET was performed with 3D acquisitions, using a 64-slice PET/CT scanner (Siemens). Low-dose CT scans were used for attenuation correction. Six PET images using [O-15]water were acquired at 14-min intervals. For each scan, after administration of 555 MBq [O-15]water, PET data were obtained in list mode, and acquired data were framed in six 5-s frames followed by nine 10-s frames, then summed for all frames after appearance of tracer into the

brain (beginning approximately 25 s after injection). Following the conclusion of the activation study, FDG was used to assess regional cerebral metabolism during mental rest. Subjects were scanned in the supine position, 40 min following injection of 185 MBq FDG in a dimly lit room having low ambient noise, with eyes and ears unoccluded. Effects on resting metabolism were evaluated by both standardized volume of interest (VOI) and statistical parametric methods. In sVOI analyses, mean activities in 47 standardized VOIs were quantified for each scan, and normalized to mean global activity, using a commercially available display-and-analysis software package (NeuroQ; Syntermed Inc.). Activation studies with [O-15]water PET, as well as metabolism studies with FDG PET, were also examined with statistical parametric mapping (SPM) software by methods previously described (Rasgon et al., 2005). Briefly, images were coregistered and reoriented into a standardized coordinate system (Talairach and Tournoux, 1988), using the nonlinear spatial transformation package in SPM8 (Friston et al., 2007), smoothed three-dimensionally at a full-width half-maximum of 8 mm, and normalized to mean global activity. Pooled data were then statistically assessed to identify the voxels which significantly differed between cognitive tasks (activation [O-15]water scans) and/or between treatment groups, or within treatment groups at different points of time, or which significantly correlated with a specified neuropsychologic parameter or peripheral cytokine measure. All results were reported in terms of locations of the most significant effects (regionally and/or in *x*, *y*, *z* Talairach-style millimeter coordinates; *Z* and *P* values) and, when statistical strength of peak voxels or of regional volumes was high enough to survive standard statistical correction for multiple comparisons at $p < 0.05$, also by those corrected values. Results of the cerebral blood flow activation studies will be reported separately.

2.6. Statistical methods

Patient demographic and medical characteristics were summarized for the entire study sample and stratified by chemotherapy exposure status. Chi-squared and *t*-tests were used to test for significant differences between the two treatment groups. Baseline (T1) mean scores on behavioral symptom measures, NP test domains and inflammatory markers were also compared with *t*-tests. For the bivariate relationships of chemotherapy and self-reported symptoms, to adjust for multiple comparisons, *p*-values <0.0125 are significant. 100% of plasma samples had detectable IL-1ra, sTNF-RII, and IL-6; 12% of samples were undetectable (<0.2 mg/L) for CRP. Undetectable CRP samples were assigned a value of 0.1 mg/L for analytical purposes. sTNF-RII was examined with both mean and log-transformed scores in various analyses, with similar findings. For ease of interpretation mean values are presented. NP test domains were adjusted for estimated IQ and inflammatory marker means were adjusted for patient age, BMI and radiation. For comparison of NP test domains by chemotherapy status, due to multiple comparisons, a *p*-value <0.0063 is significant. Repeated measures ANOVA controlling for age, BMI, radiation, and time since completion of chemotherapy, were conducted to compare sTNF-RII by chemotherapy status at each of the three assessments. The association between the SMQ with sTNF-RII was examined at baseline with a partial correlation, after controlling for age, BMI, radiation, time since last chemotherapy treatment among chemotherapy patients, depression, with and without the addition of fatigue to the model. To assess the association of changes in sTNF-RII with changes in SMQ over the 12-month study period, additional partial correlations were conducted for changes from T1 to T3.

3. Results

3.1. Patient characteristics at baseline (T1)

Study entry began in May 2007, and we conducted batched evaluations of inflammatory markers in February and November 2010 for the 98 patients who had entered the study. Among these patients, 3 were excluded from this analysis because they did not have any follow-up data after the baseline, and 2 were excluded because they had clinical infections at the time of sampling, leaving 93 patients with serial longitudinal data (e.g., 87 with complete case data and 6 with T1 (baseline) and either T2 (6 months after baseline) or T3 (12 months after baseline), who are the subject of this report. Forty-nine patients had received chemotherapy prior to study entry at T1, and 44 had not received chemotherapy. Table 1 presents the demographic and medical characteristics of the patients at T1. There were few differences between the patients who received chemotherapy compared with those who did not. Specifically, there was no significant difference in the subsequent use of endocrine targeted therapy (e.g., tamoxifen, aromatase inhibitors) of breast cancer during the following 12 months. (The examination of endocrine effects will be the focus of the main study results for the full cohort; the four cells examining endocrine therapy with or without chemotherapy, no chemotherapy or endocrine therapy, and chemotherapy alone were too small, with insufficient power for examination in this analysis.)

The chemotherapy treated patients entered the study at a longer time after diagnosis due to this additional therapy (8.7 months vs. 5.0 months, $p < 0.0001$). The study participants were largely white, married, highly educated, and employed, with high household income. There were no significant differences in cancer treatments (type of surgery, radiation receipt, or subsequent endocrine therapy use); however, as expected, patients receiving chemotherapy had more advanced stage disease ($p < 0.0001$), with 37% of the no chemotherapy patients being stage 0. Among the chemotherapy treated patients, 29% ($n = 14$) received an anthracycline containing regimen (doxorubicin, cyclophosphamide, and a taxane, $n = 12$; or fluorouracil, epirubicin, cyclophosphamide, $n = 2$). The distribution of regimens in the remaining 35 patients were as follows: docetaxel and cyclophosphamide, $n = 25$; a taxane and carboplatin, $n = 9$; gemcitabine with cisplatin, $n = 1$. These regimens reflect standard patterns of contemporary adjuvant chemotherapy treatment. As expected, chemotherapy-induced amenorrhea occurred in 47% of those treated; however, the number of women who were

Table 2
Self-reported behavioral symptoms at baseline by chemotherapy status.

	Total ($n = 93$)		Chemo ($n = 49$)		No chemo ($n = 44$)		*p*-Value
	Mean	STD	Mean	STD	Mean	STD	
FSI severity	3.88	2.12	4.49	2.26	3.20	1.74	0.0030
SMQ	−12.34	15.64	−19.29	17.41	−4.75	8.58	<0.0001
PSQI	8.15	4.02	9.16	3.88	7.11	3.94	0.0157
BDI-II	9.14	7.36	10.92	6.73	7.20	7.59	0.0148

FSI = Fatigue Symptom Inventory; SMQ = Squire Memory Questionnaire; PSQI = Pittsburgh Sleep Quality Index; BDI-II = Beck Depression Inventory-II.

postmenopausal in each group before and after treatment were similar (see Table 1).

3.2. Relationship of self-reported behavioral symptoms to chemotherapy treatment exposure

Table 2 provides the scores on the self-reported behavioral symptoms at baseline, after treatment with surgery, and/or radiation, and/or chemotherapy and prior to the initiation of endocrine therapy (if planned). Patients exposed to chemotherapy had significantly greater fatigue severity ($p = 0.003$) and memory complaints ($p < 0.0001$). Sleep problems and depressive symptoms were marginally significant at the Bonferroni-corrected $p = 0.0125$. Fatigue, memory complaints, and depressive symptoms are all highly correlated with each other in these patients: FSI severity with SMQ: $r = -0.51601$, $p < 0.0001$; FSI severity with BDI-II: $r = 0.50254$, $p < 0.0001$; SMQ with BDI-II: $r = -0.54698$, $p < 0.0001$, suggesting the possibility of a common underlying biological process mediating these symptoms.

3.3. NP test results at baseline by chemotherapy exposure

We examined the relationship between the 8 domains of NP functioning and chemotherapy exposure at baseline, adjusting for the WTAR score. As can be seen in Table 3, the chemotherapy exposed patients had somewhat lower, but non-significant differences in performance in the psychomotor domain ($p = 0.07$). There were no differences in the other domains examined. Also, it is worth noting that both of the treatment groups performed above average in reference to available age-matched normative data. This is not particularly surprising, given the above average estimated IQ for the participants in this study, and their high educational attainment (see Table 1). We also examined the relationship between each of the NP domains with each of the four proinflammatory cytokines, controlling for age, BMI, WTAR, radiation, and chemotherapy, and there were no significant relationships found (data not shown).

3.4. Proinflammatory cytokine results and their relationship to cognitive complaints

After controlling for patient age, BMI and radiation, the mean plasma level of sTNF-RII was significantly higher among chemotherapy-treated patients at baseline (T1) compared to those patients who did not receive chemotherapy (2629 vs. 2149 pg/ml, $p = 0.0015$) (see Table 4). There were no differences in mean IL-1ra, IL-6 or CRP between the two groups at baseline (Table 4), and none of these three markers changed significantly over the following 12 months (data not shown). To determine whether greater sTNF-RII levels were associated with greater cognitive complaints, we also examined the correlations between sTNF-RII at baseline and self-reported memory complaints, and found that higher levels of sTNF-RII were significantly correlated with greater memory complaints after controlling for age, BMI, radiation and depression, and time since last chemotherapy treatment among chemotherapy patients (SMQ $r = -0.21$, $p = 0.05$). To determine whether the same variance in sTNF-RII levels might contribute to both cognitive complaints and previously observed elevations in fatigue, we controlled for the sTNF-RII relationship to fatigue (FSI severity) and found that associations between sTNF-RII and memory complaints were rendered nonsignificant, although still positively correlated ($r = -0.17$, $p = 0.13$).

Table 3
NP test domains[a] at baseline by chemotherapy status adjusted for IQ ($n = 93$).

	Chemo ($n = 49$)		No chemo ($n = 44$)		p-Value
	Mean	SE	Mean	SE	
Psychomotor	0.37	0.10	0.63	0.10	0.0728
Executive function	0.29	0.09	0.49	0.10	0.1419
Verbal learning	0.42	0.10	0.59	0.10	0.2299
Verbal memory	0.68	0.09	0.74	0.09	0.6066
Visual learning	0.10	0.09	0.24	0.10	0.2789
Visual memory	0.03	0.11	0.16	0.12	0.4622
Visuo-spatial	0.46	0.10	0.59	0.11	0.3947
Motor speed	−0.41	0.16	−0.30	0.17	0.6396

[a] Detailed description of NP test domains is included in the Appendix.

Table 4
Inflammatory cytokines at baseline by chemotherapy status adjusted for age and BMI.

	Chemo ($n = 49$)		No chemo ($n = 44$)		p-Value
	Mean	SE	Mean	SE	
Il-1ra (pg/mL)	261	51	331	53	0.3484
IL-6 (pg/mL)	1.6	0.1	1.6	0.2	0.8303
CRP (mg/L)	1.99	0.43	2.30	0.45	0.6230
sTNF-RII (pg/mL)	2580	100	2113	104	0.0019

The level of sTNF-RII declined significantly in the chemotherapy treated patients from T1 to T3 ($p = 0.003$), but remained unchanged in those who were not treated with chemotherapy (see Fig. 3). Examination of the longitudinal change over time in sTNF-RII by chemotherapy exposure status revealed significantly higher levels in the chemotherapy patients at baseline and 6 months, but no significant difference at 12 months (see Fig. 3). Finally, in the chemotherapy exposed patients, we examined the relationship between the change sTNF-RII between T1 and T3 (a decline), and the change in SMQ, adjusting for age, BMI, radiation, depressive symptoms, and time since last chemotherapy treatment among chemotherapy patients (see Fig. 4). The two were significantly correlated ($r = -0.34$, $p = 0.04$) suggesting that the decline in sTNF-RII was significantly associated with improvements in self-reported memory. There was no significant correlation found among those patients who did not received chemotherapy.

3.5. Relationship of plasma sTNF-RII with regional distribution of cerebral metabolism

During the MBS recruitment and follow-up period included in this analysis, we enrolled 17 women at baseline (12 chemotherapy and 5 no chemotherapy) and 16 women had completed follow-up

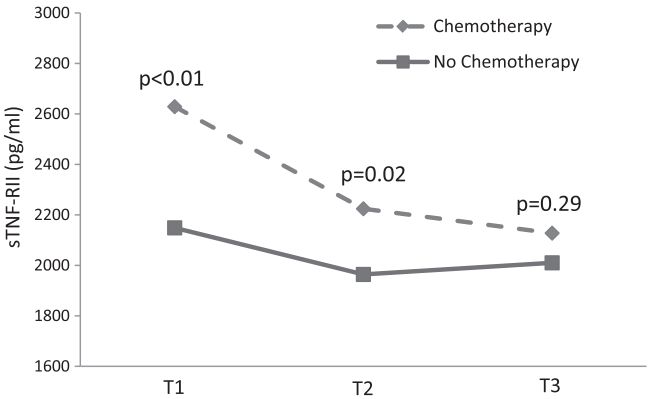

Fig. 3. Mean plasma sTNF-RII levels over 12 months by chemotherapy exposure status. Comparisons between chemotherapy exposed (dotted line) and no chemotherapy (solid line) treatment groups, controlling for age, BMI, and radiation, showed significantly higher levels of sTNF-RII in the chemotherapy treated group at T1 and T2.

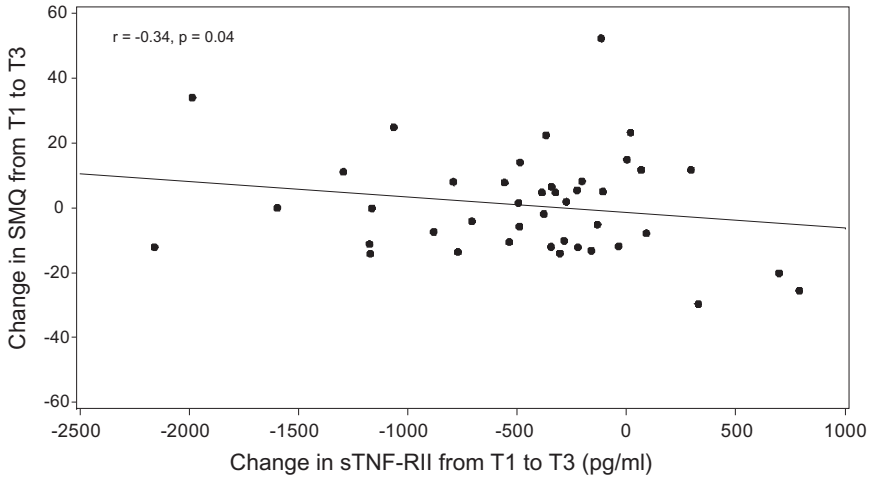

Fig. 4. Scatter plot of change in Squire Memory Questionnaire (SMQ) vs. change in plasma sTNF-RII from baseline (T1) to 12 months (T3) among chemotherapy patients ($n = 43$). Correlation adjusted for age, BMI, radiation, depressive symptoms, and time since last chemotherapy treatment. Result: $r = -0.34$, $p = 0.04$.

scans at T3 (11 chemotherapy and 5 no chemotherapy). We used this sample to explore the potential impact of proinflammatory cytokines on cerebral function as reflected by alterations in brain metabolism. This was accomplished through assessment of relationships between peripheral cytokine markers and the distribution of FDG measured using brain PET, focusing for the purposes of the present analysis particularly upon sTNF-RII. We previously identified the region of the brain having diminished resting metabolism bearing the strongest correlation with relatively diminished cognitive function in adjuvant chemotherapy-exposed patients as being located in the inferior frontal gyrus, in the vicinity of Broca's area (Silverman et al., 2007); this region thus served as the basis for an *a priori* hypothesis about where to look for a negative correlation with the relatively elevated sTNF-RII marker identified in the current adjuvant chemotherapy-exposed cohort. Within the group undergoing PET, 9 of the 12 chemotherapy-exposed subjects demonstrated baseline plasma sTNF-RII above the mean level found for all non-chemotherapy exposed subjects; across these subjects, as well as across all subjects undergoing PET, a significant negative baseline correlation ($r = -0.55$; $p = 0.02$) was in fact observed between plasma sTNF-RII and mean inferior frontal metabolism (Fig. 5). Statistical parametric mapping analysis (see Section 2.5) further revealed a relationship between plasma sTNF-RII and cerebral metabolism of the chemotherapy-exposed subjects that spanned the 1-year longitudinal time-frame of the present study. The single most significant, and concomitantly most extensive, brain region demonstrating metabolism at 1 year positively correlating with baseline plasma sTNF-RII levels was found in inferior frontal cortex, along the anteromedial aspects of the bilateral frontal lobes (Fig. 6).

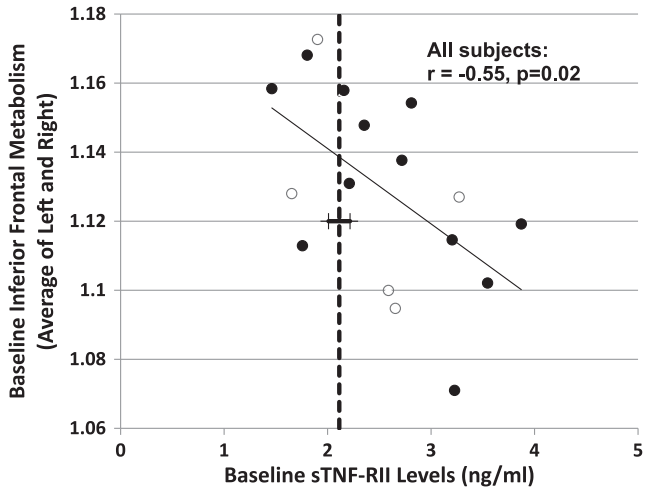

Fig. 5. Relationship of plasma sTNF-RII to inferior frontal metabolism, both measured at baseline ($r = -0.55$, $p = 0.02$) in 17 subjects who participated in the PET scan substudy, 12 chemotherapy exposed (filled circles) and 5 without chemotherapy (open circles). The mean baseline value of sTNF-RII for all non-chemotherapy treated patients in the full sample ($n = 44$) is included as a reference (bold dashed line). Regional metabolism values reflect the mean of standardized volumes of interest assessing the left and right inferior frontal gyri, as described in the text.

4. Discussion

Cognitive dysfunction after cancer treatment is common, and the causal mechanisms are uncertain (Ahles and Saykin, 2007). This dysfunction can be manifested by self-reported increased cognitive complaints after treatment (most often memory and executive functioning problems), or with documented declines in NP test performance in certain domains, or with changes in brain function through various imaging strategies (i.e., MRI, PET). Relatively few studies have examined all of these components in the same study population (Kesler et al., 2011; Deprez et al., 2012). The MBS focused, in part, on the impact of treatment associated inflammation as a potential risk factor for cognitive dysfunction after primary adjuvant therapy. The final assembled cohort for the MBS is 190 women entered after the end of primary treatment, recruited over a 4 year period. Rather than delaying all analyses until the entire cohort completed the 12 month assessment, we performed this evaluation of the inflammatory markers to determine whether or not the hypothesized relationships between post-chemotherapy treatment, inflammation and symptoms were on target. As we have reported elsewhere, plasma sTNF-RII was significantly correlated with fatigue at the baseline assessment, but not sleep or depressive symptoms (Bower et al., 2011). In this report, we extend these initial findings to examine the relationship of the inflammatory markers to cognitive dysfunction, examining results from self-reported cognitive complaints, formal NP testing, and brain metabolism. We examined both baseline (cross-sectional) and longitudinal changes in inflammatory markers after exposure to chemotherapy and other primary breast cancer treatments. We identified significant correlations between self-reported memory complaints and plasma sTNF-RII levels at baseline and longitudi-

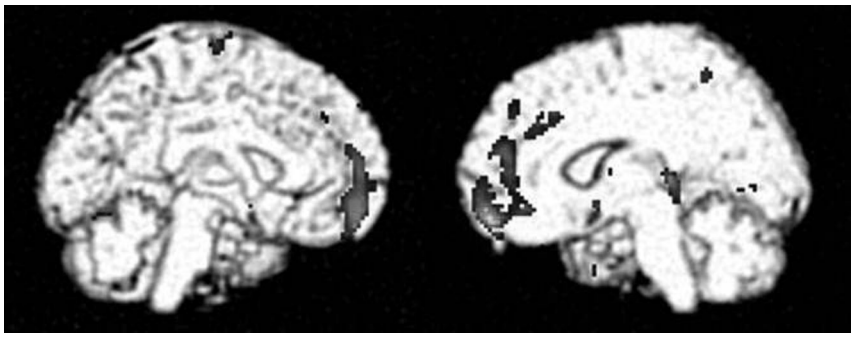

Fig. 6. Statistical parametric map of areas in brains of chemotherapy-exposed subjects having metabolism after 1 year longitudinal follow-up ($n = 11$ chemotherapy exposed patients) that is significantly correlated with baseline plasma sTNF-RII. Colorscale reflects location of significantly correlated voxels, mapped upon co-registered grayscale structural template for anatomical reference; peak voxel $p < 0.0005$, is located at (14, 56, −14) mm, and is part of a cluster of 501 contiguous voxels having $p < 0.01$.

nally in patients treated with chemotherapy. In addition to demonstrating an association between TNF dynamics and self-reported memory complaints, these analyses suggest that both memory complaints and fatigue might share a common underlying source in pro-inflammatory cytokine signaling, in that controlling for covariance between sTNF-RII and fatigue reduced associations between sTNF-RII and memory complaints to nonsignificant levels. Thus both symptom components appear to share a common underlying contribution from TNF signaling.

Significant correlations were also noted for plasma sTNF-RII and cerebral metabolic changes in the hypothesized regions of the brain on FDG scans. However, no significant relationships were seen between NP test performance and either chemotherapy exposure or the inflammatory markers. The other markers of inflammation (CRP, IL-6, and IL1ra) were not significantly associated with chemotherapy exposure in this sample at baseline or longitudinally. It is possible that inflammatory cytokines, although correlated, may have distinct associations with the central nervous system (CNS) and physical health. For example, sTNF-RII, but not IL-6, was correlated with stress-induced changes in the CNS (Slavich et al., 2010), and sTNF-RII predicted coronary heart disease independent of CRP (Shai et al., 2005). Taken together, these findings suggest that elevations in TNF-α after adjuvant chemotherapy exposure in breast cancer patients may play an important role in cognitive dysfunction. These effects may be subtle, as manifested through self-report and brain imaging studies, and not be routinely detected in standardized NP tests in this high functioning population.

In this analysis, chemotherapy exposure at baseline was significantly associated with increased memory complaints and fatigue; insomnia and depressive symptoms were marginally significant. NP test results did not significantly differ by chemotherapy exposure, and may reflect the overall high level of function in this patient population and insensitivity of the standardized NP tests in this setting and patient population. Given their high level of NP function, the increased self-reported memory complaints may be a more sensitive indicator of subtle cognitive dysfunction associated with chemotherapy exposure. However, the observational design, and lack of a pre-post-treatment comparison is a study limitation.

While most chemotherapy agents used in contemporary breast cancer adjuvant treatments do not cross the blood brain barrier (as opposed to methotrexate and fluorouracil used in the CMF regimen popular two decades ago), recent animal model studies using the anthracycline doxorubicin suggest that this agent increases the level of systemic inflammation via reactive oxygen species, and the systemic inflammation crosses the blood brain barrier to have direct effects on microglial cells, increasing local brain inflammation, especially with TNF-α (Joshi et al., 2005, 2010). Demyelination and death of progenitor cells may be a mechanism by which cognitive changes occur in association with chemotherapy exposure, as has been shown in animal studies with fluorouracil (Han et al., 2008), but more animal studies need to be conducted with contemporary chemotherapy drugs to fully understand the mechanisms by which systemic exposure leads to CNS changes (Meyers, 2008). In our final study sample evaluation, we will have a sufficient number of anthracycline exposed patients to examine whether or not there are differences in cognitive function based on specific chemotherapy exposures.

The key limitation of this study is that our findings related to proinflammatory cytokines and cognitive function are correlational. We have not as yet examined NP test outcomes beyond the baseline assessment, as we will require the full sample to examine sub-groups with regard to the addition of targeted endocrine therapy—the main exposure of interest in this prospective cohort study. The MBS did not include concurrent non-cancer control subjects, and thus we need a sufficient number of non-chemotherapy/non-endocrine participants for the reference control group that will be available in the full sample. Nevertheless, our finding of an association between chemotherapy exposure, plasma sTNF-RII and cognitive complaints at baseline and in longitudinal outcomes, provides support for continued examination of post-chemotherapy inflammation as a causal factor in this syndrome. The present results implicating TNFα in chemotherapy-related cognitive complaints are consistent with recent analyses from our laboratory linking cognitive complaints to high-expressing genetic variants of the TNF-α-308 promoter SNP (Ganz et al., 2011a, 2012).

The course of cognitive complaints after cancer treatments is not known, as few long-term longitudinal studies have been conducted to date (Phillips et al., 2012; Jenkins et al., 2006; Syrjala et al., 2011). Animal studies also suggest the possibility of a biphasic pattern of injury with acute and then delayed toxicities (Han et al., 2008). As with many other late effects of cancer treatments, some complications may only emerge several years after cancer treatments end, while others may be present at the time of treatment and persist. Our earlier cross-sectional study of breast cancer survivors who were greater than 5 years after diagnosis (Silverman et al., 2007) showed PET scan abnormalities in the same anatomic areas of the brain we find acutely in this study, suggesting that some cerebral function abnormalities might persist (Silverman et al., 2007). We are continuing to follow the MBS cohort of patients beyond the first post-treatment year (T3), and we hope to be able to track the course of cognitive functioning and its relationship to inflammation in this cohort over a longer period of time.

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Conflict of Interest

The authors of this manuscript have nothing to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2012.07.015.

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Reduced hippocampal volume and verbal memory performance associated with interleukin-6 and tumor necrosis factor-alpha levels in chemotherapy-treated breast cancer survivors

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ABSTRACT

Many survivors of breast cancer show significant cognitive impairments, including memory deficits. Inflammation induced by chemotherapy may contribute to hippocampal changes that underlie these deficits. In this cross-sectional study, we measured bilateral hippocampal volumes from high-resolution magnetic resonance images in 42 chemotherapy-treated breast cancer survivors and 35 healthy female controls. Patients with breast cancer were, on average, 4.8 ± 3.4 years off-therapy. In a subset of these participants (20 breast cancer, 23 controls), we quantified serum cytokine levels. Left hippocampal volumes and memory performance were significantly reduced and interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα) concentrations were significantly elevated in the breast cancer group compared to controls. In the breast cancer group, lower left hippocampal volume was associated with higher levels of TNFα and lower levels of IL-6 with a significant interaction between these two cytokines suggesting a potential modulatory effect of IL-6 on TNFα. Verbal memory performance was associated with cytokine levels and left hippocampal volume in both groups. These findings provide evidence of altered hippocampal volume and verbal memory difficulties following breast cancer chemotherapy that may be mediated by TNFα and IL-6.

1. Introduction

Cognitive impairments are a common late effect of breast cancer and its treatments affecting as many as 75% of survivors (Janelsins et al., 2011; Wefel et al., 2011). Memory impairments are among the most consistently observed difficulties following breast cancer treatment (Jansen et al., 2005; Correa and Ahles, 2008; Janelsins et al., 2011; Wefel et al., 2011) and may involve abnormality of the hippocampus, a region critical for memory function (Squire et al., 2010; Squire and Wixted, 2011). Animal models suggest that chemotherapy-related and/or pro-inflammatory cytokine neurotoxicity may specifically cause damage to the hippocampus (Tangpong et al., 2006; Winocur et al., 2006; Fardell et al., 2010; Joshi et al., 2010; Aluise et al., 2011; Seigers and Fardell, 2011).

Although chemotherapeutic agents typically have restricted direct access to brain tissue due to the blood–brain barrier (BBB), disease states such as cancer, radiation treatment, genetic variations and other factors may make the BBB more permeable to chemotherapy, or may induce alterations in endothelial cells that trigger microenvironment changes within the brain (Ahles and Saykin, 2007; Deeken and Loscher, 2007; Wefel et al., 2008). The hippocampus is unique in that it is one of the only brain regions of continued neural stem cell proliferation throughout the lifespan (Eriksson et al., 1998; Gage, 2000). Neural stem cell proliferation is very tightly regulated and highly sensitive to microenvironment changes. Although chemotherapeutic agents such as doxorubicin (one of the most common breast cancer treatments) are not known to readily cross the BBB, these drugs are associated with reduced hippocampal neurogenesis (Janelsins et al., 2010). Specifically, many chemotherapeutic agents cause neural progenitor cells to lose their capacity for self-renewal even after an initial dose, particularly in the hippocampus (Dietrich et al., 2006, 2010; Winocur et al., 2006; Seigers et al., 2008, 2009). Repetitive dosing, as most patients with breast cancer experience, causes persistent suppression of cell division in the hippocampus (Dietrich et al., 2006; Dietrich, 2010; Hyrien et al., 2010). Both dividing and non-dividing neural and glial cells are significantly vulnerable to chemotherapies (Dietrich et al., 2006, 2010; Winocur et al., 2006; Seigers

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et al., 2008, 2009) and even small amounts of chemotherapy in the brain may cause long-term damage by disrupting cellular plasticity (Dietrich, 2010).

Chemotherapy also may cause indirect neurotoxic brain injury via pro-inflammatory cytokine pathways. Breast cancer chemotherapy and radiation treatments have been shown to elevate peripheral cytokine levels (Vardy et al., 2007; Seruga et al., 2008; Bower et al., 2009; Vardy, 2009; Janelsins et al., 2012). Cytokines readily cross the BBB via active transport mechanisms as well as through circumventricular regions where the BBB is less rigid (Wilson et al., 2002). Binding of cytokines to endothelial receptors in the brain vasculature with subsequent release of other mediators (e.g. chemokines, prostaglandins) leads to impairment of BBB integrity (Wong et al., 1996; Anthony et al., 1997; Konsman et al., 2004). Cytokines activate microglia and astrocytes, stimulate local inflammation and induce oxidative and nitrosative brain damage, particularly in the hippocampus (Tangpong et al., 2006, 2007; Joshi et al., 2007, 2010; Lynch, 2010; Aluise et al., 2011). Cytokine receptors are abundantly expressed in the hippocampus and increased peripheral cytokine levels have been associated with disrupted hippocampal stem cell function, reduced hippocampal volume and reduced memory performance (Das and Basu, 2008; Marsland et al., 2008; McAfoose and Baune, 2009).

Women with breast cancer are at increased risk for psychiatric distress and dysfunction of the hypothalamic–pituitary-adrenal (HPA) axis (Giese-Davis et al., 2004, 2006; Spiegel et al., 2006). HPA dysfunction in patients with breast cancer is associated with immunosuppression and impairment in cytokine regulation (Sephton et al., 2009). Abnormal diurnal cortisol patterns (indicative of HPA dysfunction) have been consistently observed among women with breast cancer (Abercrombie et al., 2004; Spiegel et al., 2006). Glucocorticoids such as cortisol tend to mediate the effects of cytokines on hippocampal memory and plasticity (Yirmiya and Goshen, 2011). Chronic stress and exposure to endogenous cortisol elevations can result in decreased hippocampal volumes (Erickson et al., 2003; Brown et al., 2004). Cortisol can also impair memory performance at low or high concentrations (Erickson et al., 2003; Lupien et al., 2005).

Although abnormal hippocampal structure and function have been noted in previous studies of breast cancer chemotherapy (McDonald et al., 2010; Bergouignan et al., 2011; de Ruiter et al., 2011), to date, there have been no investigations of the relationships between hippocampal structure and peripheral cytokine levels in breast cancer survivors. We therefore measured several serum cytokines, hippocampal volume and verbal memory performance in chemotherapy-treated breast cancer survivors and matched healthy female controls. We hypothesized that increased pro-inflammatory cytokine levels would be associated with decreased hippocampal volumes and verbal memory scores in the breast cancer group compared to the control group.

2. Methods

2.1. Participants

This study included 44 female survivors of primary (stage I-IIIA) breast cancer who all underwent surgery and adjuvant chemotherapy treatment (doxorubicin + cyclophosphamide or paclitaxel = 36; cyclophosphamide + 5-fluorouracil and paclitaxel or methotrexate = 6) who were, on average, 4.8 ± 3.4 years off-therapy (range = 1–12 years). Survivors were excluded for history of relapse or prior chemotherapy treatment and were all disease and relapse free at the time of evaluation. Also included were 38 healthy female controls. There was no significant difference between groups in terms of age (range = 41–73 years), education, global intelligence and minority status but the breast cancer group had significantly more postmenopausal women (Table 1). BC survivors were recruited via the Army of Women (http://www.armyofwomen.org/), community-based BC support groups and local media advertisements. Healthy controls were recruited via the Army of Women and local media advertisements. The Army of Women advertisement indicated that researchers at Stanford were seeking to better understand "problems related to attention, memory, depression, and anxiety" following breast cancer and its treatments. Participants were excluded for previous chemotherapy treatment, neurological, psychiatric, or medical conditions known to affect cognitive function as well as any magnetic resonance imaging (MRI) contraindications. This study was approved by the Stanford University Institutional Review Board and all participants provided informed consent.

2.2. Cognitive assessment

Memory function was measured using the Multifactorial Memory Questionnaire Ability Scale (MMQ) (Troyer and Rich, 2002) and the Hopkins Verbal Learning Test Revised (HVLT) (Wefel et al., 2011). The MMQ provides subjective assessment of one's memory abilities. The HVLT is a measure of verbal memory and learning. The HVLT Total score represents the total number of words recalled across three list learning trials and HVLT Delayed score indicates the number of words recalled following a 20 min delay. Global intelligence (IQ) was measured using a composite of the Matrix Reasoning and Information subtests of the Wechsler Adult Intelligence Scale 4th Edition (Wechsler, 2008). Participants were also administered other cognitive measures including the Wisconsin Card Sorting, Categories, Behavioral Rating Inventory of Executive Function and Verbal Fluency tests which are not reported here given that the focus of this study was on memory.

2.3. Psychiatric function

Although participants were excluded for diagnosed psychiatric disorders, the Clinical Assessment of Depression (CAD), which measures depression, anxiety and cognitive/physical fatigue (Aghakhani and Chan, 2007), was included given the known effect of these symptoms on cognitive status, hippocampal physiology and cytokine levels (Seguin et al., 2009; You et al., 2011).

2.4. MRI Acquisition

MRI scanning was performed on a research-dedicated, GE Discovery MR750 3.0 Tesla whole body scanner (GE Medical Systems, Milwaukee, WI). High-resolution T1-weighted images were acquired with 3D spoiled gradient echo pulse sequence using the following parameters: TR = 8.5 ms, TE = 3.396, TI = 400 ms, flip angle = 15°, FOV = 220 mm, number of excitation = 1, acquisition matrix = 256 × 192, 124 contiguous coronal slices, in-plane

Table 1
Participant characteristics. Data are shown as mean (standard deviation) unless otherwise indicated.

	Breast cancer	Controls	t/c^2	p
N	42	35		
Age	54.6 (6.5)	55.5 (9.3)	0.474	0.64
Education (years)	16.3 (2.6)	17.0 (2.7)	1.09	0.28
Minority status	*N* = 3 (7%)	*N* = 4 (11%)	2.63	0.62
Postmenopause	*N* = 33 (79%)	*N* = 18 (51%)	9.11	0.004
Tamoxifen	*N* = 22 (52%)			
Radiation	*N* = 29 (69%)			
Time off-therapy (years)	4.8 (3.4)			
Stage	2.12 (0.72)			

resolution = 0.859 mm × 0.859 mm. Participants also underwent T2* (BOLD) and diffusion-weighted MRI scans during this session.

2.5. Hippocampal volume measurement

Data from 3 controls and 2 breast cancer survivors were excluded from the analysis due to incidental detection of neuroanatomic abnormality. Preprocessing and hippocampal volume measurement were performed in Freesurfer v4.5 (http://surfer.nmr.mgh.harvard.edu/). Briefly, the preprocessing steps involved interpolation of images to an isotropic voxel size of 1 mm^3, non-uniform intensity correction, intensity normalization, removal of non-brain tissue and spatial normalization to standardized Talairach space. Next, segmentation of the left and right hippocampal regions for each participant was performed (Fischl et al., 2002, 2004). We then utilized Freeview, a visualization and editing tool (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeviewGuide), to manually inspect and correct the hippocampal regions of interest for errors in automated delineation (Fig. 1). Specifically, approximately 75% of the data required very minor adjustment (less than 0.2 cc difference pre and post editing) and less than 3% of the data required some adjustment (less than 0.35 cc difference pre and post editing). This step was performed by a certified rater who was blind to group membership and had demonstrated reliability with a gold standard (left hippocampus = 95.1%, right hippocampus = 97.5%) by intraclass correlation (two way mixed model, absolute agreement) (Bartko, 1966). The gold standard hippocampal delineation method was created by our group (Kates et al., 1997) and has been successfully utilized in our previous studies (Kesler et al., 2004, 2009b; Reiss et al., 2004; Aye et al., 2011). Hippocampal volume measurements included combined gray and white matter tissue.

2.6. Cytokine measurement

A subset of 23 controls and 20 breast cancer survivors underwent venipuncture for cytokine analysis. These subgroups were also matched for age, education, global intelligence and minority status but differed in terms of menopausal status. Between 9:00 and 10:00 AM, before the MRI and cognitive assessments, whole blood was collected (non-fasting) using 10 ml Vacutainer serum separator tubes (Becton Dickinson, Franklin Lakes, NJ). Following 30 min of clotting time at room temperature, serum was spun at 1300 x g for 15 min at 4 °C, aliquotted, frozen and stored at −80 °C until assayed. Interleukin (IL)-6, IL-8, IL-10, IL-12, IL-1-beta (IL-β interferon-gamma (IFNγ and tumor necrosis factor-alpha (TNFα) were quantified (pg/mL) using a high sensitivity multiplexed sandwich immunoassay (Mesoscale Discovery, Gaithersburg, MD). The blood draw was begun later in the study following the acquisition of additional funding to support these methods. Any new participants enrolled at that time were asked to provide a blood sample and none declined.

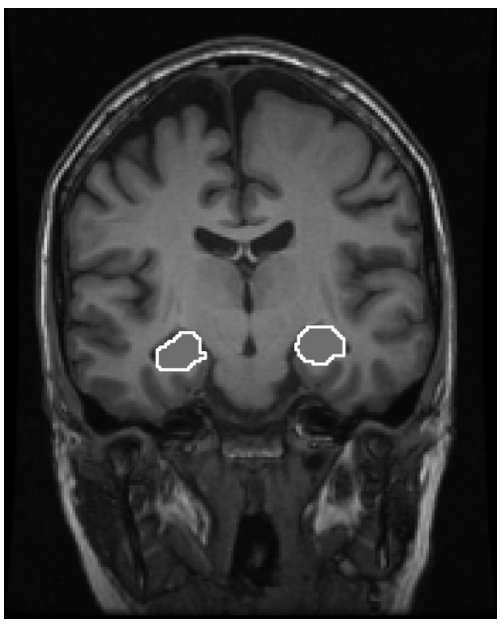

Fig. 1. Illustration of hippocampal delineation using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). The image is shown in radiologic convention where the left hippocampus is shown on the right side of the image and right hippocampus is shown on the left.

2.7. Statistical analyses

For the main group (breast cancer = 42, control = 35) the following analyses were conducted. Univariate analyses of covariance in SPSS 19.0 (IBM, Armonk, NY) were used to determine group differences in IQ, total brain volume and CAD scores, controlling for age, tamoxifen (1 = yes, 0 = no), radiation (1 = yes, 0 = no) and menopausal status (1 = postmenopausal, 0 = premenopausal). Multivariate analysis of covariance (MANCOVA) was conducted to determine group differences in (1) HVLT Total and Delayed recall conditions controlling for age, tamoxifen, radiation and menopausal status, and (2) left and right hippocampal volumes controlling for total brain volume, age, tamoxifen, radiation and menopausal status. For the subgroup that had cytokine data MANCOVA was performed to examine group differences in cytokine levels controlling for age, radiation (1 = yes, 0 = no), tamoxifen, body mass index and menopausal status. We included menopausal status and tamoxifen as covariates in all analyses to conservatively account for group differences in estrogen levels (Boulware et al., 2011), particularly since groups differed in terms of menopausal status. Radiation has also been shown to impact cognitive function in breast cancer patients, including verbal memory (Quesnel et al., 2009; Phillips et al., 2012). The effects of local radiation on brain volume are unknown and therefore this relationship was also examined in exploratory analyses as described below. Cytokine levels were not normally distributed and therefore log transformed. IFNγ and IL-β levels could not be detected in 14 participants (8 controls, 6 breast cancer) and therefore these data were excluded from the analyses.

Multiple linear regression analyses (forced entry) within each subgroup (participants who had cytokine data) were used to examine effects of cytokines on hippocampal volumes and the combined effects of cytokines and hippocampal volume on memory performance. Only those cytokine, cognitive-behavioral and hippocampal variables that differed between groups as well as their interaction terms were modeled, controlled for age and total brain volume. Additionally, exploratory analyses of the effects of host (age, education, menopausal status) treatment (time off-therapy, radiation, tamoxifen), psychiatric (CAD score) and disease (stage at diagnosis) variables on cytokine levels, hippocampal volumes and memory performance were conducted using stepwise linear regressions. Backward selection was utilized and the model with the highest adjusted R^2 was selected as the best fitting. Independent variables were mean centered for all regression analyses (Kraemer and Blasey, 2004). Interaction terms were defined by multiplying the respective mean-centered variables. Finally, we conducted exploratory two-tailed Pearson correlational analyses in the main group (all participants) to examine the relationship between HVLT indices and MMQ.

3. Results

MANCOVA analyses for hippocampal volume and cytokine levels showed significant ($p < 0.05$) omnibus F statistics and therefore, results of individual variables are reported here. As shown in Table 2, left hippocampal volume was significantly reduced in the breast cancer group compared to controls ($p = 0.01$). Right hippocampal volume was marginally decreased ($p = 0.07$). Only total brain volume and age were significant covariates in the model ($p < 0.05$). Regarding cytokine levels, TNFα ($p < 0.0001$) and IL-6 ($p = 0.003$) were significantly elevated in the breast cancer group compared to controls but no other cytokine levels differed between groups (Table 3).

MANCOVA analysis for memory function assessments demonstrated significant ($p < 0.05$ omnibus F statistics and therefore, results of individual variables are reported here. The breast cancer group showed reduced HVLT Total ($p = 0.03$) and Delayed recall performances ($p = 0.02$) as well as reduced MMQ scores ($p < 0.0001$) compared to controls (Table 4).

As illustrated by Fig. 2, in the breast cancer group, lower left hippocampal volume was associated with higher levels of TNFα ($\beta = -1.56$ $p = 0.04$) and lower IL-6 ($\beta = 0.734$, $p = 0.03$). The interaction term for TNFα and IL-6 was also significant ($\beta = 1.23$, $p = 0.04$), with the total model explaining 51% of the variance (adjusted $R^2 = 0.511$, $F = 4.34$, $p = 0.02$). In controls, left hippocampal volume was not significantly associated with cytokine levels ($p > 0.20$).

Also shown in Fig. 2, lower HVLT Total performance was significantly associated with cytokine levels and left hippocampal volume (adjusted $R^2 = 0.482$, $F = 3.48$, $p = 0.04$). Only the interaction between IL-6 and TNFα ($\beta = -2.46$, $p = 0.006$) and the interaction between TNFα and left hippocampus ($\beta = 3.28$, $p = 0.05$) contributed significantly to the model. HVLT Delayed and MMQ were not associated with cytokines or hippocampal volume. In controls, increased HVLT Delayed performance was related to reduced TNFα ($\beta = -0.642$, $p = 0.009$) and increased left hippocampal volume ($\beta = 0.455$, $p = 0.05$; adjusted $R^2 = 0.268$, $F = 4.66$, $p = 0.02$). HVLT Total and MMQ performance showed no associations in the control group. HVLT indices were not significantly correlated with MMQ score in breast cancer or controls.

Exploratory analyses indicated that, in the breast cancer group, higher IL-6 was associated with older age ($\beta = 0.673$, $p = 0.004$; adjusted $R^2 = 0.414$, $F = 11.62$, $p = 0.004$). Higher TNFα was associated with older age ($\beta = 0.916$, $p = 0.001$) and shorter time off-therapy ($\beta = -0.535$, $p = 0.02$; adjusted $R^2 = 0.553$, $F = 10.29$, $p = 0.002$). There were no associations between cytokine levels and CAD score, menopause, tamoxifen, radiation or disease stage. There were no associations between CAD score, host (age, menopausal status) or disease (radiation, tamoxifen, stage, time off-therapy) variables and hippocampal volume or verbal memory function. In controls, there were no significant relationships between age, menopause, CAD score and cytokine levels, hippocampal volumes or memory function in controls.

Table 2
Total brain and hippocampal volumes (cubic centimeters).

	Breast cancer	Controls	F	*p*
N	42	35		
Total brain	1176 (97)	1184 (101)	0.159	0.69
Left hippocampus	4.37 (0.40)	4.68 (0.49)	6.88	0.01
Right hippocampus	4.36 (0.41)	4.61 (0.53)	3.35	0.07

Data are shown as marginal means after removing the effects of covariates and (standard deviation).

Table 3
Serum cytokine levels (pg/mL).

N	BC 20		CON 23		*F*	*p*
	Log trans.	Raw	Log trans.	Raw		
IL-6	0.21 (0.32)	1.1 (1.1)	−0.28 (0.21)	1.4 (2.8)	10.33	0.003
IL-8	0.83 (0.21)	9.0 (4.5)	0.99 (0.21)	10.2 (6.7)	1.46	0.24
IL-10	0.42 (0.55)	7.9 (16.7)	0.31 (0.35)	14.9 (2.9)	0.146	0.71
IL-12	0.30 (0.66)	10.8 (32.5)	0.31 (0.48)	3.8 (5.6)	0.001	0.98
TNFα	0.93 (0.23)	6.7 (7.5)	0.56 (0.08)	5.1 (1.1)	16.70	<0.0001

Cytokine levels were log transformed for analyses and are shown here as mean after removing the effects of covariates and (standard deviation). Statistics are shown for log transformed data only.

Table 4
Cognitive-behavioral data.

	Breast cancer	Controls	*F*	*p*
N	42	35		
HVLT-R Total recall	49.3 (8.0)	57.1 (9.6)	4.85	0.03
HVLT-R Delayed recall	49.8 (6.4)	56.0 (8.1)	6.31	0.02
MMQ	42.2 (11.2)	59.3 (7.4)	30.3	<0.0001
IQ	112 (11)	115 (13)	1.12	0.29
CAD	50.6 (12.0)	45.9 (8.1)	1.62	0.21

Data are shown as marginal means after removing the effects of covariates and (standard deviation). HVLT-R = Hopkins Verbal Learning Test Revised, MMQ = Multifactorial Memory Questionnaire Ability Scale, IQ = Matrix Reasoning and Information subtests of the Wechsler Adult Intelligence Scale 4th Edition, CAD = Clinical Assessment of Depression.

4. Discussion

The present study demonstrated significantly reduced left hippocampal volume and reduced memory performance as well as significantly elevated IL-6 and TNFα in chemotherapy-treated breast cancer survivors compared to healthy female controls. Cytokine levels were associated with left hippocampal volume in the breast cancer group. Verbal memory performance was associated with cytokine levels and hippocampal volume in both groups. These findings provide novel information regarding the mechanisms underlying memory impairment following breast cancer chemotherapy.

Our findings of reduced hippocampal volume are consistent with previous studies that utilized alternate but complementary neuroimaging analysis methods (McDonald et al., 2010; Bergouignan et al., 2011). The McDonald et al. (2010) study indicated that reduction of gray matter volume in mesial temporal regions (including hippocampus) occurred by 1 month post-chemotherapy and did not show recovery unlike other brain regions (McDonald et al., 2010). Likewise, the present findings show evidence of reduced hippocampal volume in survivors who were, on average, 5 years off-therapy. Due to the limitations of these neuroimaging methods, it is unknown whether the hippocampal volume reduction was related to reduced stem cell proliferation. Microscopic and/or fMRI studies are required to examine the dentate gyrus (hippocampal region of neurogenesis) more specifically. Hippocampal volume was not associated with time off-therapy and therefore does not likely continue to decline (or recover) over time. Importantly, our findings uniquely contribute to and extend the cancer and cognition literature by showing relationships between

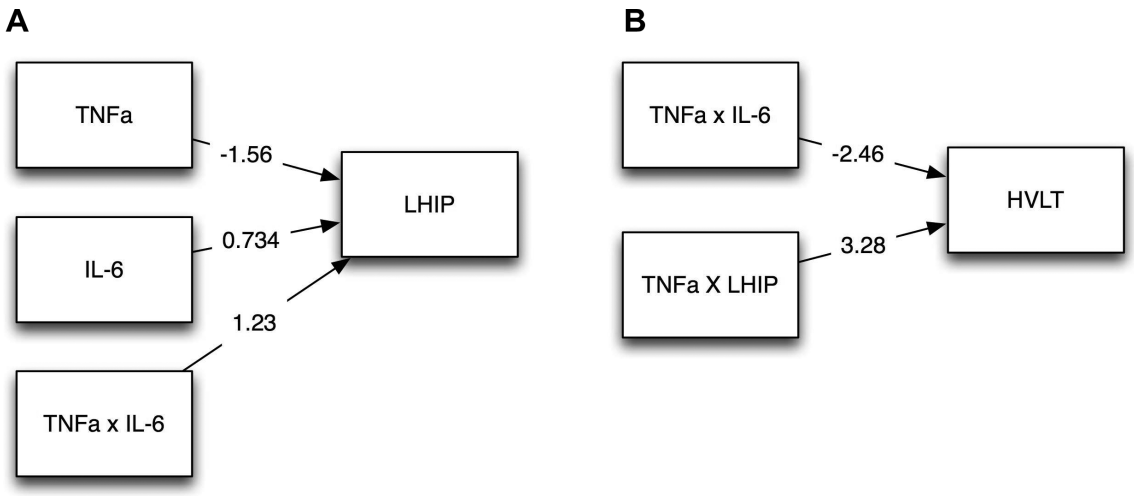

Fig. 2. In breast cancer survivors, (A) lower peripheral interleukin-6 (IL-6) and higher tumor necrosis factor-alpha (TNFα) levels were associated with lower left hippocampal (LHIP) volume. (B) Interactions between cytokines and left hippocampal volume were associated with reduced performance on the Hopkins Verbal Learning Test (HVLT).

inflammatory cytokine levels, hippocampal volume and verbal memory function.

The majority of participants in our breast cancer sample received doxorubicin based chemotherapy, which is not known to cross the blood–brain barrier but has been associated with increased cytokine expression compared to other chemotherapy regimens (Janelsins et al., 2012). Doxorubicin has been shown to elevate TNFα in animal models, particularly in the hippocampus, through oxidative modification of key plasma proteins that inhibit pro-inflammatory cytokine expression (Tangpong et al., 2006; Joshi et al., 2010; Aluise et al., 2011). Elevated TNFα has been associated with hippocampal damage in animals and may play a particularly detrimental role in neurotoxicity during early stages of brain injury (Pickering and Oconnor, 2007). Accordingly, we observed significantly elevated TNFα levels compared to healthy controls as well as an association between lower left hippocampal volumes and higher TNFα in the breast cancer group. Additionally, we found a relationship between shorter time off-therapy and increased TNFα, consistent with a potentially earlier detrimental effect of TNFα (Pickering and Oconnor, 2007).

Some patients received cyclophosphamide, fluorouracil and/or methotrexate therapies which are known to cross the blood-barrier and reduce hippocampal neurogenesis (Seigers et al., 2009; Dietrich, 2010; Janelsins et al., 2010; Seigers and Fardell, 2011). Methotrexate has some anti-inflammatory properties and has been shown to modulate IL-6 and TNFα secretion (Phillips et al., 2003). Methotrexate therapies may show less cytokine inducement compared to doxorubicin, cyclophosphamide and fluorouracil (Janelsins et al., 2012). Thus, chemotherapeutic agents may have differential effects on hippocampal physiology depending on their specific combination of neurotoxic and cytokine modulation properties. Removing the six participants who had methotrexate chemotherapy from the cytokine analysis did not change our results. Further study regarding the effects of specific chemotherapeutic agents on cytokine expression and hippocampal volume is required.

However, lower left hippocampal volume was associated with *lower* IL-6 despite IL-6 also being elevated in breast cancer survivors compared to controls. These findings are in contrast to a previous study of healthy adults showing an inverse relationship between IL-6 and hippocampal volume (Marsland et al., 2008). However, IL-6 has both pro-inflammatory and anti-inflammatory functions following brain injury and may demonstrate altered patterns of influence in individuals with histories of significant disease (Bauer et al., 2007; McAfoose and Baune, 2009). Previous studies have indicated diminished neuroprotection in the hippocampus among IL-6 deficient mice as well as a modulatory effect of IL-6 on TNFα-induced hippocampal injury (Jean Harry et al., 2003; Funk et al., 2011). Interestingly, we found a significant interaction for TNFα and IL-6 in the breast cancer group suggesting that IL-6 may modulate the effects of TNFα on the hippocampus following breast cancer and chemotherapy.

The impact of treatment-induced cytokine expression has been theorized for the past decade (Ahles and Saykin, 2007; Vardy et al., 2007; Seruga et al., 2008; Wefel et al., 2008; Vardy, 2009; Janelsins et al., 2011). Previous studies have also reported elevated pro-inflammatory cytokines in breast cancer survivors at long-term follow-up. For example, Bower and colleagues demonstrated significantly elevated markers of pro-inflammatory cytokine activity in survivors who were also an average of 5 years off-therapy (Bower et al., 2002). However, this study represents the first to identify an association between elevated cytokines and brain changes following breast cancer chemotherapy in humans. Our findings suggest that the mechanism underlying verbal memory dysfunction in breast cancer survivors may involve treatment-induced pro-inflammatory cytokine elevation that directly or indirectly damages the hippocampus which then has reduced ability to support verbal memory function. This is consistent with animal studies suggesting that cancer treatments increase cytokine levels and these cytokines cross the blood–brain barrier and induce damage to the hippocampus (Tangpong et al., 2007; Joshi et al., 2010; Lynch, 2010). Shorter time off-therapy, but not disease stage, was associated with increased TNFα in the present study indicating that during later stages, treatment rather than the disease itself is a critical inducer of pro-inflammatory cytokines and their deleterious effects. Accordingly, previous studies have shown specific effects of chemotherapy on brain structure and function as well as memory performance in breast cancer survivors after accounting for the effects of disease (Ahles et al., 2010; McDonald et al., 2010; Deprez et al., 2011, 2012; Hedayati et al., 2011; Kesler et al., 2011).

Hippocampal volume has been shown to be more strongly related to delayed verbal memory (Wolk and Dickerson, 2011). Immediate verbal memory tends to rely more on distributed brain regions including prefrontal and parietal cortices in addition to hippocampus (Wolk and Dickerson, 2011). The control group showed the expected relationship between hippocampal volume and delayed rather than immediate verbal memory but the breast

cancer group showed the reverse. It is unclear why this was the case but could suggest that elevated cytokine levels disrupt typical hippocampal mechanisms in patients with breast cancer. Our previous research indicates altered neurobiology underlying different stages of verbal memory among breast cancer survivors (Kesler et al., 2009a). Further studies are required to help dissociate the different neural mechanisms involved in various stages of verbal memory encoding and recall.

A previous study showed that radiotherapy alone was associated with reduced verbal memory in breast cancer patients (Quesnel et al., 2009). A majority of participants in the present study received radiation treatment but analyses revealed no relationships between radiation, hippocampal volume or cytokine levels. There were likely too few participants in the present sample who did not have radiation to appropriately power these analyses, particularly with respect to cytokine levels. A previous study showed no effects of local radiation on cytokine levels in patients with breast cancer (Geinitz et al., 2001) although other studies have demonstrated increased inflammatory markers during radiotherapy in patients with breast or prostate cancers (Bower et al., 2009; Lopes and Callera, 2011). Further studies are required to determine the contribution of radiation therapy to cytokine levels and neurobiologic outcome following breast cancer.

Approximately 79% of our breast cancer sample was postmenopausal and postmenopausal women often show increased cognitive deficits and hippocampal atrophy attributed to estrogen deficiency (Eberling et al., 2003; Goto et al., 2011). Postmenopausal women with breast cancer taking tamoxifen (a selective estrogen receptor modulator) were shown to have reduced hippocampal volumes and greater cognitive impairment compared to control groups in previous studies (Eberling et al., 2004; Schilder et al., 2010). Additionally, estrogen is believed to play a role in cytokine modulation (Czlonkowska et al., 2005). Approximately 52% of our BC sample had a history of tamoxifen treatment and six of these participants were still taking tamoxifen at the time of evaluation. Our analyses indicated that menopausal status and tamoxifen treatment were not associated with cytokine levels, hippocampal volume or memory performance. Other studies have also demonstrated a lack of association between tamoxifen and cognitive outcome (Ahles et al., 2002; Tchen et al., 2003; Hermelink et al., 2008; Jenkins et al., 2008; Phillips et al., 2012). However, measurement of actual estrogen levels may be important for future studies of hippocampal physiology, inflammation and memory function in breast cancer.

An age-related increase in cytokine levels was noted in the breast cancer group but not in controls. Hippocampal physiology is altered in individuals with age-related neurodegenerative conditions (Hanseeuw et al., 2011; Sabuncu et al., 2011) and may mediate normal age-related cognitive decline (Ta et al., in press; Tsukiura et al., 2011; Wimmer et al., in press; Giovanello and Schacter, 2012). Previous studies have indicated a shift to a more pro-inflammatory state associated with normal aging that may alter hippocampal function (Lynch, 2010; Viviani and Boraso, 2011). For breast cancer survivors, treatments may interact with age to increase cytokine levels or may accelerate the age-related imbalance in pro-inflammatory cytokine expression.

Our present findings suggest immune dysregulation and increased pro-inflammation may contribute to the increased vulnerability of older breast cancer patients for cognitive impairment that has been observed in previous studies (Ahles et al., 2010; Kesler et al., 2011). This fits with the age-based hypothesis of neurodegeneration proposed by Herrup (Herrup, 2010). Within this model, cancer and/or its treatments could represent a precipitating injury or deviation from the normal aging process. In an age-weakened brain, this injury results in a chronic shift to a pro-inflammatory state that in turn results in accelerated neuronal loss and cognitive decline (Herrup, 2010). Herrup's model was designed to explain Alzheimer's dementia but there is some evidence (although controversial) that chemotherapy-treated breast cancer survivors are at increased risk for developing dementia compared to non-chemotherapy treated survivors (Heck et al., 2008; Du et al., 2010). Additionally, the presence of the apolipoprotein E4 allele is a common risk factor for dementia as well as chemotherapy-related cognitive dysfunction (Ahles et al., 2003). The interaction between age, cancer, chemotherapy, immune dysregulation and cognitive decline is an essential area of continued research.

An accelerated shift to a chronic inflammatory state may help explain why cytokine levels would remain elevated so long after treatment has ended. Another possibility is that cancer and/or its treatment specifically alter immune system function such that cytokine levels do not return to normal. Initial cytokine elevation may increase symptom/side effect burden (e.g. cognitive dysfunction, depression, fatigue, pain) resulting in further cytokine release with symptoms and cytokines perpetuating each other. Continued research in this area is required to determine the mechanisms of chronic cytokine elevation following breast cancer and chemotherapy.

There are several limitations of this study. The cross-sectional design limits interpretations regarding treatment effects on hippocampal volume, cytokine levels and memory function. The small sample size (especially of cytokine levels) may have reduced statistical power for detecting certain smaller effects and thus these results should be considered preliminary. Our study advertisements included statements regarding a focus that included memory problems and therefore the sample may have been biased towards survivors who were concerned about their memory function. As with many studies of breast cancer survivors, our sample was very heterogeneous in terms of disease, host and treatment variables. Information regarding specific treatment regimens (e.g. number of cycles, doses) and endocrine function (e.g. estrogen levels, years post-menopause) that may be important mediators of main effects were not available. Peripheral cytokine levels have been shown to relate to hippocampal physiology in previous studies as noted above but do not provide assessment of actual brain levels of inflammatory markers. It is unknown why IFNγ and IL-β levels could not be detected in this sample. Also, screening instruments such as the CAD may not be sufficient for detecting psychiatric disorders following breast cancer (Palmer et al., 2011). Longitudinal studies that combine neuroimaging with cytokine quantification are required in larger samples to further investigate the effects of chemotherapy and inflammation on neurobiology and cognitive outcome.

Despite these limitations, this study provides further evidence of brain injury following breast cancer and chemotherapy as well as novel, preliminary support for the role of pro-inflammatory cytokines in this brain injury and subsequent verbal memory deficit. Increased cytokine levels and reduced left hippocampus volume were observed in chemotherapy-treated breast cancer survivors even after controlling for other breast cancer treatments including radiation and tamoxifen. Cytokine levels and hippocampal volumes were associated with verbal memory functioning and therefore, these results contribute important information regarding the mechanisms underlying cognitive deficit following breast cancer chemotherapy. Continued research could potentially identify patients at high risk for cytokine dysfunction and guide treatment protocols for helping to improve or prevent cognitive difficulties in cancer survivors.

Conflicts of interest

All authors declare that there are no conflicts of interest.

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Frontal gray matter reduction after breast cancer chemotherapy and association with executive symptoms: A replication and extension study

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ABSTRACT

Cognitive changes related to cancer and its treatment have been intensely studied, and neuroimaging has begun to demonstrate brain correlates. In the first prospective longitudinal neuroimaging study of breast cancer (BC) patients we recently reported decreased gray matter density one month after chemotherapy completion, particularly in frontal regions. These findings helped confirm a neural basis for previously reported cognitive symptoms, which most commonly involve executive and memory processes in which the frontal lobes are a critical component of underlying neural circuitry. Here we present data from an independent, larger, more demographically diverse cohort that is more generalizable to the BC population. BC patients treated with ($N = 27$) and without ($N = 28$) chemotherapy and matched healthy controls ($N = 24$) were scanned at baseline (prior to systemic treatment) and one month following chemotherapy completion (or yoked intervals for non-chemotherapy and control groups) and *APOE*-genotyped. Voxel-based morphometry (VBM) showed decreased frontal gray matter density after chemotherapy, as observed in the prior cohort, which was accompanied by self-reported difficulties in executive functioning. Gray matter and executive symptom changes were not related to *APOE* ε4 status, though a somewhat greater percentage of BC patients who received chemotherapy were ε4 allele carriers than patients not treated with chemotherapy or healthy controls. These findings provide confirmatory evidence of frontal morphometric changes that may be a pathophysiological basis for cancer and treatment-related cognitive dysfunction. Further research into individual risk factors for such changes will be critical for development of treatment and prevention strategies.

Cognitive changes related to breast cancer and its treatment have been an area of increasing study, with numerous reports demonstrating cognitive impairment in patients relative to controls. These changes have been differentially attributed to chemotherapy, radiation, and anti-estrogen treatment (Agrawal et al., 2010; Ahles et al., 2010; Collins et al., 2009; Jim et al., 2009; Quesnel et al., 2009), and have been reported most prominently in executive functions (e.g., working memory) and processing speed, cognitive processes largely subserved by frontally mediated brain systems (impairment in other cognitive domains has also been noted; for review and meta-analysis see (Anderson-Hanley et al., 2003; Correa and Ahles, 2008; Stewart et al., 2006)). A higher than expected incidence of impaired cognitive performance has also been found in patients prior to systemic treatment (Ahles et al., 2008; Wagner et al., 2006; Wefel et al., 2004), suggesting that host factors and/or the cancer disease process itself may play a role. This prior work demonstrates the continued need for further investigation of the effects of cancer treatment and the disease process on cognition in vulnerable individuals (McDonald and Saykin, 2011; Vardy et al., 2008).

The neural mechanisms underlying these cognitive changes have likewise been the subject of increasing investigation. Several cross-sectional, retrospective structural MRI studies have utilized voxel-based morphometry (VBM) to assess gray matter changes after breast cancer treatment quantitatively, in an automated, unbiased manner (de Ruiter et al., in press; Hakamata et al., 2007; Inagaki et al., 2007; McDonald et al., 2008; Saykin et al., 2003; Yoshikawa et al., 2006). Those studies comparing gray matter between patients who did and did not receive chemotherapy have demonstrated residual gray matter deficits in the chemotherapy-treated group, even several years after treatment completion (de Ruiter et al., in press; Inagaki et al., 2007; McDonald et al., 2008; Saykin et al., 2003). We recently reported the first prospective VBM study examining such gray matter changes relative to pre-treatment baseline (McDonald et al., 2010). We predicted that

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these changes would be detectable in the short-term but would recover at least partially over time, given prior cognitive studies suggesting longitudinal improvement in brain function after chemotherapy (Ahles et al., 2010; Collins et al., 2009; Jansen et al., 2011; Jenkins et al., 2006; Schagen et al., 2002). Findings were consistent with study hypotheses, demonstrating reduced gray matter in chemotherapy-treated patients one month after chemotherapy completion in bilateral frontal, medial temporal, and cerebellar regions. One year later gray matter density had returned to baseline levels in some regions, though not all. No between-group differences were found at baseline, and changes were not seen in patients who did not receive chemotherapy or healthy controls.

The purpose of the current investigation was to assess gray matter alterations related to breast cancer and its treatment prospectively in an independent cohort of patients treated with and without standard-dose systemic chemotherapy and demographically matched healthy controls, in order to replicate our previous findings. Given the prominence of executive function changes among the cognitive domains affected in cancer patients after treatment (Anderson-Hanley et al., 2003), and the recent finding of a relationship between self-reported executive functioning and altered brain activation after breast cancer chemotherapy (Kesler et al., 2011), we also sought to examine the relationship of these gray matter changes to self-reported executive functioning. Finally, a large body of research has shown a significant relationship between the apolipoprotein E (*APOE*) ε4 allele and Alzheimer's disease and its precursors, and has demonstrated a role for *APOE* in other neurocognitive disorders (for reviews see (Bookheimer and Burggren, 2009; Smith, 2000)). Given prior work demonstrating decreased cognitive functioning in cancer survivors treated with chemotherapy who carried the ε4 allele vs. those who did not (Ahles et al., 2003), we further evaluated possible risk factors for gray matter changes after chemotherapy by investigating their relationship to presence or absence of the *APOE* ε4 allele.

1. Participants

Written informed consent was obtained from all participants according to the Declaration of Helsinki under a protocol approved by the Indiana University Institutional Review Board. Participants were female breast cancer patients treated with (CTx+, $N = 27$) and without (CTx−, $N = 28$) systemic chemotherapy and healthy controls ($N = 24$). Patients had non-invasive (stage 0) or non-metastatic invasive (stages I, II, or III) disease, and were treated with common standard-dose chemotherapy regimens which all included a taxane (see Table 1 for demographic and treatment data). Exclusion criteria for all groups were: (1) prior treatment with cancer chemotherapy, CNS radiation, or intrathecal therapy; (2) current or past alcohol or drug dependence; (3) neurobehavioral risk factors including neurologic, medical, or psychiatric conditions known to affect brain structure or function, except history of depression or anxiety in breast cancer patients. Potential participants for all groups were excluded for current diagnosis of any DSM-IV Axis I disorder or a history of any psychiatric disorder requiring hospitalization. Anxiety and depression symptoms were assessed at each study visit with the Center for Epidemiologic Studies-Depression Scale (CES-D) (Radloff, 1977) and the State-Trait Anxiety Inventory-State subscale (STAI-S) (Spielberger, 1983).

2. Methods

Study measures were completed at baseline (after surgery but before radiation, chemotherapy, and/or anti-estrogen treatment) and approximately one month following the completion of chemotherapy (M1), or yoked intervals for the CTx− and control groups, for all participants except nine CTx+ patients who received neoadjuvant chemotherapy prior to surgery and additional treatment. For these nine participants the baseline study visit was prior to both cancer surgery and systemic treatment, and the second study visit was approximately one month after chemotherapy completion. For CTx+ patients the baseline visit was conducted on average 9.9 days (SD 11.0) prior to the start of chemotherapy (range 1–43 days). One CTx− participant began tamoxifen about three weeks prior to her baseline scan. Of note, data reported here are drawn from a larger study in which participants undergo a comprehensive assessment including structural and functional neuroimaging, objective and subjective cognitive evaluation, and genetic and other biomarkers at three time-points. Data collection is ongoing, particularly for the final study visit (not reported here, given our current partial sample), and the present findings therefore represent an interim analysis of a subset of the larger study.

2.1. Self-reported executive function

Self-report of executive functioning was obtained with the Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A) (Roth et al., 2005), which includes an overall composite score (the Global Executive Composite, or GEC) and two major index scores: the Behavioral Regulation Index (BRI), composed of the Inhibit, Shift, Emotional Control and Self-Monitor scales, and the Metacognition Index (MI), which includes the Initiate, Working Memory, Plan/Organize, Task Monitor, and Organization of Materials scales. Between-group differences on BRIEF-A scale and index T-scores were compared using the general linear model in SPSS (SPSS Statistics 19, IBM Corporation, Somers, NY) to examine differences in self-reported executive function at M1 controlling for baseline levels. Of note, higher T-scores on this measure indicate greater levels of executive complaints.

2.2. APOE genotyping

APOE alleles were determined using standard assays for the two single nucleotide polymorphisms (SNPs) coding for the ε4 (rs429358) and ε2 (rs7412) vs. more common ε3 allele of *APOE*. Participants who were carriers of one or two copies of the ε4 allele were considered *APOE* ε4 positive. Within the CTx+ group, differences between *APOE* ε4 positive and negative patients for significant gray matter clusters and BRIEF-A scales were compared using the general linear model in SPSS (SPSS Statistics 19, IBM Corporation, Somers, NY) to examine differences at M1 controlling for baseline levels.

2.3. MRI scan acquisition

All scans were acquired on the same Siemens Tim Trio 3T scanner using a 12-channel head coil. A T1-weighted three-dimensional magnetization prepared rapid gradient echo (MPRAGE) volume was used for VBM, with the following parameters: TR = 2300 ms, TE = 2.98 ms, FOV = 256 mm, FA = 9 deg, 160 1.2 mm thick sagittal slices with no skip, 256×256 matrix, in-plane resolution of 1 mm^2. This MPRAGE sequence has been extensively tested and validated via the multicenter, international Alzheimer's Disease Neuroimaging Initiative (ADNI) study (see http://adni.loni.ucla.edu/ for additional information). T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences were also acquired to rule out incidental pathology.

2.4. Image analysis

Locally developed MATLAB (R2009b, Mathworks, Inc., Natick, MA) scripts were used to implement optimized VBM methods

Table 1
Sample demographics.

	CTx+ (*N* = 27)	CTx- (*N* = 28)	Control (*N* = 24)
Age at baseline (yrs.)	49.9 (7.6)	52.4 (9.1)	47.0 (9.2)
Education (yrs.)	15.5 (2.8)	15.4 (2.3)	15.4 (2.4)
Estimated full scale IQ (Barona Index (Barona et al., 1984))	110.1 (6.5)	111.3 (6.1)	110.6 (6.5)
Handedness (R,L/Amb)	26, 1	26, 2	22, 2
Percent Caucasian, Non-hispanic	78	89	83
CES-D raw score: Baseline	10.8 (9.5)	8.6 (8.6)	7.8 (7.6)
M1	14.6 (9.3)	9.3 (9.5)	7.4 (7.2)
STAI-S raw score: baseline	35.3 (15.2)	28.9 (7.7)	31.6 (10.3)
M1	35.4 (12.4)	32.3 (12.2)	32.5 (11.9)
Inter-scan interval (days)	158.7 (68.9)	204.3 (151.7)	160.8 (28.9)
Cancer stage: 0 (DCIS)	0	7	
I	11	18	
II	12	3	
III	4	0	
Received radiotherapy[a]	22	18	
Number on anti-estrogen therapy[a,b]: baseline	0	1 TAM	
M1	2 ANA 1 TAM	12 TAM 5 LET 2 ANA 1 EXE 1 RAL	
Chemotherapy regimen[a,c]:			
Doxorubicin/cyclophosphamide/paclitaxel	9		
Docetaxel/cyclophosphamide	9		
Docetaxel/carboplatin	5		
Docetaxel/doxorubicin/cyclophosphamide	1		
Docetaxel/cisplatin	1		
Paclitaxel	1		

Values are Mean (SD).
CES-D = Center for Epidemiologic Studies-Depression Scale.
STAI-S = State-Trait Anxiety Inventory-State subscale.
M1 = one month post chemotherapy completion (or yoked intervals).
[a] Details regarding radiation, chemotherapy regimen, and anti-estrogen treatment were not available for one CTx+ patient.
[b] ANA = anastrozole; TAM = tamoxifen; LET = letrozole; EXE = exemestane; RAL = raloxifene.
[c] Nine CTx+ patients were also treated with trastuzumab; one was also treated with sunitinib; one was also treated with bevacizumab.

(Ashburner and Friston, 2000; Ashburner and Friston, 2001; Good et al., 2001) using SPM (Version 8, Wellcome Department of Imaging Neuroscience, London, UK), similar to our prior longitudinal study (McDonald et al., 2010). Briefly, after reconstruction MPRAGE follow-up scans were registered to the baseline scan for each subject. Scans were then registered to the Montreal Neurological Institute (MNI) T1-weighted template and segmented into gray matter, white matter, and cerebrospinal fluid compartments using the MNI T1-weighted template and corresponding tissue probability maps. Gray matter maps were then spatially normalized to MNI space, resampled to 1 mm isotropic voxels, and smoothed using an isotropic Gaussian spatial filter (FHWM = 10 mm) to reduce residual inter-individual variability. The smoothed, normalized gray matter maps were subjected to statistical parametric mapping on a voxel-by-voxel basis using the general linear model as implemented in SPM8. The SPM8 prior probability gray matter template was used to restrict the statistical comparisons to the gray matter compartment. As multiple prior structural and functional MRI studies in breast cancer patients have consistently shown alterations in frontal brain regions (Cimprich et al., 2010; de Ruiter et al., 2011; Inagaki et al., 2007; Kesler et al., 2009; Kesler et al., 2011; McDonald et al., 2010; Scherling et al., 2011; Silverman et al., 2007), all imaging analyses were restricted to the frontal lobes using a mask composed of frontal lobe subregions from the WFU PickAtlas toolbox in SPM8, which was included as an explicit mask in the SPM8 design matrix.

Random effects analyses were conducted using analysis of variance (ANOVA) to construct contrast maps of voxels in which local gray matter density differed between groups and over time. Comparisons were conducted within an omnibus group (three independent levels: CTx+, CTx−, control) by time (two non-independent levels: baseline, M1) ANOVA. The critical significance threshold (P_{crit}) was set to 0.001. Cluster extent (k) for all analyses was set to limit results only to regions that survived an unbiased search of the entire frontal region of interest at a cluster-level threshold of $P_{FWE\text{-}corrected} < 0.05$. Within the omnibus SPM8 ANOVA design matrix between-group comparisons were conducted using weighted contrast vectors. For example, pair-wise comparisons of gray matter density at baseline (CTx+ vs. CTx−, CTx+ vs. control, CTx− vs. control) were conducted by entering values of 1 and -1 in the appropriate columns in the matrix. In this manner examination of regions where controls showed greater gray matter density than CTx+ at baseline would be conducted by entering 1 in the control baseline column and −1 in the CTx+ baseline column. Group-by-time interactions were conducted in a similar fashion. For example, to evaluate regions in which the control and CTx+ groups showed significant differences from baseline to M1, values of 1 would be entered in the CTx+ baseline and control M1 columns, and values of −1 would be entered in the CTx+ M1 and control baseline columns (and vice versa for the inverse interaction).

We hypothesized that gray matter decreases would be seen from baseline to M1 in the CTx+ group, consistent with our prior findings in an independent cohort (McDonald et al., 2010). Mean values for significant clusters in the analysis of regions showing decreasing gray matter density from baseline to M1 in the CTx+ group were extracted using MarsBaR v0.42 (http://marsbar.sourceforge.net/). The general linear model in SPSS was used to investigate the relationship of these mean gray matter change values in

Table 2
Regional gray matter changes (P_{crit} < 0.001, cluster-level $P_{FWE\text{-}corr}$ < 0.05).

MNI coordinates (x y z)	Cluster extent (k)	Cluster-level $P_{FWE\text{-}corrected}$	T	Z	Region description (for cluster peak)
Between-Group Analyses					
Control > CTx− at baseline					
−22 −14 43	1566	0.001	5.78	5.49	L cingulate gyrus (BA24)
Interaction Control > CTx+ from baseline to M1					
−49 41 30	1357	0.002	4.18	4.06	L middle frontal gyrus (BA46)
Within-Group (CTx+) Analyses					
Gray matter decline from baseline to M1					
−12 36 50	717	0.04	4.41	4.27	L superior frontal gyrus (BA8)
−45 37 29	4320	<0.001	4.36	4.22	L middle frontal gyrus (BA46)
Correlation with BRIEF-A initiate scale adjusted T-score					
−27 52 −7	1410	0.035	7.19	5.24	L middle frontal gyrus (BA10)

BA = Brodmann area.

the CTx+ group to *APOE* status, examining between-group differences in M1 gray matter density accounting for baseline levels. To evaluate relationships between self-reported executive function symptoms and gray matter linear regression in SPSS was used to calculate adjusted T-scores for M1 accounting for baseline score. These were then entered as a covariate into the SPM8 design matrix separately for each group (CTx+, CTx−, control) to assess positive and negative correlations with gray matter density at M1.

3. Results

As expected and consistent with conventional treatment patterns, CTx+ patients had significantly higher stage disease than CTx− patients (χ^2 = 18.08, df = 3, P < 0.001). There were no other between-group demographic differences, and no group-by-time interactions were observed for depression or anxiety symptoms (CES-D, STAI-S; P > 0.05, Table 1). The second scan session was about six months after the baseline visit on average, and interscan intervals did not differ between groups (P > 0.05, Table 1). MNI coordinates, cluster extents, P values, T and Z scores, and region descriptions are presented in Table 2. Imaging analyses described below were repeated including age as a covariate to control for possible gray matter density decline with aging, without a significant change in the pattern of findings.

3.1. Between-group analyses

At baseline the only significant between-group difference was a single cluster in the left cingulate gyrus in which controls showed greater gray matter than CTx− patients (Table 2). Group-by-time interaction analyses showed reduced gray matter density in CTx+ patients relative to controls at M1 relative to baseline in the left middle frontal gyrus (Fig. 1). This pattern of change over time was not apparent for the CTx- group. There were no regions where the control group showed lower gray matter than either cancer group at M1 relative to baseline, nor were there any regions where a significant group-by-time interaction was found between the two cancer groups from baseline to M1.

3.2. Within-group analyses

At M1 relative to baseline the CTx+ group showed decreased gray matter density in the left middle and superior frontal gyri (Fig. 2), including in the same middle frontal gyrus regions shown to be significant in the interaction analyses above. Within the control and CTx− groups there were no gray matter regions which showed significant decline from baseline to M1. There were also no regions showing increased gray matter from baseline to M1 for any group.

3.3. Self-reported executive function changes and relationship to gray matter density

There were no between-group differences in BRIEF-A T-scores at baseline for any scale or index (Table 3). Longitudinal analysis of BRIEF-A T-scores revealed a significant difference in the Initiate scale (P = 0.011), with CTx+ patients showing increased scores over time, indicating more self-perceived symptoms in the area of ability to initiate problem-solving or activity. A trend in the same direction was also evident on the BRIEF-A Working Memory scale (P = 0.054). No significant differences were evident on other scales. Graphical representation of raw T-score changes (Fig. 3) demonstrates that for many BRIEF-A scales, particularly those which make up the Metacognition Index, CTx+ patients showed the greatest score increase at M1 relative to baseline, indicative of greater increase in self-perceived executive difficulties.

When BRIEF-A Initiate scale adjusted T-score was entered as a covariate into the SPM8 design matrix, a significant negative correlation with M1 gray matter density was seen in the left middle frontal gyrus for the CTx+ group (Table 2, Fig. 4), indicating that

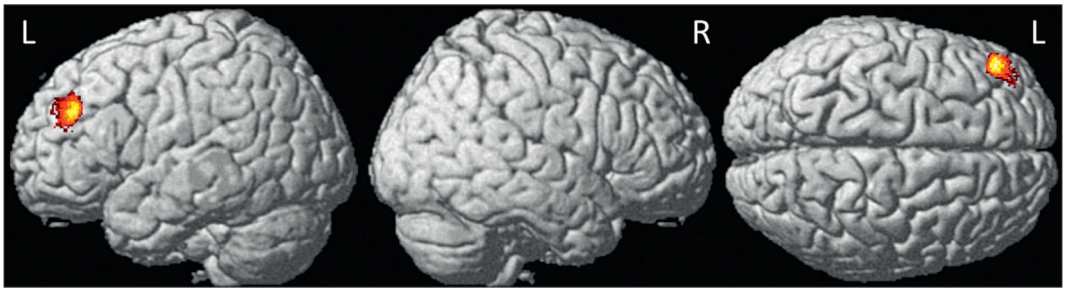

Fig. 1. Between-group interaction analyses of regional gray matter density declines in chemotherapy-treated breast cancer patients relative to healthy controls from baseline to one month after chemotherapy (P_{crit} < 0.001, cluster-level $P_{FWE\text{-}corr}$ < 0.05, see Table 2 for region descriptions).

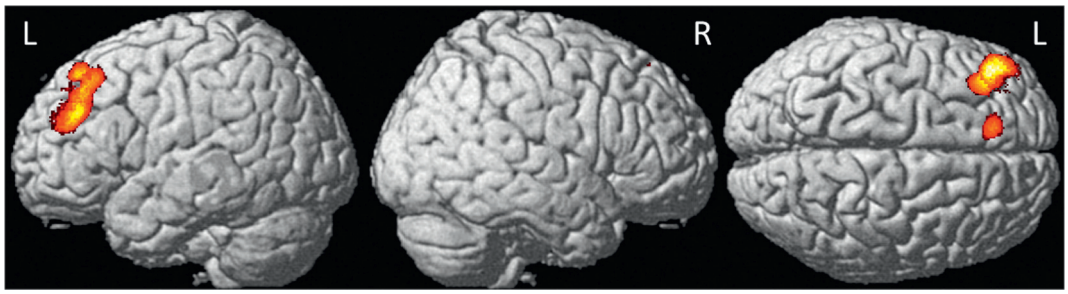

Fig. 2. Regional gray matter density declines in chemotherapy-treated breast cancer patients from baseline to one month after chemotherapy ($P_{crit} < 0.001$, cluster-level $P_{FWE\text{-}corr} < 0.05$, see Table 2 for region descriptions).

Table 3
Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A) T-scores.

	CTx+ ($N = 27$)		CTx− ($N = 28$)		Control ($N = 24$)[a]	
	Baseline	M1	Baseline	M1	Baseline	M1
Inhibit	51.2 (7.4)	51.0 (9.0)	49.8 (10.6)	50.8 (8.4)	49.0 (8.6)	50.0 (7.6)
Shift	51.2 (10.0)	53.7 (9.2)	51.6 (12.3)	53.6 (11.1)	52.8 (11.1)	53.0 (11.1)
Emotional control	51.2 (10.3)	52.6 (10.4)	51.4 (11.2)	52.8 (11.1)	50.2 (10.9)	51.8 (11.5)
Self-monitor	47.9 (8.1)	48.4 (8.5)	48.0 (11.7)	47.9 (8.4)	48.8 (7.4)	48.4 (7.8)
Behavioral regulation index	50.6 (9.3)	52.0 (10.7)	50.3 (12.2)	51.9 (10.1)	50.0 (8.8)	51.0 (9.7)
Initiate*	49.8 (11.0)	56.3 (11.1)	51.6 (12.6)	50.6 (11.0)	47.5 (7.8)	48.3 (7.1)
Working memory^	54.0 (9.4)	59.5 (10.3)	53.0 (12.4)	54.0 (11.5)	52.1 (10.7)	52.4 (10.4)
Plan/organize	51.2 (9.2)	54.3 (11.1)	51.0 (11.6)	51.9 (10.4)	50.5 (9.3)	53.3 (11.4)
Task MOnitor	52.9 (9.2)	56.0 (9.7)	52.2 (11.2)	53.8 (9.5)	53.0 (9.4)	52.0 (9.3)
Organization of materials	50.3 (9.5)	53.0 (11.7)	51.1 (11.0)	53.2 (9.7)	52.8 (12.9)	53.1 (11.9)
Metacognition index	51.7 (9.3)	56.4 (11.1)	51.9 (12.4)	53.0 (10.5)	51.4 (9.9)	52.1 (10.2)
Global executive composite	51.2 (9.5)	54.9 (10.6)	51.2 (12.7)	52.8 (10.5)	50.7 (9.5)	51.8 (10.0)

Values are mean (sd) of scale/index T-scores, higher score reflects greater complaints.
[a] One control participant did not complete the BRIEF-A at M1.
* Significant between-group difference at M1 controlling for baseline ($P = 0.011$), CTx+ group showing greatest change.
^ Trend for between-group difference at M1 controlling for baseline ($P = 0.054$), CTx+ group showing greatest change.

reduced gray matter density was associated with higher levels of executive complaints in this domain. There were no positive correlations between Initiate scale adjusted T-score and gray matter density at M1 in the CTx+ group, and no significant relationships in either direction were apparent at this threshold for the CTx− or control groups.

3.4. Relationship of gray matter and BRIEF-A changes to APOE status

Regions of gray matter which showed significant decline from baseline to M1 and BRIEF-A initiate scale T-scores (where a significant difference over time was seen in the CTx+ group, as noted above) were compared between *APOE* ε4 positive and negative CTx+ patients, but no significant between-group differences were observed (all $P > 0.05$). Of note, a higher percentage of patients in the CTx+ group were ε4 positive than in the other two groups (CTx+ 42%, CTx− 21%, control 25%; *APOE* status was not available for one CTx+ patient), though this difference was not statistically significant.

4. Discussion

These findings replicate our previous work showing decreased frontal gray matter shortly after chemotherapy completion in breast cancer patients. Relative to our prior study (McDonald et al., 2010), the current cohort is larger, more racially and ethnically diverse, includes patients receiving neoadjuvant chemotherapy, and was conducted on a new generation 3T vs. older 1.5T magnet. Demonstration of reduced frontal gray matter in this cohort provides independent confirmation of the prior results, strengthening the evidence that breast cancer chemotherapy is associated with frontal gray matter changes. Also consistent with our prior work, such changes were not evident in controls or patients who received anti-estrogen treatment but not chemotherapy, suggesting that these frontal gray matter decreases are specific to chemotherapy treatment, rather than solely reflecting host factors, the cancer disease process, or effects of other cancer treatments. Gray matter changes in the current study were also consistent with frontal regions in which prior work has demonstrated structural and functional abnormalities in breast cancer patients prior to adjuvant treatment (Scherling et al., 2011, 2012), post-treatment (de Ruiter et al., 2011; Kesler et al., 2009; Kesler et al., 2011; McDonald et al., 2010; Silverman et al., 2007), and longitudinally (McDonald et al., in press), further supporting the importance of frontal abnormalities in the observed subjective and objective cognitive changes.

These findings extend our prior prospective work by demonstrating self-reported executive complaints that follow the same pattern as gray matter changes. Across BRIEF-A subscales, and particularly in the area of metacognitive functioning, chemotherapy-treated patients were more likely to show increased T-scores from baseline to one month post-treatment, indicative of greater perceived executive dysfunction. The CTx+ group also showed the only significant increase in symptoms over time (on the Initiate scale), and a trend in the same direction on the Working Memory scale. Change in Initiate scale adjusted T-score showed a negative correlation with gray matter density one month after chemotherapy completion (reduced gray matter density at M1 was correlated with greater executive complaints). No such correlation was seen

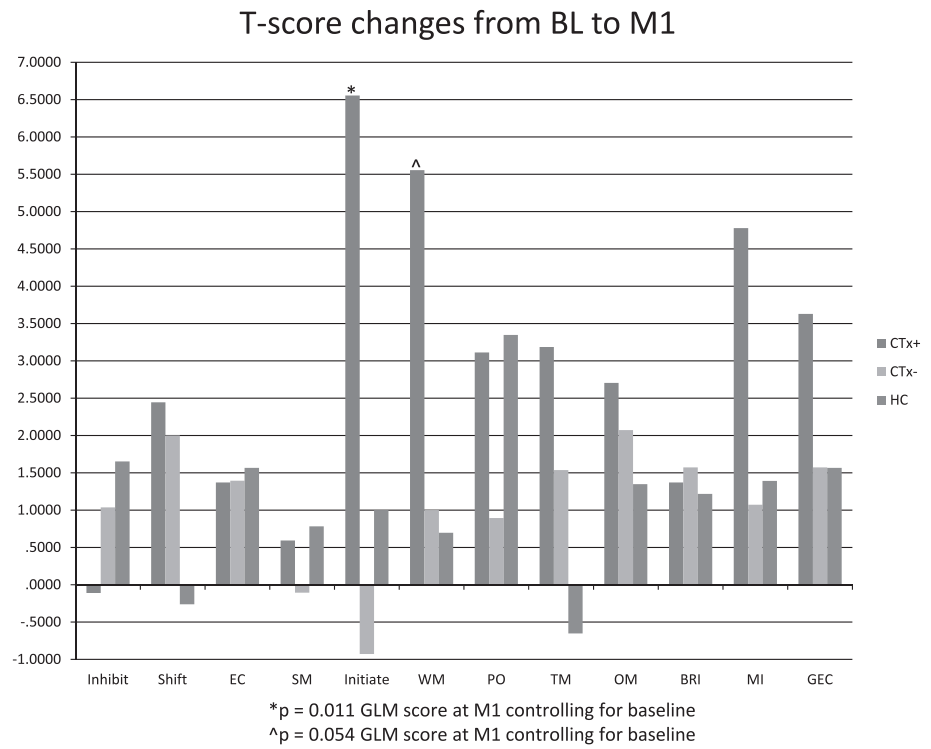

Fig. 3. T-score changes for BRIEF-A scales and indexes from baseline to M1. Note relatively greater increases for CTx+ patients on scales which make up the Metacognition Index (Initiate, WM, PO, TM, OM). (BRIEF-A = Behavior Rating Inventory of Executive Function-Adult Version, BL = baseline, M1 = one month after chemotherapy completion, CTx+ = chemotherapy-treated, CTx− = nonchemotherapy-treated, HC = healthy control, EC = Emotional Control, SM = Self-Monitor, WM = Working Memory, PO = Plan/Organize, TM = Task Monitor, OM = Organization of Materials, BRI = Behavioral Regulation Index, MI = Metacognition Index, GEC = Global Executive Composite).

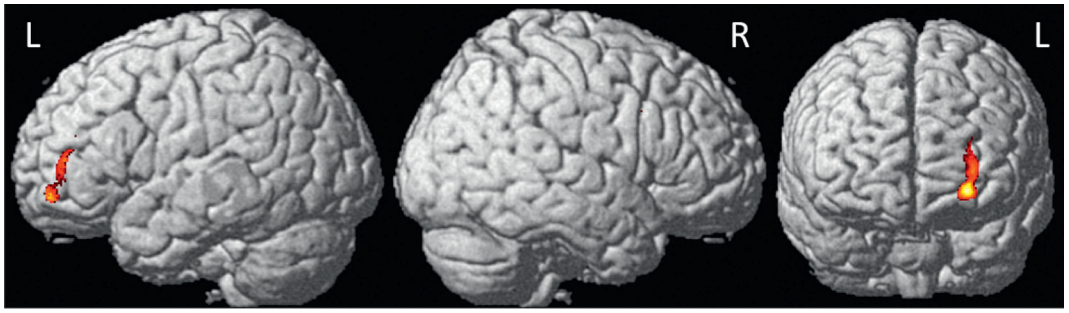

Fig. 4. Negative correlation of BRIEF-A Initiate scale adjusted T-score with gray matter density one month after chemotherapy completion ($P_{crit} < 0.001$, cluster-level $P_{FWE\text{-}corr} < 0.05$, see Table 2 for region descriptions, BRIEF-A = Behavior Rating Inventory of Executive Function-Adult Version).

in patients who did not receive chemotherapy or controls. These findings are consistent with prior cognitive studies showing greater symptoms in the short-term following chemotherapy treatment. In addition, the frontal regions in which we found significant gray matter changes over time and correlations with executive complaints (Brodmann areas 8, 10, and 46) are the same regions where a recent study demonstrated functional abnormalities in breast cancer patients post-treatment and found relationships between brain activation and self-perceived executive functioning as measured by the BRIEF-A (Kesler et al., 2011). Our findings therefore provide independent support for Kesler et al.'s previous results. In addition, previous work has demonstrated that BRIEF-A measures correlate with frontal lobe volume in schizophrenia (Garlinghouse et al., 2010). Our findings therefore offer additional support for the BRIEF-A as a measure sensitive to functionally meaningful brain changes in neuropsychiatric populations.

In our prior cohort there were no between-group differences apparent at baseline. In the current study, the only baseline difference was a single cluster in the left cingulate gyrus in which CTx− patients showed lower gray matter density than controls. This finding seems unlikely to be related to cancer per se, as no such group difference was seen in the CTx+ group. In addition, our primary interest was in examination of changes over time. As this region showed no significant change over time in within-group or interaction analyses, it remains of uncertain clinical significance. We also examined self-reported symptoms of depression and anxiety (CES-D, STAI-S). As in our prior cohort, there were no significant group-by-time interactions on these factors, and group means were below levels typically considered to be clinically significant, suggesting that these psychosocial factors do not account for the observed differences in gray matter density or self-reported executive dysfunction.

While at present the systemic effects of chemotherapy and other cancer treatments remain poorly understood, we and others have proposed possible mechanisms for chemotherapy-induced cognitive and brain changes, including chemotherapy-induced DNA damage (directly or through increases in oxidative stress), individual variation in genes related to neural repair and/or

plasticity, and chemotherapy-induced hormonal changes (Ahles and Saykin, 2007). The question of whether chemotherapy-related cognitive and brain changes are related to direct cytotoxic effects of chemotherapeutic agents crossing the blood–brain barrier has not been conclusively addressed. All but one of the CTx+ participants in the current study received cyclophosphamide, carboplatin, or cisplatin, and all CTx+ patients in the prior cohort received cyclophosphamide. As these agents are believed to cross the blood–brain barrier to some degree, this remains a possible explanation for the observed decreases in gray matter density.

Prior work has also suggested that such cancer and treatment-related changes are likely to affect only a subgroup of cancer patients, who may be more vulnerable to these effects for as yet undetermined reasons. Previous studies have suggested that patients who have more advanced stage disease, are older at the time of breast cancer diagnosis, have lower baseline cognitive reserve, or are *APOE* ε4 positive may be at increased risk for cognitive changes related to cancer and its treatment (Ahles et al., 2008; Ahles et al., 2010; Ahles et al., 2003). To examine a potential biological mechanism for the observed gray matter and executive symptom changes we investigated their relationship to *APOE* status, but did not find a significant effect of *APOE* ε4 carrier status on either gray matter change or increase in self-reported executive symptoms. This may reflect relatively low power to detect genetic influences in the present study. It was noteworthy that a relatively greater percentage of chemotherapy-treated patients were ε4 positive than in the other two groups. This is consistent with prior work showing an association between breast cancer and ε4 status (Chang et al., 2005; Moysich et al., 2000; Porrata-Doria et al., 2010), though other studies have failed to find such an effect (Chang et al., 2006; Niemi et al., 2000; Yaylim et al., 2003). By comparison, in a meta-analysis Farrer et al. (1997) found that 25.7% of a group of 6262 Caucasian healthy older adults were *APOE* ε4 positive, in contrast to patients with Alzheimer's disease, of whom 58.5% were *APOE* ε4 positive. While these figures were drawn from an older population, recruited for comparison to individuals with Alzheimer's disease, we note that the percentages of *APOE* ε4 positive participants in our CTx− and control groups (21% and 25%, respectively) are similar to the control group of Farrer et al., while our CTx+ group had a notably higher percentage of *APOE* ε4 positive individuals (42%).

Some limitations of the current work should be considered. First, while group sizes were larger than in our prior cohort, they remain relatively small, particularly for exploration of genetic or other risk factors for cognitive changes related to cancer and treatment. This is also a highly educated cohort. As noted above, previous work has shown that patients with lower baseline cognitive reserve (for which level of education is sometimes used as a proxy) appear to be at greater risk for cancer- and treatment-related cognitive changes. It may therefore be that even greater treatment-related changes in gray matter density would be apparent in a less educated cohort. Alternatively, it may be that individuals with greater education are more likely to be aware of literature regarding cognitive effects of cancer treatment, and may therefore report subjective changes more frequently (see (Schagen et al., 2009) for examination of priming effects in cancer patients). Also, as noted above, CTx+ patients had higher disease stage, on average, than CTx− patients. This is consistent with standard clinical care, as patients with more advanced disease are more likely to receive chemotherapy; however, it does also prevent separation of potential effects of treatment from disease stage. While there was significant commonality in treatment regimen for CTx+ patients (all received a taxane, most also received cyclophosphamide) and anti-estrogen treatment for CTx− patients (over half received tamoxifen), variation in treatment may potentially contribute to data variability (e.g., more patients in the CTx+ than CTx− group received local radiation). The inclusion of patients who received neoadjuvant treatment (one third of the CTx+ group) also potentially increases data variability; this group has not yet been exposed to some surgery-related variables at the M1 visit, but also may have somewhat more advanced disease than patients receiving standard adjuvant treatment.

It is also possible that changes in hormonal status (e.g., chemotherapy-induced ovarian failure) might play a role in the functional and structural brain changes noted in this population. In the CTx+ group 44% (12 of 27 patients) reported that periods were regular at the baseline visit, but had stopped or begun to stop at M1, a change they most commonly attributed to chemotherapy. However, as might be expected, patients who were menstruating at study entry were on average 10 years younger than those who were postmenopausal at study entry (mean age (SD) 44.6 (4.7) and 54.1 (6.9), respectively, $P < 0.001$), such that change in menstrual status is confounded with age. Comparison of values for the regions where significant gray matter changes were found from baseline to M1 between patients with and without changes in menstrual status showed no group-by-time interaction ($P > 0.05$), suggesting that chemotherapy-induced ovarian failure did not account for the observed structural changes. However, it will be important to continue to examine the effects of changes in hormonal (estrogen) status on brain functioning in future studies while also considering potential confounds.

Regarding these limitations, it will be advantageous in future work to pool samples when possible, to allow further investigation of *APOE* and/or other genetic or other biological factors thought likely to convey risk for these changes, as well as examination of individual contributions of specific cancer treatments and demographic factors (e.g., education, cognitive reserve). It will also be beneficial in the future to examine the relationship of these gray matter changes to objective psychometrically defined cognitive functioning. While prior work has consistently shown increases in both objective and subjective cognitive impairment after cancer chemotherapy, objective cognitive performance and subjective complaints are often not directly correlated, highlighting the need to examine both factors. It will also be helpful to examine other potential genetic factors which may be contributory (e.g., *COMT* (Small et al., 2011)). In our prior cohort (McDonald et al., 2010), reductions in gray matter density in the CTx+ group showed partial but not complete recovery to baseline levels at a follow-up scan conducted one year after the M1 visit. Other recent work (Koppelmans et al., 2012) has shown persistent decreases in total brain and gray matter volume in breast cancer survivors on average 21 years post-treatment. These findings suggest that while some improvement may be expected over time, persistent brain changes may be apparent, at least for a subgroup of patients. We anticipate being able to investigate these and other factors in this cohort in the future, as well as to examine longer-term outcome in terms of gray matter density, when members of this cohort complete additional follow-up visits.

In summary, the current findings replicate and extend our prior work and that of others demonstrating structural brain changes related to breast cancer chemotherapy and concurrent changes in perceived cognitive functioning. This pattern of gray matter change was not observed in breast cancer patients who did not receive chemotherapy or healthy controls, and was found in frontal regions important for attentional and executive functioning, domains commonly found to be affected by cancer and its treatment. These findings therefore provide additional supportive data for a structural neuroanatomic basis for the cognitive problems most commonly reported during and after chemotherapy.

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Conflict of Interest

The authors of this manuscript have nothing to declare.

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Cortisol and inflammatory processes in ovarian cancer patients following primary treatment: Relationships with depression, fatigue, and disability ☆

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ABSTRACT

Elevations in the pro-inflammatory cytokine interleukin-6 (IL-6) and alterations in the anti-inflammatory hormone cortisol have been reported in a variety of cancers. IL-6 has prognostic significance in ovarian cancer and cortisol has been associated with fatigue, disability, and vegetative depression in ovarian cancer patients prior to surgery. Ovarian cancer patients undergoing primary treatment completed psychological self-report measures and collected salivary cortisol and plasma IL-6 prior to surgery, at 6 months, and at 1 year. Patients included in this study had completed chemotherapy and had no evidence of disease recurrence. At 6 months, patients showed significant reductions in nocturnal cortisol secretion, plasma IL-6, and a more normalized diurnal cortisol rhythm, changes that were maintained at 1 year. The reductions in IL-6 and nocturnal cortisol were associated with declines in self-reported fatigue, vegetative depression, and disability. These findings suggest that primary treatment for ovarian cancer reduces the inflammatory response. Moreover, patients who have not developed recurrent disease by 1 year appear to maintain more normalized levels of cortisol and IL-6. Improvement in fatigue and vegetative depression is associated with the normalization of IL-6 and cortisol, a pattern which may be relevant for improvements in overall quality of life for ovarian cancer patients.

1. Introduction

Inflammation has recently been described as the 7th hallmark of cancer (Colotta et al., 2009) and is widely considered a critical factor in the pathogenesis of ovarian cancer (Clendenen et al., 2011; Ness et al., 2000; Wu et al., 1992). Both tumor and stromal cells in the tumor microenvironment produce high levels of the inflammatory cytokine interleukin-6 (IL-6) (Watson et al., 1990; Nilsson et al., 2005; Offner et al., 1995; Obata et al., 1997) which serves to promote tumor growth and dissemination. In the tumor microenvironment, IL-6 promotes angiogenesis (Nilsson et al., 2005), invasion, and attachment (Obata et al., 1997), and the generation of tumor-associated macrophages (Jeannin et al., 2011) Plasma levels of IL-6 in ovarian cancer are quite elevated (Lutgendorf et al., 2008; Tempfer et al., 1997) and are thought to reflect levels of IL-6 in the tumor microenvironment (Edgell et al., 2010). Elevations of IL-6 are also associated with decreased time

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to recurrence and shorter survival time in ovarian cancer patients (Lane et al., 2011; Plante et al., 1994; Scambia et al., 1995).

In addition to direct effects of inflammatory cytokines such as IL-6 on tumor growth, elevated systemic levels of cytokines such as IL-6 have known effects on the central nervous system, eliciting the classical inflammatory repertoire described as "sickness behaviors" such as reduced food intake, fatigue, anhedonia, and lethargy (Dantzer et al., 2002; Dantzer, 2006; Musselman et al., 2001). In animal models of ovarian and breast cancer, plasma and/or central nervous system (CNS) IL-6 has been associated with reduced locomotion and depression-like behaviors (Lamkin et al., 2011; Pyter et al., 2009). We have previously reported an association between plasma IL-6 and vegetative depression, disability, and fatigue in ovarian cancer patients at the time of surgery (Lutgendorf et al., 2008). However, the effects of ovarian cancer treatment on this important cytokine, as well as the association of IL-6 with vegetative symptoms have not been characterized in a longitudinal analysis.

Cortisol is a glucocorticoid hormone released by the hypothalamic–pituitary–adrenal (HPA) axis in response to inflammation (Rhen and Cidlowski, 2005), stress and other stimuli (Chrousos and Gold, 1998), and serves as an important regulator of metabolic function. In healthy individuals, cortisol secretion follows a diurnal rhythm characterized by elevated levels in the morning which decline over the afternoon and evening, and reach a nadir during the first half of the night (Dallman et al., 2000; Tsigos and Chrousos, 2002) Dysregulated patterns of cortisol secretion, often characterized by elevated nocturnal cortisol, have been noted in diverse populations of cancer patients including breast, ovarian, and cervical cancers and lymphoma (Abercrombie et al., 2004; Jehn et al., 2010; Mormont and Lévi, 1997; Palesh et al., 2008)

Elevated nocturnal cortisol and blunted diurnal cortisol slope have been observed in ovarian cancer patients prior to surgery, a pattern that has been hypothesized to be secondary to tumor-derived inflammation (Lutgendorf et al., 2008; Musselman et al., 2001; Weinrib et al., 2010) The specific effects of abnormal diurnal cortisol patterns on tumor physiology in humans are not known, although disruption of cortisol rhythms resulting in a flattened cortisol slope has been associated with shortened survival time in breast cancer patients (Sephton et al., 2000). In animal models, the disrupted release of cortisol in response to inflammation is of substantial importance, as altered glucocorticoid receptor expression secondary to elevated cortisol has been identified as a potential mechanism in the initiation of ovarian cancer (Rae and Hillier, 2005), the failure of cancer cells to undergo apoptosis (Melhem et al., 2009; Pan et al., 2011; Schlossmacher et al., 2011), the development of chemotherapy resistant ovarian cancer cells (Pang et al., 2006), and accelerated tumor growth (Filipski and Lévi, 2009). In addition to its association with tumor growth, cortisol dysregulation has been linked with quality of life of cancer patients. Abnormal cortisol rhythms have been associated with poor performance status (Touitou et al., 1996), fatigue (Bower et al., 2005), and depression (Jehn et al., 2010) in cancer patients. In addition, we have previously reported links between abnormal cortisol rhythms and vegetative symptoms of depression at the time of surgery in ovarian cancer patients (Lutgendorf et al., 2008; Weinrib et al., 2010).

Primary treatment for ovarian cancer typically involves surgical resection of the tumor, followed by six cycles of platinum-based chemotherapy, and lasts approximately 6 months (Bristow et al., 2002). Despite the prognostic and functional importance of cortisol and IL-6, little attention has been paid to how primary treatment affects these substances, their trajectory following primary treatment, and their relationship with patients' self-reported functional ability. One small study reported reductions in IL-6 and C-reactive protein in 38 patients with ovarian carcinoma immediately following chemotherapy (Zakrzewska and Poznanski, 2001) but did not examine psychological correlates or glucocorticoid levels. Another study reported reduced serum cortisol levels immediately after platinum-based chemotherapy (Morrow et al., 2002) as a correlate of nausea, but did not examine long term effects of primary treatment.

Using a prospective longitudinal design, we examined patterns of cortisol secretion, levels of IL-6, and self-reported measures of fatigue, disability and vegetative depression prior to surgery, at 6 months, and at 1 year among ovarian cancer patients who had completed primary treatment, had no evidence of disease recurrence, and were not receiving chemotherapy at 1 year. Our model was designed to examine the relationship between changes in inflammatory pathways following primary treatment and associated changes in clinically relevant measures of self-reported functioning. We hypothesized that levels of IL-6 would be reduced and diurnal patterns of cortisol normalized following primary treatment for ovarian cancer. Furthermore, we hypothesized that these changes would be maintained 1 year after surgery in patients whose disease had not recurred. We hypothesized that these reductions would be associated with decreases in self-reported fatigue, vegetative depression, and disability.

2. Methods

2.1. Participants

Consecutive gynecologic oncology patients with suspected ovarian cancer were prospectively recruited during their pre-surgical clinic appointment. Eligibility was restricted to patients with primary epithelial ovarian, peritoneal, or fallopian tube carcinomas. Histological diagnosis for inclusion in the study was confirmed by pathology. Patients with benign disease, non-epithelial malignancies, tumors of low malignant potential (LMP), history of previous cancer, use of systemic corticosteroids in the last month, receipt of neo-adjuvant chemotherapy and those below 18 years of age were excluded. Only patients who had been enrolled in the study long enough to have reached their 1 year follow-up were included in the present analyses. To avoid confounding influences of cancer recurrence and/or chemotherapy on inflammatory processes and diurnal cortisol, data was only used from patients who had no evidence of disease recurrence and were not receiving chemotherapy at the time of the 6 months or 1 year follow-up.

The final sample is described in Fig. 1. Of 510 potentially eligible patients, 180 had benign disease, 39 had tumors of low malignant potential, 41 had tumors of non-ovarian pathology, and 15 did not have surgery, received neo-adjuvant chemotherapy regimen, or did not meet another inclusion factor. Thirty-two patients withdrew from the study before surgery. Thus 202 ovarian cancer patients were eligible for inclusion. Of these patients, 31 did not have peripheral blood sampling before surgery. Four patients who died shortly after surgery were not included in the pre-surgical analyses as no longitudinal information could be collected. Of the 168 patients eligible for inclusion before surgery, 117 also collected salivary cortisol. The lower number of patients who collected cortisol is most likely due to the more burdensome and time consuming nature of the procedure (9 different collection times over 3 days) which exigent surgery sometimes rendered impossible. At the 6 months follow-up, peripheral blood samples were available for 114 patients without evidence of recurrence and who were not receiving chemotherapy at the time of follow-up; 58 collected salivary cortisol at this time-point. At 1 year, peripheral blood samples were available for 92 patients without evidence of recurrence and not receiving chemotherapy and 47 collected sali-

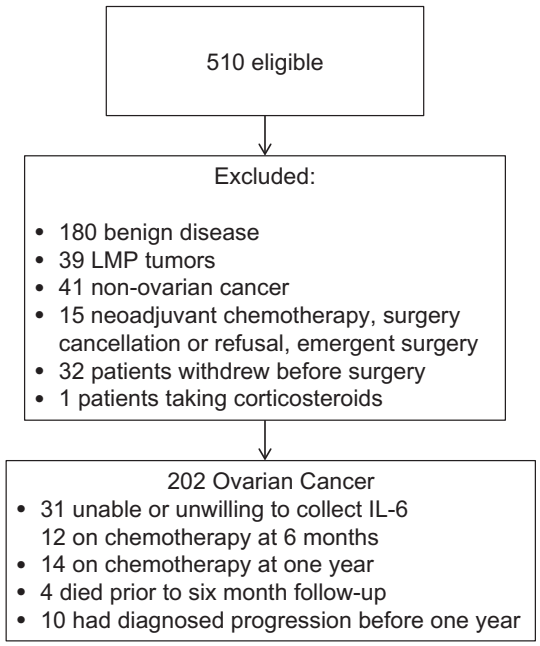

Fig. 1. Patient flow diagram.

vary cortisol. Because mixed models can estimate a missing time-point, patients were included if they had IL-6 collections at any two of these time-points.

2.2. Procedure

Informed consent was obtained during the pre-surgical visit, and participants received questionnaires and instructions for home collection of salivary cortisol. To ensure the best adherence to instructions, a research assistant contacted the participants prior to sampling to repeat instructions. Samples were refrigerated by patients until brought to the clinic at the time of surgery, where they were stored at −80 °C until analysis. If samples were not brought to follow-up visits, patients were requested to return them by mail and mailers were supplied. Peripheral blood was collected in the morning prior to surgery, and at the 6 months and 1 year follow-up clinic visits. Samples of pre-surgical blood were generally obtained before 12 p.m. (>85%); follow-up bloods were obtained at the time of the patient's appointment; more than 70% of these samples were obtained prior to 12 p.m. at each follow-up time-point. This level of homogeneity was desirable as IL-6 undergoes a slight degree of circadian variation (Vgontzas et al., 2005). All procedures were approved by the institutional review boards at participating institutions. At the time of surgery, participants had not yet received a definitive cancer diagnosis. The 6 months visit was usually performed 1 month following the completion of chemotherapy, and the 12-month assessment was performed at the routine 12 months follow-up visit.

2.3. Measures

2.3.1. Fatigue

The Profile of Mood States Short Form (POMS-SF; Curran et al., 1995) is a 37-item self-report scale assessing mood over the past week which has been used previously in cancer patient populations (Thornton et al., 2008). It is composed of 6 subscales: anxiety, dysphoria, anger, vigor, fatigue and confusion. The fatigue subscale consists of five words or phrases (i.e. "worn out," "exhausted," "weary") that are endorsed on a scale from 0 (not at all) to 4 (extremely). While fatigue is a heterogeneous concept, the POMS-fatigue subscale has been shown to correlate highly with other fatigue scales designed for cancer patients (Giacalone et al., 2010; Locke et al., 2007). In this study the fatigue subscale was used as an outcome measure in longitudinal analyses. The anxiety subscale was used as predictor of outcome variables in secondary longitudinal analyses.

2.3.2. Vegetative symptoms of depression

The Center for Epidemiological Studies Depression scale (CES-D; Radloff, 1977) is a 20 item self-report scale measuring frequency of depressive symptoms over the last week. Four subscales representing facets of depressive symptoms have been confirmed by factor analysis. These are depressed affect, vegetative depression, positive affect, and interpersonal relationships (Sheehan et al. 1995) Vegetative depression is characterized by reduced motivation and somatic complaints (i.e. "could not get 'going'", "had trouble keeping my mind on tasks," "sleep was restless"). Items were endorsed on a 0–3 point scale, with 0 representing symptoms occurred rarely, and 3 representing that symptoms occurred most or all of the time.

2.3.3. Performance status

Prior to surgery patients completed a measure of their Gynecologic Oncology Group performance status. Functional ability is rated from 0 (fully active) to 4 (completely disabled), with higher scores indicating less functional ability.

2.3.4. Sleep

A single item, "how many hours of sleep did you get on average in the last week?" was used in secondary analyses. Hours of sleep, rather than a measure of overall sleep quality, was used because most research linking sleep and glucocorticoid disruption has focused on hours of sleep deprivation rather than global sleep quality (Vgontzas et al., 1999; Weitzman et al., 1983).

2.3.5. Demographic, medical, and health information

Demographic data were obtained by patient self-report. Clinical information, including histology, stage, grade of tumor, and treatment history was abstracted from medical records.

2.3.6. Cortisol

Samples were collected upon awakening, in the afternoon between 16:00 and 18:30 p.m., and at bedtime for the 3 days before surgery, and 3 days following the 6- and 12-month clinic visit. Participants wrote the time of collection on the salivettes, a practice that has been shown to be reliable in previous studies (Kraemer et al., 2006). Salivary cortisol is stable at room temperature (Kirschbaum and Hellhammer, 1989). Assays were performed using a commercial chemiluminescence immunoassay (IBL, Hamburg, Germany) in the laboratory of Clemens Kirschbaum at the Technical University of Dresden. The lower detection limit is 0.41 nmol/L, and inter-assay and intra-assay coefficients of variance are <10%.

2.3.7. Interleukin-6

Detection of IL-6 in plasma was performed by enzyme-linked immunosorbent assay (R&D Diagnostics, Minneapolis, MN), with results interpolated from the standard curve provided with the kit. The minimum detectable level is less than 0.7 pg/mL and inter-assay variability for this assay ranges from 3.3% to 6.4%. IL-6 samples below the sensitivity of the regular assay were quantitated with the R&D High-Sensitivity ELISA. The obtained intra-assay coefficient of variance was less than 10%. Values were log (10) transformed to normalize their distribution.

2.4. Statistical analyses

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 19.0. All distributions were examined for outliers and normality before analysis.

2.4.1. Data reduction strategy

Before analyses, sampling time outliers for cortisol were removed. The first sample was collected in conjunction with personal awakening time, which is associated with a rise in cortisol (Kirschbaum and Hellhammer, 2000). Acceptable ranges of sampling times were determined to fit the maximum number of participants while maintaining a certain amount of homogeneity. Acceptable ranges were 0400 to 0900 h for morning cortisol, 1600 to 1830 h for afternoon cortisol, and 2000 to 2400 h for nocturnal cortisol. Samples which were more than four standard deviations above the mean of their particular time point were excluded (37 out of 1590 samples). Previous research has supported the use of aggregated cortisol measures to increase reliability of data (Kirschbaum and Hellhammer, 1989; Kraemer et al., 2006). Mean cortisol values at each time-point were calculated and values were transformed using the natural logarithm (ln) to normalize their distribution.

Because glucocorticoid dysregulation is characterized not only by elevations of salivary cortisol at particular times of day, but also by changes in diurnal slope, the slope of diurnal change in cortisol level was calculated in accordance with past research (Sephton et al., 2000, 2009). The regression of the 9 cortisol values on the hour of sample collection was calculated, with data pooled over the 3 days for each patient. Steeper slopes, reflected by smaller (more negative) slope values, indicate more rapid salivary cortisol declines over the course of the day.

2.4.2. General linear model

To test for differences in interleukin-6 and cortisol levels between patients with low (stage 1 and 2) versus high stage disease (3 and 4), general linear models controlling for disease grade and patient age were used.

2.4.3. Mixed models

All longitudinal analyses used mixed effects models (SPSS MIXED procedure) with fixed slopes and intercept terms. This approach was used because such an analysis can handle unbalanced data and because it allowed us to control for correlated error where relevant (Laird and Ware, 1982). Since aging has been associated with alterations in the hypothalamic–pituitary–adrenal (HPA) axis (Deuschle et al., 1997) and with increased levels of IL-6 (Ershler et al., 1993), all analyses controlled for age. All analyses also controlled *a priori* for disease stage and grade as the best measures of disease severity which may be independently linked to inflammation (Macciò et al., 2009). Both IL-6 and nocturnal cortisol were included in all analyses to disaggregate their effects. As we have previously reported a strong relationship between nocturnal cortisol, fatigue, disability and vegetative depression at the time of surgery (Weinrib et al., 2010) we focused on nocturnal cortisol levels for longitudinal analyses involving the association of these quality of life measures with cortisol and IL-6.

Because associations have been reported between inflammatory processes and BMI (Fain, 2010; Illan-Gomez et al., 2012; Siervo et al., 2012), as well as with hours of sleep (Redwine et al., 2000; Vgontzas et al., 1999; Weitzman et al., 1983), in secondary analyses we tested models using BMI and hours of sleep, individually, as predictors of change in outcome variables. As improvements in inflammatory parameters and self-reported measures may be sensitive to anxiety (Campbell and Ehlert, 2012) change in anxiety was also tested independently as a predictor of change in all outcome variables. Finally, optimal vs. suboptimal surgical resection of tumor was examined to determine if reductions in inflammatory parameters were associated with better surgical outcome.

All models were first evaluated with an auto-regressive covariance structure. If residual correlations were non-significant, a diagonal covariance structure was employed. A diagonal covariance structure implies that there is no further relationship between variables beyond those specified in the model and that observations made on the same individual may be independent (Littell et al., 2000). Because of the significant time lag between measurements, approximately 6 months, this covariance structure was considered. An autoregressive covariance structure specifies that residual errors of observations on the same individual are correlated (Littell et al., 2000). Model fit was evaluated by Wald Z tests of rho covariance parameters and by comparison of Schwarz's Bayesian Criterion (BIC) to models with other covariance structures. Pairwise comparisons of estimated marginal means, using a Sidak adjustment, were conducted to examine changes from baseline to 6 months and from 6 months to 1 year.

2.4.4. Change in cortisol and IL-6 over time

Residual correlations in models of afternoon and nocturnal cortisol changes over time were not significantly different from zero according to Wald tests (both p values >0.089), suggesting a diagonal correlational structure is more appropriate. The use of a diagonal covariance structure for these models was further supported by a lower BIC value and highly significant diagonal variances (all p values <.0001), suggesting better model fit. In contrast, residual correlations in the model of change in morning cortisol concentrations and IL-6 were significantly different from zero according to Wald tests (p values <.0001); thus, an auto-regressive covariance structure was employed. This approach was justified by lower BIC values compared to the diagonal covariance structure

2.4.5. Change in self-reported measures with change in cortisol and IL-6

The models of the association of changes in vegetative depression, disability and fatigue with changes in IL-6 and cortisol had significant *rho* values according to the Wald test (all p<.036) so we employed an auto-regressive covariance structure. This approach was supported by lower (BIC) suggesting better model fit. Compared to models using auto-regressive heterogeneous covariance structures, Schwarz's Bayesian Criterion (BIC) was lower for our models, suggesting better fit.

3. Results

3.1. Patient characteristics

As seen in Table 1, the average age of participants at the time of surgery was 58 years (SD = 12.2). Most had advanced stage disease (72%), high grade neoplasms (85%) and serous histology (74%).

3.2. Covariates and potential confounding factors

Levels of IL-6 and nocturnal cortisol did not vary significantly between patients with early versus advanced stage disease (IL-6: $p = 0.176$; cortisol: $p = 0.741$) at baseline. Neither stage nor grade showed a significant association with changes in cortisol or IL-6 over time (all p values >0.174). Older patients showing significantly less pronounced changes in cortisol at each time-point between surgery and one-year (all p values <0.026); however, age did not have a significant association with changes in IL-6 over time ($p = 0.944$). In contrast, older patients reported significantly greater reductions in vegetative depression over time ($p = 0.006$). In secondary analyses, BMI, change in hours of sleep, change in

Table 1
Patients' demographic and clinical characteristics.

Measure	Cancer patients $N = 163$
Age in years	
Mean (SD)	57.5 (12.2)
Marital status	
Single	11.3%
Divorced/separated	12%
Widowed	12.7%
Married/living with partner	64%
Race	
American Indian/Alaskan native	1.2%
Asian	0.6%
African American	1.9%
Caucasian	96.3%
Ethnicity	
Hispanic	6.9%
Non-hispanic	93.1%
Cancer stage	
Stage I	20.3%
Stage II	7.6%
Stage III	63.3%
Stage IV	8.9%
Cancer grade	
Low	14.2%
High	85.8%
Tumor histology	
Serous	73.5%
Endometrioid	11.7%
Mucinous	4.9%
Clear cell	3.7%
Unknown/other	6.2%
Cytoreduction	
Optimal	75.9%
Suboptimal	24.1%

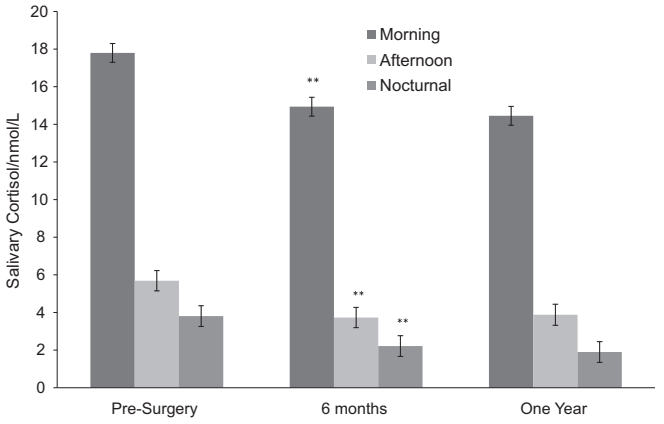

Fig. 2. Estimated marginal means of salivary cortisol concentrations pre-surgery, at 6 months and 1 year controlling for disease stage, grade, and age of patients. **Indicates a significant change ($p < .01$) from previous time point. Log transformed values are back-transformed.

anxiety and presence/absence of residual tumor, all had non-significant relationships with the outcome variables (all p values >0.17).

3.3. Changes in cortisol and IL-6 over time

Morning, afternoon and nocturnal cortisol levels dropped significantly over the course of the year following surgery (morning cortisol: $F_{2,93} = 5.75$, $p = .004$; afternoon cortisol: $F_{2,98} = 10.30$, $p < .0001$; nocturnal cortisol: $F_{2,95} = 17.21$, $p < .0001$). IL-6 decreased significantly over the year following surgery as well ($F_{2,201} = 66.21$, $p < .0001$). Pairwise comparisons of cortisol levels as a function of follow-up time-point revealed highly significant decreases for morning, afternoon and evening cortisol levels between surgery and 6 months (all p values <0.007), but no significant differences between 6 months and 1 year (both p values >0.633), suggesting relative stability during months 6–12. Diurnal slope became significantly steeper between surgery and 1 year ($F_{2,109} = 8.91$, $p < .001$) indicating that following primary treatment, a more normalized pattern of diurnal cortisol concentrations was observed. Similarly, IL-6 decreased between surgery and 6 months ($p < .0001$) but levels were relatively consistent between 6 months and 1 year ($p = .989$). Estimated marginal means are presented in Figs. 2 and 3. (Estimated marginal means provide a more accurate estimate of values than raw means when covariates are included in the model).

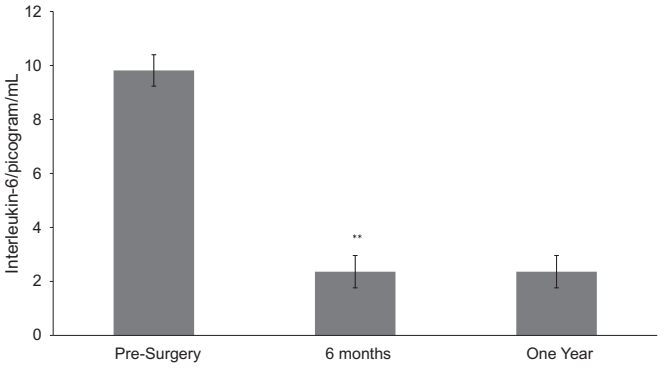

Fig. 3. Estimated marginal means of IL-6 in peripheral blood pre-surgery, at 6 months, and at 1 year controlling for disease stage, grade, and age of patients. **Indicates a significant change ($p < .01$) from previous time point. Log transformed values are back-transformed.

Table 2
Self report measures (estimated marginal means).

Measure	Pre-surgery $N = 168$	6-month $N = 114$	One-year $N = 92$
Self-Reported Measures Mean (standard error)			
POMS[a] fatigue subscale	7.93(.68)	6.76(.68)*	6.82(.74)
CES-D[b] vegetative subscale	6.79(.43)	4.51(.45)*	4.54(.48)
GOG[c] performance status	.86(.09)	.60(.10)*	.46(.10)

*Represents a significant change from previous time-point ($p < .05$), pair-wise comparisons with Sidak adjustment.
[a] Profile of mood states.
[b] Center for epidemiological studies-depression.
[c] Gynecological oncology group.

3.4. Relationships of changes in cortisol and IL-6 over time with fatigue and vegetative depression

During the period from surgery to 1 year, decreased IL-6 was significantly associated with decreased fatigue ($F_{1,120} = 5.24$, $p = 0.024$) and vegetative depression ($F_{1,188} = 12.20$, $p < 0.001$) and was marginally associated with decreased self-reported disability ($F_{1,180} = 2.91$, $p = 0.090$). Decreased nocturnal cortisol was significantly associated with decreased self-reported disability ($F_{1,172} = 7.41$, $p = 0.007$) and fatigue ($F_{1,116} = 3.96$, $p = 0.049$) and was marginally associated with decreased vegetative depression ($F_{1,180} = 3.30$, $p = 0.071$). Estimated marginal means are presented in Table 2. Parameter Estimates are presented in Tables 3–5.

4. Discussion

The key finding of this study is that following primary treatment of patients diagnosed with epithelial ovarian cancer, diurnal

Table 3
Parameter estimates, change in profile of mood states fatigue subscale.

Parameter	β	p
Disease stage		
1	−.44	.825
2	3.75	.136
3	1.73	.302
4	0	
Grade		
Low	−.046	.978
High	0	
Age	−.064	.132
Nocturnal cortisol[a]	1.05	.049
Interleukin-6[b]	1.75	.024

[a] Natural log-transformed.
[b] Log_{10}-transformed.

Table 4
Parameter estimates, change in gynecologic oncology group patient performance status.

Parameter	β	p
Disease Stage		
1	−.58	.035
2	−.51	.126
3	−.26	.260
4	0	
Grade		
Low	.24	.280
High	0	
Age	−.01	.223
Nocturnal cortisol[a]	.26	.007
Interleukin-6[b]	.20	.090

[a] Natural log-transformed.
[b] Log_{10}-transformed.

Table 5
Parameter estimates, change in self-reported center for epidemiologic studies – depression vegetative depression subscale.

Parameter	β	p
Disease Stage		
1	−.97	.419
2	−.67	.643
3	−1.37	.180
4	0	
Grade		
Low	−.68	.507
High	0	
Age	−.07	.005
Nocturnal cortisol[a]	.66	.071
Interleukin-6[b]	1.75	.001

[a] Natural log-transformed.
[b] Log_{10}-transformed.

cortisol rhythms become more normalized, and IL-6, a key indicator of inflammation, markedly decreases. Morning, afternoon and nocturnal cortisol levels decreased significantly between the time of surgery and 6 months follow-up; peripheral blood IL-6 followed the same pattern. The diurnal cortisol slope became steeper between surgery and 6 months follow-up; this more normalized slope was also maintained at 1 year. Prior to surgery afternoon salivary cortisol concentrations in ovarian cancer patients were 33% higher than those of a group of healthy women previously studied in our lab, and nocturnal concentrations were 51% higher (Weinrib et al., 2010). In contrast, at the six-month clinic visit, afternoon and nocturnal levels were comparable to the healthy community sample (Weinrib et al., 2010). Pre-surgical IL-6 levels of ovarian cancer patients were almost four times higher than ranges previously reported for healthy adults (Ferrucci et al., 1999; Sergi et al., 2011); IL-6 levels dropped to approximate the range for healthy older adults by 6 months post-surgery. Both IL-6 and cortisol levels were fairly constant between 6 months and 1 year, suggesting that the normalization of peripheral blood IL-6 and cortisol rhythms seen at 6 months was maintained at 1 year in patients who did not experience disease recurrence. These longitudinal findings extend previous cross-sectional findings reporting higher levels of IL-6 and disrupted cortisol rhythms in women with ovarian cancer (Lutgendorf et al., 2008; Tempfer et al., 1997; Zakrzewska and Poznanski, 2001; Weinrib et al., 2010) and demonstrate for the first time that successful primary treatment is associated with persistent decreases in inflammation-linked substances in ovarian cancer patients.

The most likely explanation for the reduction in markers of systemic inflammation and normalization of cortisol is reduction or elimination of the cytokine-secreting tumor mass via primary chemotherapy and surgery. Solid neoplasms such as epithelial ovarian tumors secrete IL-6 (Nilsson et al., 2005; Obata et al., 1997; Offner et al., 1995; Watson et al., 1990). IL-6 facilitates the action of interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) on the hypothalamus (Perlstein et al., 1991; Sparkman et al., 2006; Zhou et al., 1996) thus ultimately increasing cortisol secretion (Alesci et al., 2005; Mastorakos et al., 1993). The majority (76.3%) of the patients in this study experienced optimal cytoreduction, indicating the presence of less than 1 cm of residual disease following surgery. Further reduction/elimination is thought to have been achieved through chemotherapy, and participating patients were considered disease-free at the time of 6 months and/or 1 year follow-ups.

In previous work we found no differences in pre-surgical anxiety or distress between women whose pelvic mass was subsequently determined to be benign and those with ovarian cancer. Despite this, the ovarian cancer group had higher levels of evening cortisol and a flatter diurnal slope (Weinrib et al., 2010). We have also previously reported that levels of plasma IL-6 are much higher in ovarian cancer patients than in those with tumors of low malignant potential (LMP) prior to surgery (Lutgendorf et al., 2008), despite equivalent pre-surgical anxiety. In the current data, change in anxiety did not predict change in any of the outcome variables. Taken together, these findings support our contention that the normalization of inflammatory parameters and improved quality of life are not due to reductions in cancer-related anxiety but to primary treatment for ovarian cancer. This lends support to the hypothesis that glucocorticoid disruption and elevated IL-6 in ovarian cancer patients is due, at least in part, to tumor-derived inflammation.

Decreases over time in nocturnal cortisol and IL-6 were associated with decreases in self-reported fatigue and vegetative depression over the same time period. These findings extend our previous cross-sectional observations of relationships between these factors at the time of surgery (Weinrib et al., 2010) and are consistent with reports of associations of glucocorticoid dysregulation with conditions characterized by fatigue and vegetative depression (Marques et al., 2009; Neeck and Crofford, 2000; Vegiopoulos and Herzig, 2007). Moreover, our findings indicate that reductions in self-reported impairments are maintained at 1 year in patients who remain disease-free. Previous research has demonstrated improvement in QOL measures in ovarian cancer patients following chemotherapy (Von Gruenigen et al., 2006; Wenzel et al., 2007). Vegetative depression and fatigue have been found to greatly influence overall QOL in this population (Fox and Lyon, 2007). Our findings suggest that part of this improvement may be linked to the reduction of tumor-associated inflammation and

normalization of cortisol patterns, which appear to be related to primary treatment.

It is likely that several processes contribute simultaneously to changes in vegetative depression in ovarian cancer patients. The interactions of pro-inflammatory cytokines such as IL-6 with the central nervous system have been well characterized. (Conti et al., 2004; Terreni and De Simoni, 1998) and IL-6 has been linked to depressive spectrum behaviors characterized as "sickness behaviors" (Dantzer et al., 2002; Dantzer, 2006). Both IL-6 and hypercortisolemia have been linked to depression (Dowlati et al., 2010; Howren et al., 2009; Liu et al., 2011; Lopez-Duran et al., 2009; Stetler and Miller, 2011) and disruption of cortisol rhythms has been linked to depression in breast and ovarian cancer populations (Abercrombie et al., 2004; Jehn et al., 2006); Lutgendorf et al., 2008; Weinrib et al., 2010) It is possible that the residual tumor mass may have affected both inflammatory pathways and self-reported functional outcomes. However, extent of surgical resection was not significantly associated with reduction of inflammation. It is also possible that physical discomfort and or pain associated with the tumor mass and/or ascites may have affected inflammatory pathways and functional outcome measures independently (De Jongh et al., 2003; de Oliveira et al., 2011). Because the current study does not include substantive measures of pain we were unable to examine the relationship of pain with variables of interest. Future research should consider the role of pain in inflammation reduction.

4.1. Limitations

These findings are correlational, and even though longitudinal analyses have several advantages over cross-sectional designs, no causal relationships can be inferred. Our findings are limited to a subset of patients who were without diagnosed disease progression at 6 months and 1 year. Levels of inflammation and their associations with vegetative depression, disability and fatigue in patients with disease progression cannot be inferred from these results. Imminent surgery precluded quite a few patients from collecting cortisol at the pre-surgical time-point, and participation at 6 months and 1 year follow-ups was diminished by the burden of sample collection; thus the reduced sample of patients who collected cortisol may not be fully representative of the whole spectrum of ovarian cancer patients. As patients included in this study did not require supplemental treatments (i.e. additional chemotherapy) they may represent a healthier subset of all ovarian cancer patients with more normalized levels of biomarkers. Additionally, a larger patient sample may have allowed comparisons of biomarkers in the present sample to levels in patients with disease recurrence. Associations with disability should be interpreted with caution; we do not propose any specific mechanisms by which cortisol or IL-6 might induce physical limitations. It is possible that disability reported at the time of surgery was secondary to the physical burden of tumor and ascites and associated discomfort and that decreases of self-reported disability are not necessarily the consequence of reductions in IL-6 and cortisol normalization. Our model did not allow for discrimination of within-person changes from between-person, static effects. It is therefore not possible to distinguish which effects drove the significant coefficients.

5. Conclusions

These findings extend previous cross-sectional observations by demonstrating normalized levels of IL-6 and cortisol in epithelial ovarian cancer patients following primary treatment. Normalization of inflammation and diurnal cortisol patterns was paralleled by improvements in functional abilities of patients. Inflammatory cytokines and cortisol may contribute to patterns of vegetative symptoms. Further research may focus on the trajectory of other angiogenic and inflammatory molecules following primary treatment and the relationship of IL-6 reduction and cortisol normalization to disease course in ovarian cancer patients. As alterations in glucocorticoid secretion are associated with changes in expression of the glucocorticoid receptor these findings may have important implications for disease mechanisms. Characterizing the trajectory of mediators of inflammation may lead to innovative therapies in the treatment of epithelial ovarian cancer.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Early impact of social isolation and breast tumor progression in mice

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ABSTRACT

Evidence from cancer patients and animal models of cancer indicates that exposure to psychosocial stress can promote tumor growth and metastasis, but the pathways underlying stress-induced cancer pathogenesis are not fully understood. Social isolation has been shown to promote tumor progression. We examined the impact of social isolation on breast cancer pathogenesis in adult female severe combined immunodeficiency (SCID) mice using the human breast cancer cell line, MDA-MB-231, a high β-adrenergic receptor (AR) expressing line. When group-adapted mice were transferred into single housing (social isolation) one week prior to MB-231 tumor cell injection into a mammary fat pad (orthotopic), no alterations in tumor growth or metastasis were detected compared to group-housed mice. When social isolation was delayed until tumors were palpable, tumor growth was transiently increased in singly-housed mice. To determine if sympathetic nervous system activation was associated with increased tumor growth, spleen and tumor norepinephrine (NE) was measured after social isolation, in conjunction with tumor-promoting macrophage populations. Three days after transfer to single housing, spleen weight was transiently increased in tumor-bearing and non-tumor-bearing mice in conjunction with reduced splenic NE concentration and elevated CD11b + Gr-1+ macrophages. At day 10 after social isolation, no changes in spleen CD11b+ populations or NE were detected in singly-housed mice. In the tumors, social isolation increased CD11b + Gr-1+, CD11b + Gr-1-, and F4/80+ macrophage populations, with no change in tumor NE. The results indicate that a psychological stressor, social isolation, elicits dynamic but transient effects on macrophage populations that may facilitate tumor growth. The transiency of the changes in peripheral NE suggest that homeostatic mechanisms may mitigate the impact of social isolation over time. Studies are underway to define the neuroendocrine mechanisms underlying the tumor-promoting effects of social isolation, and to determine the contributions of increased tumor macrophages to tumor pathogenesis.

1. Introduction

The emotional stress experienced by cancer patients can be associated with increased tumor progression (Antoni et al., 2006), but the biological pathways involved in stress-induced tumor progression are only beginning to be understood. In animal models of cancer, exposure to stressors potentiates tumor growth and metastasis in a variety of tumors (Hermes et al., 2009; Saul et al., 2005; Shakhar and Ben-Eliyahu, 1998; Sloan et al., 2010; Thaker et al., 2006; Williams et al., 2009) suggesting that therapies targeting stress biochemical pathways may be effective in reducing tumor progression. Here we examine the impact of social isolation of adult mice, an ethologically relevant stressor, on breast tumor growth. Social isolation in rodents elicits anxiety and other fearful behaviors (Hermes et al., 2009; Williams et al., 2009). Furthermore, chronic social isolation as experienced by humans has been linked to cancer (Reynolds and Kaplan, 1990), and is a risk factor for cancer mortality and other diseases (Hawkley and Cacioppo, 2003).

The sympathetic nervous system (SNS) is a major stressor pathway characterized by release of the catecholamines norepinephrine (NE) and epinephrine (EPI) from sympathetic noradrenergic nerves and from the adrenal medulla. Several lines of evidence point to a role for the SNS in modulating tumor progression. Regional ablation of sympathetic nerves depleted NE and reduced tumor growth (Raju et al., 2007). Stress-induced increase in tumor growth and/or metastasis can be prevented by pre-treatment with a β-AR blocker prior to stressor exposure or mimicked using β-AR agonists *in vivo* (Shakhar and Ben-Eliyahu, 1998; Sloan et al., 2010; Thaker et al., 2006). Furthermore, using an ovarian cancer model, Thaker and colleagues showed that β-AR expression by the tumor cells was necessary for stressor-induced tumor growth (Thaker et al., 2006). It is interesting to note the variety of targets in stress- and

Abbreviations: IL-6, Interleukin 6; SNS, sympathetic nervous system; NE, norepinephrine; EPI, epinephrine; β-AR, beta-adrenergic receptors; VEGF, vascular endothelial growth factor.

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or SNS-induced potentiation of tumor progression including cells of the immune system (for example, macrophages and natural killer cells) (Shakhar and Ben-Eliyahu, 1998; Sloan et al., 2010), angiogenesis (Thaker et al., 2006), and direct stimulation or inhibition of tumor proliferation (Slotkin et al., 2000). In addition to understanding the stress-induced neuroendocrine mediators/receptors that modulate tumor pathogenesis, it is also important to identify the target cells in order to predict the outcome of stress exposure and to develop therapies with minimal side effects.

The MDA-MB-231 cell line is a human mammary tumor adenocarcinoma representative of the more aggressive triple negative human breast cancer. MB-231 cells express high levels of β-AR, as detected by standard radioligand binding assay, but other breast cancer cell lines displayed a low level of β-AR expression and minimal responsiveness to NE *in vitro* (Madden et al., 2011). By contrast, NE stimulation of MB-231 cells inhibits VEGF production, and dramatically increases IL-6 production *in vitro*. With the view that MB-231 serves as a model of breast cancers expressing high levels of β-AR, we have begun testing the impact of stressor exposure on MB-231 tumor growth, using social isolation as an established model of a psychological stressor that can promote tumor progression, including spontaneous mammary tumor progression (Hermes et al., 2009; Thaker et al., 2006; Williams et al., 2009).

We report here that social isolation transiently increased tumor growth only when social isolation was initiated when tumors were palpable. These changes were associated with alterations in tumor macrophage populations early after social isolation and not associated with elevated tumor or peripheral NE concentration. The results demonstrate the complexity of the response of breast tumors to stressor exposure that needs to be better characterized before targeting stress hormones in the therapeutic treatment of breast cancer.

2. Materials and methods

2.1. Mice

Female severe combined immunodeficiency (SCID) (NOD.CB17-*Prkdc*^{scid}/J) mice were purchased from The Jackson Laboratory, Bar Harbor, ME between 6 and 8 weeks of age, and were housed 5 per cage with food and water ad lib on a 12:12 light:dark cycle. SCID mice were provided acid water ad lib upon arrival. The mice were housed using microisolator technology to effect a biological barrier at the level of the individual cage. Upon initiation of the experiments, the antibiotic sulfamethoxazole and trimethoprim (Hi-Tech Pharmacol. Co., Amityville, NY) was included in the drinking water throughout the duration of the experiment. The antibiotic treatment was necessary in order to prevent the occasional pneumonia that developed in these immunodeficient mice. All experimental protocols were approved by the University of Rochester University Committee on Animal Resources.

2.2. Cell lines

MB-231 tumor cells (American Tissue Type Collection; Manassas, VA) were maintained in Dulbecco's Modified Essential Medium (DMEM) containing 4.5 g/L glucose, L-glutamine, penicillin/streptomycin and 10% fetal calf serum (FCS) (all from Gibco, Invitrogen Inc., Carlsbad, CA). MB-231 cells were employed experimentally within 3 months of acquiring and/or thawing, and were regularly tested for the absence of mycoplasma contamination.

2.3. Social isolation

SCID mice were allowed to adapt to group housing (5 per cage) for at least two weeks before being housed singly. Both group- and single-housed mice were housed in cages measuring 7.5" × 11" × 5".

2.4. Tumor implantation and measures

MB-231 (2–4 × 10^6 cells) was injected into a single mammary fat pad of NOD/SCID female mice in mice anesthetized with 90 mg/kg ketamine and 9 mg/kg xylazine. Mice were palpated weekly until tumors were detected. The shortest and longest diameters of each tumor were measured with calipers. Tumor volumes was calculated using the equation: 0.5 × length × width^2.

2.5. Flow cytometry

Single suspensions from spleen or tumors were prepared by pressing tissue through a metal mesh into ice-cold PBS containing 10% fetal calf serum. Red blood cells were lysed using ammonium lysis buffer. After washing, the cells were counted and resuspended in phosphate buffered saline containing 1% bovine serum albumin and 0.25% sodium azide (flow wash). Macrophages were stained using three-color immunofluorescence. Cells (1.5 × 10^6) were incubated in 25 μl FcBlock (anti-CD16, diluted 1:50; BD Biosciences, bdbiosciences.com) for 15 min at 4 °C. Rat anti-F4/80 (clone BM8; FITC-conjugated; Abcam Inc.; abcam.com), rat anti-CD11b (clone M1/70; Alexafluor 647-conjugated, BD Biosciences) and rat anti-Gr-1 (anti-Ly-6G and Ly-6C; clone RB6-8C5; PE-conjugated; BD Biosciences) were diluted 1:50 in flow wash. Antibodies (100 μl) were incubated 30 min at 4 °C. Cells incubated in flow wash only served as autofluorescent controls. Cells were washed two times in flow wash, fixed in 0.5 ml PBS containing 1% paraformaldehyde, and stored in the dark at 4 °C for no longer than 2 weeks before analysis. Fluorescence was analyzed in the University of Rochester Flow Cytometry Core on a BD LSR II 18-Color flow cytometer. Forward scatter and side scatter gating was used to eliminate non-lymphoid cells from the analysis. Analysis gates were set based on the autofluorescent controls.

2.6. Cytokine and norepinephrine determination

For cytokines, tumor homogenates at a concentration of 4% w/v were prepared in RIPA buffer containing protease inhibitors (HALT Protease Inhibitor Cocktail, Thermoscientific; Thermofisher.com). To measure NE, tumors were homogenized at a concentration of 1% w/v in 1 N HCl. Protein in homogenates was measured colorimetrically using a Pierce BCA Protein Assay kit (Thermoscientific). NE and tumor cytokines in the homogenates were measured by ELISA according to the manufacturer's instructions. A NE ELISA kit was purchased from Rocky Mountain Diagnostics. Mouse- and human-specific VEGF and IL-6 Quantikine kits (R and D Systems, Minneapolis, MN) are highly species specific with little or no cross-reactivity detected with the corresponding analyte from other species. As reported by the manufacturer and confirmed in our laboratory, the only (minimal) cross-reactivity detected is 0.2% cross-reactivity of human VEGF in the mouse VEGF ELISA. Serial dilutions of the tumor homogenates were tested to determine the optimal homogenate dilution for each analyte. Absorption was measured at 450 nm using a multiwell plate reader (Synergy HT, Biotek Instruments Inc., Winooski, VT). Curve fitting and sample concentration calculations were conducted with Gen5 software (Biotek). Results were normalized based on protein concentration or tissue wet weight.

2.7. Statistical analysis

Statistically significant differences between groups were determined using GraphPad Prism software. For all analyses, $p < 0.05$ is considered statistically significant. When comparing two groups, *F*-test for equality of variance was used to determine if the variance differed significantly. If variance between the two groups was

equal, Student's *t*-test was used. For non-equal variance, non-parametric Mann–Whitney was used as indicated. To compare more than two groups, a significant main effect by one-way ANOVA was followed by post hoc Newman–Keuls analysis. Tumor volume over time was analyzed using a two-way repeated measures ANOVA, and significant main effects or interactions were analyzed using Bonferroni's post hoc analysis.

3. Results

3.1. Social isolation prior to MB-231 tumor cell injection

We have investigated the impact of stressor exposure in the form of social isolation on orthotopic growth of the human breast tumor cell line, MB-231, in SCID female mice. Mice were separated into single housing one week prior to MB-231 tumor cell injection (2×10^6 cells) into the mammary fat pad. Fig. 1 represents results from three experimental repetitions in which tumor growth was measured over time. In these experimental repetitions, tumor NE, human and mouse VEGF and IL-6 were not consistently altered at the time of sacrifice (day 83 post-MB-231 injection in Fig. 1; data not shown). No difference in lung metastases (the only site of metastasis from the primary MB-231 tumor) was observed between the two groups (data not shown). We postulated that the inability to produce replicable changes in tumor growth was due to the fact that MB-231 is a slow growing tumor *in vivo*, allowing mice to adapt to social isolation. If true, we predicted that social isolation would have a greater impact if transfer to single housing took place closer to the exponential phase of tumor growth.

3.2. Social isolation after MB-231 injection

To test this possibility, SCID mice were injected orthotopically with MB-231 (4×10^6 cells) and tumor growth was monitored over time. One-half of the mice were singly housed when tumors were palpable in all mice (in this experiment, day 14 post-tumor injection; average tumor volume = ~25 mm^3). The other mice remained in their home cages, and tumor growth in all mice was measured over time. MB-231 tumor growth was greater in singly-housed compared to group-housed mice when tumor volume was analyzed through day 28 post-separation (Fig. 2A; repeated measures ANOVA, main effect of housing, $p < 0.03$ with no interaction by time, $p = 0.5$). By day 34 post-separation, the effect of social isolation had dissipated somewhat. When this time point was included in the analysis, the main effect was no longer significant ($p = 0.07$) with no interaction by day post-separation ($p = 0.6$). Mice were sacrificed at this time point (day 34 post-separation). Tumor weight did not differ between groups (data not shown). Interestingly, a trend towards reduced tumor NE in the singly-housed group was noted (Fig. 2B, Mann–Whitney, $p = 0.1$). Tumor human VEGF concentration did not differ between groups (data not shown), but a trend towards increased human IL-6 was noted in tumors from singly-housed mice (Fig. 2C; Mann–Whitney, $p = 0.1$). Mouse IL-6 did not differ between groups (data not shown). No difference in lung metastases was observed between the two groups (data not shown). These results demonstrate that social isolation facilitated tumor progression, but the duration of the effect on tumor growth was limited.

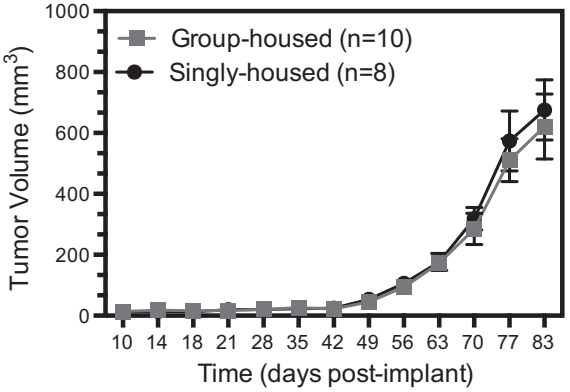

Fig. 1. MB-231 tumor growth is not altered by social isolation prior to tumor cell injection. SCID female mice were singly housed seven days prior to orthotopic injection of MB-231 cells. Tumor diameter was measured with calipers on the days indicated. Tumor volume is expressed as mean ± SEM, $n = 8$–10 mice per group.

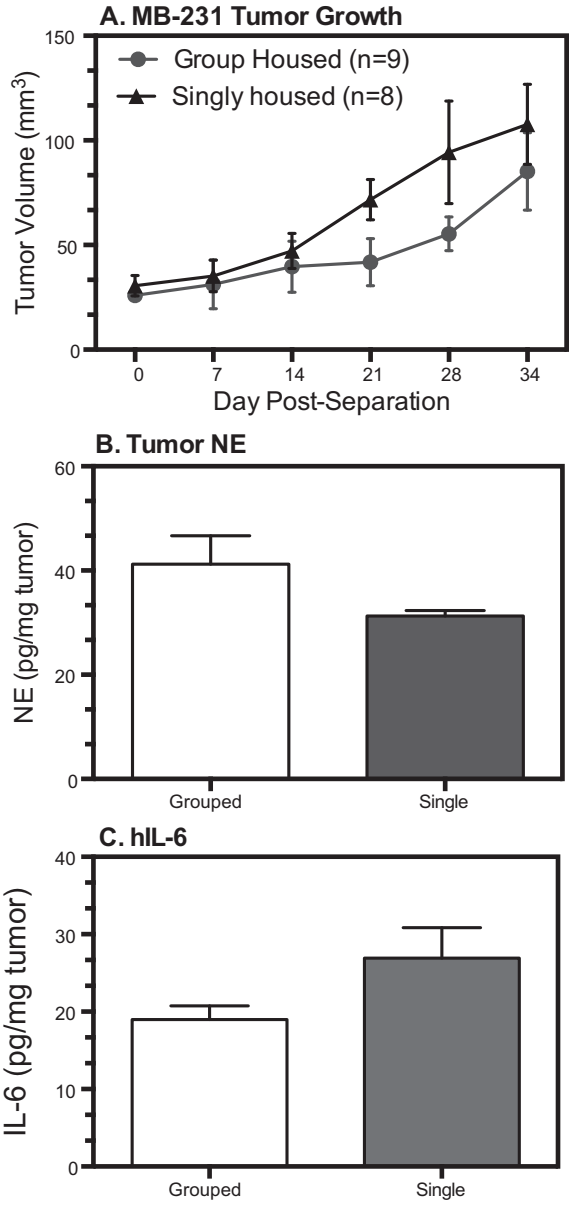

Fig. 2. MB-231 tumor growth is transiently increased by social isolation after tumor injection. SCID female were injected with MB-231 cells, and when all mice had palpable tumors, half of the mice were transferred from group to single housing. (A) Tumor volume over time. NE (B) and human IL-6 (C) were measured by ELISA in tumors harvested at day 34 post separation. Results are expressed as mean ± SEM, $n = 8$–9 mice per group. See text for statistical analysis of tumor growth. For tumor NE and human IL-6, no significant effects based on the non-parametric Mann–Whitney test, $p = 0.1$.

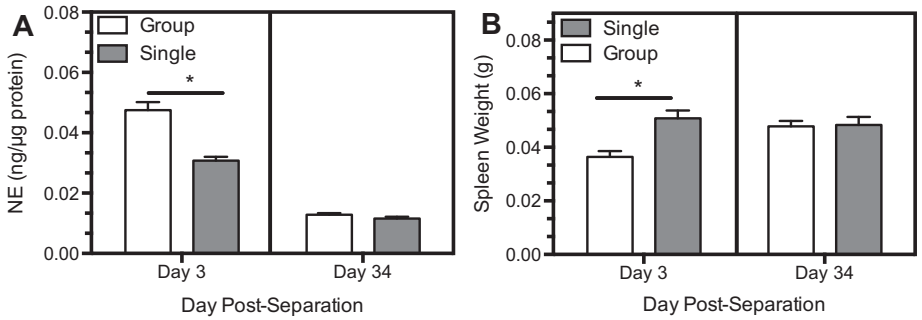

Fig. 3. Social isolation reciprocally alters spleen NE (A) and spleen weight (B) early after social isolation. A subset of mice described in Fig. 2 was sacrificed at day 3 post-separation. Results are expressed as mean ± SEM, $n = 5$ mice per group. Asterisk indicates different versus group-housed at the corresponding time point by Newman–Keuls post hoc analysis.

In this same experiment, a subset of mice was sacrificed day 3 after social isolation to determine if sympathetic activation occurred early after social isolation. At this time point, the tumors ($\leqslant$10 mg in weight) were too small to measure NE; therefore the spleen was used as a highly-innervated surrogate organ to compare NE concentration after social isolation. Day 3 post-separation, splenic NE concentration was significantly reduced in the singly-housed mice (Fig. 3A; ANOVA, housing x day interaction, $p < 0.001$). The decrease in NE concentration was associated with increased spleen weight (Fig. 3B; ANOVA, housing x day interaction, $p = 0.02$), suggesting that the increase in spleen mass reduced NE concentration in the singly housed mice. By day 34 post separation, neither NE concentration nor spleen weight was altered in singly-housed compared to group-housed mice, but compared to day 3, splenic NE concentration was significantly reduced in both groups in conjunction with increased spleen mass (ANOVA, main effect of day, $p < 0.001$).

3.3. Effects of social isolation on increased spleen mass are not dependent on tumor

In the next experiment, to determine if the early effect of social isolation on spleen mass was dependent on the presence of growing tumors, non-tumor bearing SCID female mice were socially isolated. In addition, flow cytometry was used to determine if macrophage populations were altered after social isolation, as reported for other social stressors (Engler et al., 2004). Social isolation increased spleen weight at this time point in association with decreased spleen NE concentration (Fig. 4A and B), similar to the effects at d3 in tumor-bearing mice. The percentage of splenic macrophages expressing F4/80+ and CD11b + Gr-1-cells was not significantly altered (Fig. 4C and E), but the percentage of CD11b+ macrophages that co-express Gr-1+ were significantly increased in socially isolated mice. These cells are myeloid derived suppressor cells that have potent immunosuppressive capabilities (Gabrilovich and Nagaraj, 2009). These results demonstrate that the early effect of social isolation in the spleen is independent of tumor growth, and that specific splenic macrophage populations are sensitive to neurohormonal changes elicited by social isolation. Since these macrophage populations are important regulators of tumor progression, we tested if macrophage populations are altered in spleens and tumors of socially isolated mice.

3.4. Social isolation alters spleen and tumor macrophage populations

The next experiment examined alterations in spleen and tumor NE concentration and macrophage populations 10 days after social isolation in mice bearing tumors. Social isolation was initiated when average tumor volume was ~50 mm^3. Social isolation did not alter spleen or tumor weight or NE concentration 10 days after

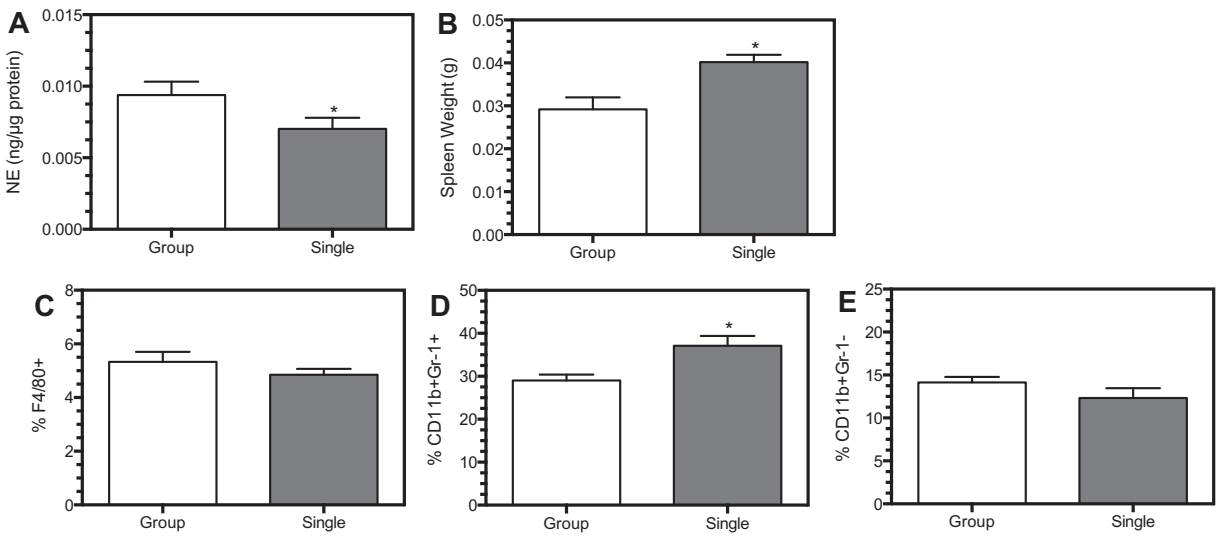

Fig. 4. Early effects of social isolation on spleen NE and spleen weight is independent of tumor growth. Three days after transfer to single housing, SCID female mice were sacrificed and spleen NE (A), weight (B), and macrophage populations (C–E) were determined. Asterisk indicates significant difference by Student's t-test, $p < 0.05$. Results are expressed as mean ± SEM, $n = 5$ mice per group.

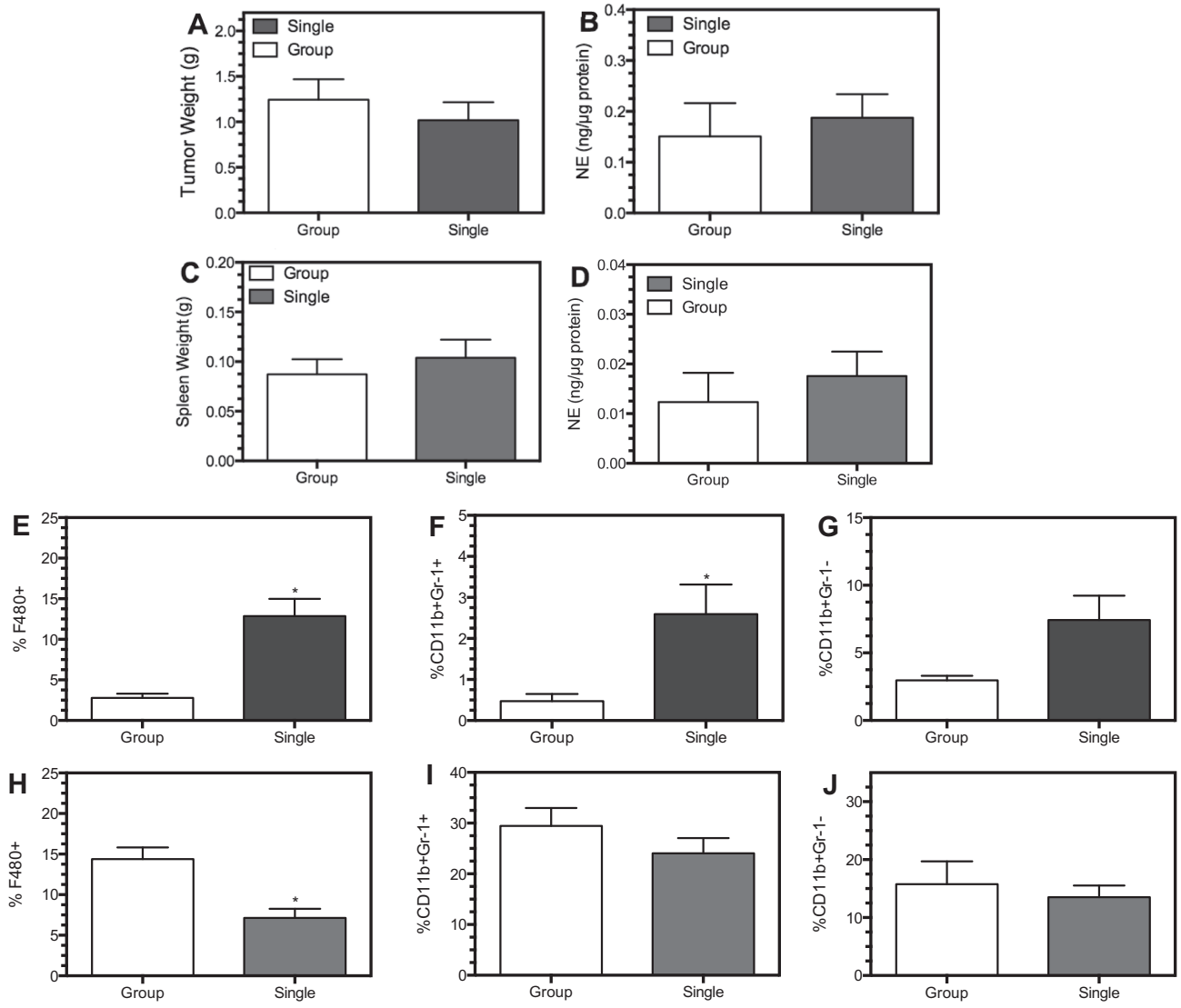

Fig. 5. Social isolation alters tumor and spleen macrophage populations. SCID female mice were injected with MB-231 in the mammary fat pad. When tumors were palpable, one-half the mice were transferred from group-housing to single housing. After 10 days, mice were sacrificed and tumor (A and B) and spleen (C and D) weight and NE concentration were determined, and macrophage populations were analyzed by flow cytometry in tumors (E–G) and spleens (H–J). Asterisk indicates significance based on the non-parametric Mann–Whitney test (A and B) or by student's *t*-test (D), $p < 0.05$. In (C), $p = 0.067$. The results are expressed as mean ± SEM of 9–10 mice per group.

social isolation (Fig. 5A–D). However, macrophage populations were increased in tumors from socially isolated mice with significant increases in the percentage of F4/80+ (Fig. 5E) and CD11b + Gr-1+ populations (Fig. 5F) and a trend toward an increase in the CD11b + Gr-1- population (Fig. 5G). In the spleen, the percentage of F480+ macrophages was reduced (Fig. 5H) with no significant changes in either CD11b+ population (Fig. 5I and J). These results demonstrate distinct changes in tumor and spleen macrophage populations with social isolation.

4. Discussion

Social isolation is a well-characterized social stressor that has several advantages to more standard laboratory-type stressors. It is a milder form of stress compared to other forms of stressor exposure. For example, in our hands singly-housed mice do not lose weight (data not shown) nor display elevated tissue NE concentration, in contrast to the weight loss (Sloan et al., 2010) and increased tissue NE concentration (Thaker et al., 2006) reported with daily restraint stress. Yet animals exposed to social isolation mimic behavioral anxiety and increased vigilance associated with social isolation observed in humans (Hermes et al., 2009; Williams et al., 2009). Here, we demonstrated that social isolation transiently increased MB-231 tumor growth, but only when social isolation was initiated when tumor growth was near exponential phase. No consistent effects of tumor growth or metastasis were observed when social isolation occurred prior to MB-231 injection, suggesting a temporal dependence in the context of a mild stressor. Indeed, early and transient changes in spleen NE concentration and macrophage populations, independent of whether or not the animals were tumor-bearing, were observed. Furthermore, increases in tumor macrophage populations took place before any indications of altered tumor growth. The changes in macrophage populations in the tumors were not associated with altered tumor NE. These results suggest that social isolation can have an impact on tumor progression, but the impact is transient and may not be associated with dramatic changes in tumor growth and metastasis.

The changes in macrophage populations in tumor and spleen indicate that social isolation facilitates leukocyte recruitment – a process described with another social stressor, social disruption

(Engler et al., 2004). Repeated social disruption increased spleen weight in concert with loss of CD11b+ myeloid cells from the bone marrow and an increase in CD11b+ cells in the spleen. Furthermore, the increase in spleen weight elicited by social disruption was mediated through β-AR stimulation, as it was prevented by propranolol pretreatment to block β-AR (Wohleb et al., 2011). The increase in the CD11b + Gr-1+ population in spleen from non-tumor bearing, singly-housed mice shown here (Fig. 4), suggests that this process also occurs with social isolation. A similar stress-induced increase in tumor macrophages has been described by Sloan and colleagues, who demonstrated elevated F4/80+ tumor macrophages and a trend toward increased myeloid derived suppressor cells with restraint stress; this effect was blocked by propranolol treatment (Sloan et al., 2010). Restraint stress did not facilitate primary tumor growth, but increased tumor angiogenesis and dramatically elevated metastasis (Sloan et al., 2010). In our hands, tumor associated macrophage populations expressing F4/80 and CD11b were increased with social isolation. The spleen is an important source of tumor associated macrophages and tumor associated neutrophils (Cortez-Retamozo et al., 2012). The social isolation-induced decrease in the splenic F4/80+ population in conjunction with an increase in this population in tumors suggests that the spleen may contribute to the increased F4/80+ tumor associated macrophages in socially isolated mice. It is likely that both the spleen and bone marrow may be targets of stress hormones that promote the migration of these macrophage populations into the tumor. Both tumor associated macrophages and myeloid derived suppressor cell populations are associated with tumor progression (Gabrilovich and Nagaraj, 2009), but we have yet to establish that the increased tumor macrophages lead to the increased tumor growth in the singly-housed mice.

Social isolation has been characterized as a stressor based on behavior (it elicits anxiety behaviors in female mice) (Palanza et al., 2001), but is less well characterized in terms of hypothalamic pituitary axis or sympathetic nervous system activation. Long-term social isolation increased development of spontaneous mammary tumors and metastasis, but these rats were socially isolated from puberty (Hermes et al., 2009), making it difficult to directly compare to the social isolation procedure here, which was begun when the mice had reached adulthood. Nonetheless, social isolation led to reduced baseline levels of plasma corticosterone at the nadir of the diurnal rhythm in social isolated rats, but an elevated and prolonged corticosterone response to a stressor (Hermes et al., 2009; Williams et al., 2009). Similarly, a 21-day period of single housing did not alter baseline plasma catecholamines, but upon exposure to an acute stressor, plasma catecholamines in socially isolated rats were significantly elevated versus group-housed (Dronjak et al., 2004). Therefore, the social isolation model will be particularly useful for examining the impact of an acute stressor in animals exposed to long-term social isolation. The results presented here suggest that social isolation alone may elicit alterations that have a transient impact. Future plans include using social isolation to understand the biological consequences of multiple stressors on tumor pathogenesis, a more likely scenario in the context of a diagnosis of breast cancer.

The effects of social isolation were not associated with increased NE concentrations in spleen or in tumor, as might be expected if social isolation activated the SNS. However, there are a few caveats in interpreting tissue NE measures. First, NE concentration in the spleen appeared to fluctuate with changes in spleen mass. One way to interpret this finding is that sympathetic nerve fibers within the spleen do not respond rapidly to a rapid expansion in tissue volume, such as the increase in spleen weight with social isolation or even in a growing tumor. We can detect sympathetic nerve fibers in MB-231 tumors independent of tumor size, however the impact of the expanding tumor architecture on NE concentration has not been systematically examined. Furthermore, measuring only tissue NE may not be an appropriate measure of SNS activation, especially under conditions of a relatively mild stressor such as social isolation where homeostatic mechanisms serve to maintain a constant tissue NE baseline (Eisenhofer et al., 2004)). Therefore, we have begun to assess normetanephrine, a product of NE metabolism by catechol-*O*-methyltransferase, as a potential additional measure of sympathetic activation and released NE. These experiments will help define the role of SNS activation and β-AR stimulation in the context of social isolation.

The results presented here demonstrate an early, but transient effect of a psychological stressor, social isolation, in both tumor-bearing and normal female SCID mice. The results imply that exposures to relatively mild stressors may promote tumor progression, depending on the timing relative to tumor growth, but also suggest the possibility that homeostatic mechanisms can mitigate the impact of social isolation. This is a potential area of investigation in terms of identifying pathways that help minimize the impact of chronic stress experienced in breast cancer patients. It is critical to understand how mild stressors interact to develop into a more severe stressor and to develop therapies that work in concert with standard breast cancer therapies to inhibit tumor progression.

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Conflict of Interest

The authors of this manuscript have nothing to declare.

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The effect of pre-transplant distress on immune reconstitution among adult autologous hematopoietic cell transplantation patients

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ABSTRACT

Myeloablative hematopoietic cell transplantation (HCT) is a common treatment for hematological malignancy. Delayed immune reconstitution following HCT is a major impediment to recovery with patients being most vulnerable during the first month after transplant. HCT is a highly stressful process. Because psychological distress has been associated with down regulation of immune function we examined the effect of pre-transplant distress on white blood cell (WBC) count among 70 adult autologous HCT patients during the first 3 weeks after transplant. The participants were on average 38 years old; 93% Caucasian, non-Hispanic and 55% male. Pre-transplant distress was measured 2–14 days before admission using the Cancer and Treatment Distress (CTXD) scale, and the Symptom Checklist-90-R (SCL-90-R) anxiety and depression subscales. WBC count was measured during initial immune recovery on days 5 through 22 post-transplant. Linear mixed model regression analyses controlling for gender and treatment-related variables revealed a significant effect of the mean pre-transplant SCL Anxiety-Depression score on WBC recovery. We found no significant effect of pre-transplant CTXD on WBC recovery. In general, higher levels of pre-treatment anxiety and depression were associated with slower WBC recovery. Psychological modulation of WBC recovery during HCT suggests a unique mechanism by which psychological distress can exert influence over the immune system. Given that WBC recovery is essential to survival for HCT patients, these data provide a rationale for treating anxiety and depression in HCT patients.

1. Introduction

Myeloablative hematopoietic cell transplantation (HCT) is a common treatment for leukemia, lymphoma and other hematological malignancies, and is also used to treat non-malignant disease such as aplastic anemia. Over 15,000 allogeneic and 30,000 autologous HCT occur each year worldwide (Copelan, 2006). The process, which was first introduced in the late 1960s, has improved to the point that 5-year survival rates can range from 80% to below 20% depending on the type of transplant and diagnosis (Copelan, 2006). But the treatment is demanding both physically (Copelan, 2006) and emotionally (Andrykowski et al., 2005; Fann et al., 2007; Hoodin et al., 2006; Syrjala et al., 2004).

During the autologous HCT process, the patient first has their own hematopoietic stem cells harvested and stored. Patients then receive supra-lethal doses of chemotherapy, sometimes combined with total body irradiation, which results in eradication of the immune system. The patient is then "rescued" from certain death with infusion of their own stored stem cells. After stem cell infusion, 10–30 days are required for the patient's circulating white blood cell (WBC) counts to rise from close to zero at the time of transplant to levels adequate to begin to protect them from acute symptoms and infection vulnerability. Immune reconstitution continues during a 3-month period with return of innate immunity followed later by the return of specific immunity. Complete immune recovery can take many months (Peggs and Mackinnon, 2004).

Transplant-related immune deficiency results in significant morbidity and mortality among HCT patients (Peggs and Mackinnon, 2004), and patients are particularly vulnerable to infection during the first weeks after transplant when WBC counts are low (Storek et al., 2003). More rapid return of immune function has been associated with fewer post-transplant side effects and better overall and disease-free (Porrata and Markovic, 2004; Auletta and Lazarus, 2005; Porrata et al., 2008).

Abbreviations: HCT, hematopoietic cell transplantation Acronyms; HCT, hematopoietic cell transplantation; WBC, white blood cell; SNS, sympathetic nervous system; HPA, hypothalamic–pituitary–adrenal; HSC, hematopoietic stem cells; CTXD, Cancer and Treatment Distress; SCL-90-R, Symptom-Checklist-90-Revised; SCL-A, SCL-90-R anxiety subscale; SCL-D, SCL-90-R depression subscale.

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The HCT process also takes an emotional toll on patients. Patients arrive at transplant with differing diseases, histories of treatment, levels of physical function, and psychological, social, informational, and financial resources. All of these factors can contribute to the distress an individual experiences before, during, and after transplant. Psychosocial predictors of outcome have been studied extensively, with clear, replicated findings that pre-transplant physical and psychological function predict post-transplant physical and psychological outcomes (Andorsky et al., 2006; Lee et al., 2001; Syrjala et al., 2004). Pre-transplant treatment-related distress, in particular, predicts acute post-transplant symptom course, especially for pain and general distress (Schulz-Kindermann et al., 2002; Syrjala and Chapko, 1995). Psychosocial needs are greatest just prior to transplant if judged by the percent of patients with clinically significant anxiety or depression at different phases of treatment and recovery (Broers et al., 2000; Fife et al., 2000; Syrjala et al., 2004). Studies also document that pre-transplant psychological status predicts survival (Andrykowski et al., 1994; Loberiza et al., 2002; Prieto et al., 2005), although the mechanisms through which increased mortality occurs remain largely unexamined.

There is a large body of literature documenting that psychological distress can impair immune function, including innate and specific immunity (e.g., Burns et al., 2003; McEwen, 1998; Yin et al., 2000). Biological mediators of the effect of psychological distress on immune function include cortisol, via activation of the hypothalamic pituitary adrenal (HPA) axis, and catecholamines and neuropeptides via activation of the sympathetic nervous system (SNS) (McEwen, 1998). There is also a literature to support the hypothesis that psychological distress could influence immune recovery post-HCT.

Hematopoietic stem cells (HSCs), which ultimately mature to form all the different cells found in peripheral blood, reside in specific niches in the bone marrow. In this specialized environment, tight control of proliferation and differentiation is accomplished through a variety of physical (cell-cell) and molecular pathways (Adams and Scadden, 2006). It has long been known that bone marrow and other immune system organs are highly innervated by the SNS (Afan et al., 1997; Bellinger et al., 1992; Ehninger and Trumpp, 2011; Felten et al., 1985; Felten and Felten, 1994; Yamazaki et al., 2011), and that this innervation has functional significance (Broome and Miyan, 2000; Kohm et al., 2000; Maestroni, 2000; Mendez-Ferrer et al., 2008; Steidl et al., 2004). For example, SNS innervation of bone marrow happens immediately before the onset of hematopoiesis in utero (Afan et al., 1997), and growth of early hematopoietic progenitor cells is impaired in patients with spinal cord injury who thus have interrupted transmission of SNS activity below the site of the injury (Iversen et al., 2000). There are now animal model data that show HSCs respond to stressors occurring at the organismal level via adrenergic signals originating from the SNS. Katayama et al. showed that signals from the SNS control the attraction of stem cells to their niche in the bone marrow (Katayama et al., 2006). Taken together, these data provide strong evidence that alterations in SNS tone (e.g., in response to a stressor) may influence proliferation and mobilization of hematopoietic stem cells.

Given the importance of immune reconstitution for HCT patients and the stressful nature of the HCT process, we wondered if psychological distress, with its well-known influences on SNS tone, might be associated with changes in WBC recovery after HCT. If psychological distress is associated with slower WBC recovery, it would strengthen the rationale for using psychological intervention prior to HCT to reduce distress and improve return of immune competence among survivors.

The purpose of the present study was to examine the relationship between psychological distress and WBC recovery in the first weeks after autologous HCT. We hypothesized that elevated psychological distress prior to the transplant would be associated with slower rates of WBC recovery.

2. Method

2.1. Participants

The present study is a secondary analysis of data from a cohort of 70 autologous patients. Demographic and treatment characteristics of the sample are presented in Table 1. The patients were from a larger sample of 405 allogeneic and autologous patients enrolled in a prospective, longitudinal study of HCT survivorship between April, 1987 and January, 1990. To be included in the study, patients had to be at least 18 years old and about to receive a first HCT (Syrjala et al., 2005). The study and all study materials were approved by

Table 1
Demographic and clinical characteristics.

Pre-transplant age (N = 70)		
Mean ± SD	37.66 ± 11.60	
Range	18–62	
	Frequency (N = 70)	Proportion
Gender		
Female	32	45.71
Male	38	54.29
Ethnicity/race		
Caucasian, non-hispanic	69	98.6
Hispanic	1	1.4
Education		
Less than high school	3	4.6
High school or GED	22	33.8
Some college, trade school, Completed college	28	43.1
Graduate degree	12	18.46
Marital status		
Single	15	22.4
Married or cohabitating	45	67.2
Separated/divorced/widowed	7	10.5
Disease status at transplant		
Relapse	38	54.3
Remission	32	45.7
Diagnosis		
Lymphoma	28	40
Acute leukemia	28	40
Hodgkin disease	8	11.4
Other	6	8.6
TBI status		
Yes	51	72.9
Mean total dose (Gy) ± SD	9.31 ± 6.03	
Range	6.00–19.60	
Stem cell dose (10^6 CD34 + cells/kg body weight)		
Mean ± SD	3.07 ± 1.69	
Range	0.35–9.22	
GM-CSF post-transplant (yes)	2	3
Corticosteroids post-transplant (yes)	44	63
Mean ± SD days corticosteroid	9.95 ± 5.42	
Range days corticosteroid tx	1–17	
Any infection day 5-day 21 (yes)	48	76.2
Pre-treatment distress		
Low (0.0–0.76)	8	12.90
Medium (0.77–1.81)	43	69.35
High ($\geqslant$1.82)	11	17.74
Pre-treatment anxiety		
Low (0.0–0.34)	24	34.29
Medium(0.35–0.95)	32	45.71
High ($\geqslant$0.96)	14	20.00
Pre-treatment depression		
Low (0.0–1.00)	45	64.29
Medium (1.01–1.73)	18	25.71
High ($\geqslant$1.74)	7	10.00

the FHCRC IRB. The patient sample for the present study was limited to autologous HCT patients because autologous patients do not receive immunosuppressive medications for graft versus host disease prophylaxis. All patient stem cells were procured from bone marrow harvest. After bone marrow harvest, the patients received conditioning regimen of Cyclophosphamide and TBI ($n = 36$), Cyclophosphamide, Busulfan and TBI ($n = 12$), Cyclophosphamide and Busulfan ($n = 9$), or other chemotherapy and TBI ($n = 3$) or other chemotherapy without TBI ($n = 4$) prior to transplant.

2.2. Self-report measures

All self-report measures, described below, were completed between 2 and 14 days before transplant at the transplant clinic. Assessment was scheduled as rapidly after arrival as possible, and within a week of arrival unless medical events interceded.

Cancer and treatment-related distress was measured with the Cancer and Treatment Distress (CTXD) scale. The CTXD scale is a 27-item measure of treatment-related distress specific to the HCT process. Items such as "nausea and vomiting", "possibility of relapse", and "being a burden to other people" are rated for the extent to which they cause distress or worry to the patient, regardless of whether they have actually occurred. Ratings are made on a 4-point scale from 0 ("no distress) to 3 ("severe distress") and a mean score for the 27 items is computed (Syrjala et al., 2004). This measure has been shown to detect unique treatment-related distress and to predict pain better than measures of generalized anxiety or depression (Schulz-Kindermann et al., 2002; Syrjala and Chapko, 1995). The scale has good internal consistency with alphas ranging from .85 to .91 (Schulz-Kindermann et al., 2002; Syrjala and Chapko, 1995; Syrjala et al., 2004). The alpha in the present sample was .87.

Anxiety and depression were assessed using the anxiety and depression subscales of the Symptom Checklist 90-Revised (SCL-90-R). The SCL-90-R anxiety subscale (SCL-A) is comprised of 10 items such as "nervousness" or "shakiness inside". The depression subscale (SCL-D) is comprised of 13 items such as "feeling blue" or "crying easily". Participants are asked to indicate how much a problem has "distressed or bothered you during the past 7 days including today". Items are rated using a scale from 0 ("not at all") to 4 ("extremely"). Subscale scores are computed as the mean of subscale item scores, thus the scores could range from 0 to 4. The SCL-A and SCL-D are highly reliable, valid, and widely utilized in psychiatric studies (Derogatis et al., 1973). For the SCL-D, a cut-off score of 1.72 has been documented as having the highest positive predictive value for diagnosing major depression (Mulrow et al., 1995). Means and standard deviations from a community normative sample for the SCL-A are .30 (.37) and for the SCL-D are .36 (.44) (Derogatis, 1994). Internal consistencies range from .85 to .90, and test–retest reliabilities range from .68 to .86 (Derogatis and Cleary, 1977). The alpha for the SCL-A in the present sample was .89. The alpha for the SCL-D in the present sample was .86.

2.3. White blood cell (WBC) counts

Complete differential blood cell counts are performed every day by a CLIA-certified laboratory during the inpatient period per the inpatient clinical protocol. Study patient total WBC counts were obtained from the research medical record database for days 5–22 of the post-transplant period as most patients have initial immune recovery by day 22 and counts before day 5 are near zero. All WBC values are the number of cells per microliter (cells/mcL) of blood.

2.4. Statistical analysis

Because of the strong correlation between pre-transplant anxiety and depression, a composite variable was created by taking the mean of the anxiety and depression scale scores for each patient. In order to control for variables that we identified, a priori, to be important predictors of WBC count, we evaluated age, sex, pre-transplant medical risk stats, stem cell dose, total body irradiation (TBI) dose, the presence of infection, if corticosteroids were given, and cytomegalovirus virus (CMV) status (positive or negative) as potential covariates for the models. As we have done in previous studies, pre-transplant medical risk categories were assigned as either low or high depending upon the patient's diagnosis and remission/relapse status (Syrjala et al., 2004). For the resultant distress model we included: age, pre-transplant medical risk status, if corticosteroids were given, and CMV status. For the resultant anxiety-depression composite models we included sex, CMV status, pre-transplant medical risk status, stem cell dose, total body irradiation (TBI) dose, and if corticosteroids were given. These covariates were chosen using the Wald test to compare with a model containing no covariates. All sets of covariates significantly improved the model at the $p < 0.05$ level. To account for the expected nonlinear (quadratic) trend in the WBC count over time, all models included time, measured continuously by day and day squared, as a predictor. The models also included interactions of the predictors (pre-transplant treatment-related distress, anxiety, and depression) with time measured by day and day squared. All mixed effects regression analyses were conducted using continuous predictors with and without covariates.

A series of two sets of linear mixed model regression analyses were conducted to investigate the effects of pre-HCT treatment-related distress and an anxiety-depression composite predictor on change in WBC counts over days 5 through 22 post-HCT, using *xtmixed* (Stata SE 10.0 for Windows, Stata Corporation, College Station, TX). In an effort to estimate as few parameters as possible we used an exchangeable covariance structure for the two models. This a priori choice assumes that each pair is equally correlated. Because of the short time duration, we assume that observations are similarly correlated over time. We rejected the use of an unstructured covariance structure as it estimates many parameters and reduces the power of our models. We chose not to pursue an autoregressive covariance structure because exponential decay is unlikely given the short time duration and influence of other factors on WBC recovery. We used mixed model methodology to adjust for the correlation of repeated measures, to allow for incomplete measurement, and to include covariates. Analyses began with day 5 post-transplant since a nadir in WBC count continued from day 0 to day 5. The number of days post-transplant was added as a random effect that varied by patient. This method provides a more accurate estimate of error variability.

For the purposes of illustrating the statistically significant model (Fig. 1), categorical variables were created for the anxiety-depression composite score using clinically meaningful cut points to plot WBC curves over time. The SCL-A and SCL-D have published population norm cut scores (Derogatis et al., 1973). Pre-transplant anxiety can be defined as: low (an SCL-A score of 0.0–0.34), medium (a score between 0.35 and 0.95), and high (a score $\geqslant 0.96$). Pre-transplant depression can be defined categorically as: low (an SCL-D score of 0.0–1.00), medium (a score between 1.01 and 1.73), and high (a score $\geqslant 1.74$). In order to create cut scores for the composite, we averaged the anxiety and depression cut scores together, and so defined the categories as: low (0.0–0.67), medium (0.68–1.34) and high ($\geqslant 1.35$).

3. Results

Means for pre-transplant treatment-related distress, anxiety, and depression by diagnosis group are shown in Table 2. Treatment-related distress, anxiety, and depression scores did not differ

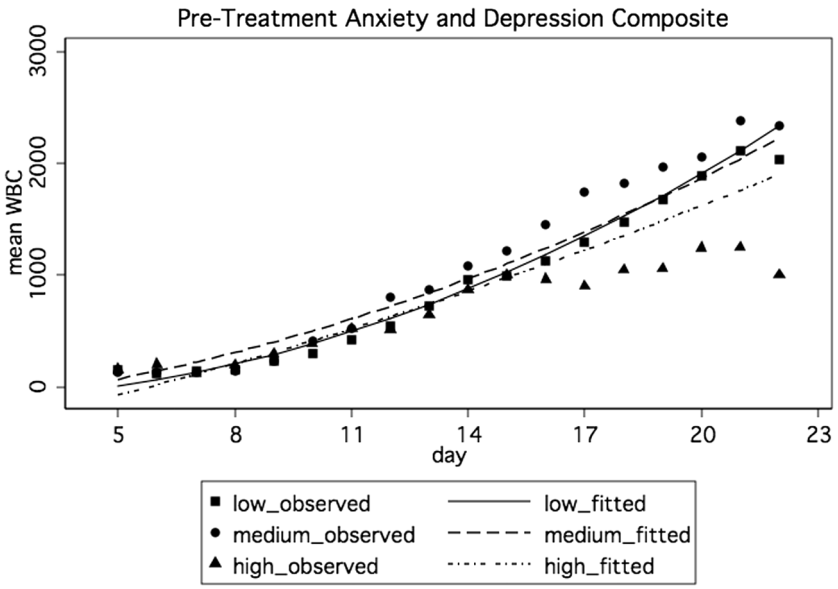

Fig. 1. Mean predicted WBC by day and pre-transplant anxiety and depression composite. The unadjusted means are also plotted for each group as a reference.

by length of time between assessment and the start of treatment measured in days.

3.1. Associations among independent variables

While the correlation between pre-transplant anxiety and depression was 0.61 ($n = 70$, $p < .001$), the correlations of pre-transplant treatment-related distress with anxiety and depression were 0.37 ($n = 62$, $p = .003$) and 0.33 ($n = 62$, $p = .008$), respectively. When treatment-related distress, anxiety and depression were entered as continuous variables in one model predicting WBC count over time, none emerged as significant predictors. For this reason and to increase parsimony, we created a composite variable by creating a mean of the SCL-Anxiety and SCL-Depression scores. We then created mixed-effects regression models for the treatment-related distress and the SCL anxiety-depression composite.

3.2. Results of mixed effects regression

3.2.1. Treatment-related distress

The mixed-effects regression model showing how pre-transplant treatment-related distress, as a continuous variable, was associated with change in WBC count over day 5 through day 22 after transplant revealed no significant interactions between treatment-related distress and time or time squared using a Wald test of significance for both terms simultaneously (p = ns). There were significant effects of age ($\beta = 12.23$, $p < .01$), corticosteroid given ($\beta = 25.91$, $p < .01$) and CMV status ($\beta = 180.47$, $p < .05$). This indicated that the change in WBC count over time was not different for different levels of treatment-related distress. Patients with higher treatment-related distress improved as fast as those with lower pre-treatment treatment-related distress.

3.2.2. SCL-anxiety and depression composite variable

The mixed-effects regression model showing how the pre-transplant anxiety-depression composite, as a continuous variable was associated with change in WBC count over day 5 through day 22 after transplant with and without treatment covariates are shown in Tables 3 and 3a. There was no significant interaction between the anxiety-depression composite variable and time in the models with or without covariates. However, there was a

Table 2
Means, standard deviations of distress, anxiety, and depression by diagnostic group.

	Distress (CTXD) Mean ± SD (n)	Anxiety (SCL-A) Mean ± SD (n)	Depression (SCL-D) Mean ± SD (n)
Lymphoma	2.17 ± 0.60 (25)	0.66 ± 0.57 (28)	0.91 ± 0.53 (28)
Acute Leukemia	2.17 ± 0.51 (22)	0.65 ± 0.67 (28)	0.72 ± 0.67 (28)
Hodgkin	2.32 ± 0.53 (7)	0.48 ± 0.51 (8)	0.82 ± 0.69 (8)
Other	2.06 ± 0.23 (4)	0.98 ± 0.92 (6)	0.88 ± 0.64 (6)

Table 3
Results of mixed effects regression model for pre-transplant anxiety and depression composite predicting WBC count.

Variable	Estimate	Std error	p-Value
Day	−25.55	32.25	0.43
Day squared	6.07	1.08	0.00
Depression	−251.06	195.03	0.20
Pre-depression by day	56.64	35.42	0.09
Pre-depression by day squared	−2.80	1.21	0.02
Constant	17.86	179.12	0.92

Table 3a
Results of mixed effects regression model for pre-transplant anxiety and depression composite predicting WBC count with treatment variable covariates.

Variable	Estimate	Std error	p-Value
Day	−19.39	32.98	0.56
Day squared	5.85	1.08	0.00
Composite	−296.27	197.71	0.13
Composite by day	50.92	36.49	0.16
Composite by day squared	−2.36	1.22	0.05
Sex	−284.13	82.28	0.001
Risk	−384.22	136.78	0.01
Stem cell dose	−73.14	24.24	0.003
TBI[1] dose	−15.20	6.86	0.03
Corticosteroid	21.95	6.29	0.000
CMV[2]	274.92	83.13	0.001
Constant	670.49	275.66	0.02

[1] Total body irradiation (TBI).
[2] Cytomegalovirus virus (CMV) status (positive or negative).

significant interaction between the anxiety-depression composite variable and time squared ($\beta = -2.80$, $p = .02$) which declined slightly but remained significant after adding treatment covariates to the model ($\beta = -2.36$, $p = .05$). All of the covariates entered into the model were significant terms. In general, higher scores on the anxiety-depression composite were associated with slower change in WBC count over time.

4. Discussion

The present study sought to investigate the effects of pre-transplant self-reported treatment-related distress, anxiety, and depression symptoms on WBC counts among 70 adult autologous HCT patients during the first 3 weeks after their transplant. The immune recovery curves did not generally differentiate until after day 11, when the WBC counts began to rise. Pre-transplant anxiety-depression significantly predicted the pace of WBC recovery. In general, patients who reported higher levels of pre-transplant anxiety and depression had slower recovery of WBC counts compared to patients reporting lower levels of pre-transplant anxiety and depression.

We did not see the same association with pre-transplant reports of treatment-related distress. When treatment-related distress was used as a continuous predictor in the analyses, the interaction between time or time squared and treatment-related distress was not significant. While treatment-related distress, anxiety, and depression symptoms do tend to co-occur, the treatment-related distress variable is qualitatively different from the composite anxiety and depression variable, which was measured with a standardized instrument for the general population. While the anxiety and depression items of the SCL-90 measure clinical symptoms of anxiety and depression that patients bring to the transplant setting with them, the treatment-related distress instrument captures a distress response to an *impending* (but not current) threat, the HCT and all the possible treatment-related threats (e.g., mucositis, nausea and vomiting, hair loss, death). One reason the pre-transplant measure of treatment-related distress did not predict WBC recovery could be because the treatment-related distress instrument was measuring distress about something that had not occurred yet. However, in other HCT studies with both autologous and allogeneic transplant patients, pre-transplant treatment-related distress was a better predictor of pain and general distress during the first month of HCT than mood as measured with standardized measures (Schulz-Kindermann et al., 2002; Syrjala and Chapko, 1995). Perhaps the standardized anxiety and depression measures are capturing a construct that is a better predictor of biological functioning (i.e., WBC count) than the treatment-related distress measure. Also, the patients in the present study were all autologous and thus less vulnerable than allogeneic patients to many of the treatment-related threats assessed in the treatment-related distress measure (e.g. GVHD).

Overall, the results in these models indicate that pre-transplant anxiety and depression (general psychological distress), but not treatment-specific distress is associated with the pace of immune recovery in HCT patients, even after adding treatment-related covariates to the models. Higher levels of anxiety and depression are consistently associated with a slower WBC recovery.

These results, while the first to document such effects in the HCT setting, are similar to studies that have found high levels of psychological distress (including anxiety and depression) associated with general down regulation of immune function in humans (Kiecolt-Glaser et al., 2002; Segerstrom and Miller, 2004). Animal studies have shown that sympathetic innervation of the bone marrow modulates proliferation and mobilization of HSC in the bone marrow microenvironment (Katayama et al., 2006). For example, Katayama et al. (2006) showed that signals from the SNS suppress osteoblast function and control the attraction of stem cells to their niche in the bone marrow. Taken together, these data provide strong evidence that alterations in SNS tone (e.g., in response to a stressor) may influence proliferation and mobilization of hematopoietic stem cells (HSC).

The present data may have important clinical implications for HCT patients. The period of immune deficiency in the weeks after transplant, and its related morbidity and mortality are a critical time in the HCT process. Patients experience increased vulnerability to infection and usually need to be hospitalized during this phase of treatment. The period of immune deficiency immediately after transplant is also associated with chronic diarrhea, stomach upset, and severe mouth pain secondary to mucositis. Supportive therapies such as administration of exogenous immune growth factors have been developed to reduce this vulnerable time as much as possible. The present data suggest that reducing pre-transplant anxiety and depression could also contribute to reducing morbidity related to immune deficiency during this early period after transplant. If the models are replicated, and if the trends for pace of immune reconstitution continue, at day 90, the difference between WBC count in a patient with low anxiety and depression and high anxiety and depression could be much greater. In the present study, most patients were discharged by day 22 and WBC counts were not collected daily. Future work should evaluate the effect of pre-transplant depressive symptoms and anxiety on infection rates, days of re-hospitalization, and WBC subset counts from day 5 post transplant to beyond day 22.

The relationship between pre-transplant depression symptoms and anxiety and recovery of WBC count is provocative for another reason. A number of studies have reported that pre-transplant depression is associated with decreased survival among HCT patients (e.g. Andrykowski et al., 1994; Grulke et al., 2008; Loberiza et al., 2002). Furthermore, pre-transplant depression was not associated with other clinical predictors, suggesting depressed mood pre-transplant was not due to clinical severity (Grulke et al., 2008). While some papers have not found this association, a recent review reported that the majority of prospective studies using reliable measures, with adequate sample sizes and robust statistical methods to control for medical and treatment confounds, did find a significant relationship between high levels of depressive symptoms and increased mortality (Hoodin et al., 2006). We did not test for survival advantages in the present sample because we felt the power was insufficient. But a more speedy return of immune function has been associated with better overall and disease-free survival in other studies (Porrata and Markovic, 2004). In fact, an absolute lymphocyte count of greater than 500 at 15 days post autologous transplant has been associated with lower recurrence rates and longer survival (Auletta and Lazarus, 2005; Porrata et al., 2008; Porrata and Markovic, 2004). While the dependent variable in the present study was total WBC count, not absolute lymphocyte count, this helps put the present data into perspective. Future work should evaluate the effects of psychological functioning on recovery of WBC subsets, such as lymphocytes, neutrophils, etc.

The present secondary data analysis study has both limitations and strengths. Limitations of the present study include the age of the data. There have been major changes in HCT procedures since the present data were collected and with these changes have come improvements in immune recovery and survival. Thus, the findings from the present study cannot be generalized to current autologous HCT patients. We conducted the study with autologous patients to limit the confounding effect of immunosuppressive therapy given to allogeneic transplant recipients. Thus, the current findings cannot be generalized to allogeneic patients. Furthermore, WBC count is a fairly non-specific indicator of immune recovery.

Future work should focus on recovery of leukocyte subsets in patients being treated with current regimens. Furthermore, it is not clear in the autologous setting whether the patient's pre-transplant anxiety and depression is directly affecting the bone marrow micro-environment before harvesting or after infusion, or whether pre-transplant distress adversely affects the hematopoietic stem cell graft itself. It would also be valuable to study the effects of anxiety and depression on immune reconstitution through the later phases of treatment. Further research is needed to determine whether the course of anxiety and depression through acute treatment and long-term follow-up continues to influence immune function, morbidity and mortality. Other potential modulators of mood and WBC count that could not be assessed in the present study include data on tricyclic anti-depressant use and data on nuetropenic fever during the post-transplant period. It is also not clear from the present study what relative role the SNS and HPA axis play in mediating the observed relationships. Strengths of the present study include the longitudinal design, the relatively homogeneous (i.e., all autologous) sample, the use of well-validated measures and sophisticated statistical methods.

In conclusion, this is the first study, to our knowledge, to document the effects of psychological distress on WBC recovery among HCT patients. The present results are consistent with the work of Katayama et al. (2006) and suggest modulation of sympathetic outflow to the stem cell niche could be a unique mechanism by which psychological distress exerts influence on immune function in general, and specifically, total WBC count in HCT patients. Psychological intervention (e.g., stress management, relaxation training) has been shown to modulate sympathetic activity in other settings (Miller and Cohen, 2001). The present findings support future research to test the effects of psychological interventions to reduce distress, improve total WBC count, and improve health outcomes in HCT patients. Given that WBC recovery is essential to survival for HCT patients, these data provide a rationale for screening patients pre-transplant to determine those who would benefit from intervention to treat psychological distress in the HCT setting.

Conflict of Interest

The authors of this manuscript have nothing to declare.

Acknowledgments

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Childhood adversity increases vulnerability for behavioral symptoms and immune dysregulation in women with breast cancer

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ABSTRACT

Women respond differentially to the stress-associated with breast cancer diagnosis and treatment, with some women experiencing more intense and/or sustained behavioral symptoms and immune dysregulation than others. Childhood adversity has been identified to produce long-term dysregulation of stress response systems, increasing reactivity to stressors encountered during adulthood. This study determined whether childhood adversity increased vulnerability for more intense and sustained behavioral symptoms (fatigue, perceived stress, and depressive symptoms), poorer quality of life, and greater immune dysregulation in women ($N = 40$) with breast cancer. Evaluation was after breast surgery and through early survivorship. Hierarchical linear modeling was used to examine intra-individual and inter-individual differences with respect to initial status and to the pattern of change (i.e. trajectory) of outcomes. At initial assessment, women exposed to childhood emotional neglect/abuse had greater perceived stress, fatigue, depressive symptoms and poorer quality of life, as well as lower natural killer cell activity (NKCA). Although these outcomes improved over time, women with greater childhood emotional neglect/abuse exhibited worse outcomes through early survivorship. No effect was observed on the pattern of change for these outcomes. In contrast, childhood physical neglect predicted sustained trajectories of greater perceived stress, worse quality of life, and elevated plasma IL-6; with no effect observed at initial assessment. Thus, childhood adversity leaves an enduring imprint, increasing vulnerability for behavioral symptoms, poor quality of life, and elevations in IL-6 in women with breast cancer. Further, childhood adversity predisposes to lower NKCA at a critical time when this immune-effector mechanism is most effective at halting nascent tumor seeding.

1. Introduction

Any cancer diagnosis evokes fear and dread, but for women a diagnosis of breast cancer is especially devastating, as breast cancer is the second leading cause of cancer death in American women (American Cancer Society, 2011). Women diagnosed with breast cancer experience increased stress perception, anxiety, and mood disturbance (Bower, 2008; Witek-Janusek et al., 2008; Witek-Janusek et al., 2007) and the emotional response to breast cancer is independent of disease stage, as women with early stage non-invasive breast cancers also react emotionally to a breast cancer diagnosis (Beatty et al., 2008; Rakovitch et al., 2003; Witek-Janusek et al., 2007). Such emotional distress is often accompanied by depressive symptoms and fatigue, which emerge at diagnosis (Witek-Janusek et al., 2007) and intensify with treatment (Maraste et al., 1992; Schreier and Williams, 2004). For most women psychological distress dissipates in the months after treatment; yet for some women depressive symptoms and fatigue continue beyond treatment and persist into survivorship (Bower et al., 2000; Ganz et al., 1998). Recently, individual differences in the trajectories (intensity and duration) of distress, depressive symptoms and fatigue have been described for women from the time of their breast cancer diagnosis through early cancer survivorship. Those studies identify individual heterogeneity in the behavioral response to breast cancer, with a sizable number of women exhibiting greater vulnerability to the emotional challenges associated with cancer than others (Dhruva et al., 2010; Dunn et al., 2011; Henselmans et al., 2010). Such findings emphasize the importance of identifying vulnerability factors that contribute to individual differences in the psychological response to and recovery from a traumatic life event, like breast cancer.

Childhood adversity has been identified as a vulnerability factor, giving rise to an adult phenotype characterized by heightened reactivity to stress (Danese and McEwen, 2011; Heim et al., 2010; Nemeroff, 2004). Multiple studies document that early life stress

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alters neurobiological processes of the brain during development, a time when the brain is more malleable and thus more susceptible to adverse environmental stimuli (Danese and McEwen, 2011; Heim et al., 2010; Nemeroff, 2004). Adults exposed to childhood adversity manifest greater emotional responsiveness to stress (McLaughlin et al., 2010a), as well as a greater physiological response to stress, including increased autonomic nervous system and dysregulated hypothalamic-pituitary-adrenocortical (HPA) axis reactivity (Heim et al., 2008). Individuals with a history of adversity during childhood are at greater risk for depression and other mood disorders later in life, especially in the context of additional challenge (Chen et al., 2010b; Heim et al., 2010; Hill et al., 2000; Nemeroff, 2004).

Prior work also demonstrates that early life adversity predisposes to a proinflammatory phenotype. For example, lower childhood socioeconomic status, and presumably more adverse early life experiences, was reported to be associated with higher circulating levels of IL-6 (Carroll et al., 2011). Moreover, in a longitudinal study, childhood maltreatment was found to predict risk for low grade inflammation in adults; and this effect was independent of other risk factors, such as adult and child socioeconomic status and health behaviors (Danese et al., 2007). In the context of stressful challenge, previous work demonstrates that childhood adversity predisposes to an exaggerated proinflammatory response. When subjected to an acute laboratory social evaluative stress test (Trier Social Stress Test – TSST), healthy adults with exposure to childhood maltreatment exhibited a greater elevation in plasma IL-6, compared to those without a history of childhood maltreatment (Carpenter et al., 2010). Consistent with this observation, older adults exposed to childhood adversity were found to have greater circulating IL-6 and TNF-alpha levels when experiencing the naturalistic and chronic stress associated with caregiving for others with dementia (Kiecolt-Glaser et al., 2010). Such a proinflammatory phenotype linked to early life adversity may emerge during young adulthood, as peripheral blood mononuclear cells (PBMC) derived from young women raised in a harsh family climate produced more IL-6 in response to *in vitro* challenge with lipopolysaccharide and in response to real life psychological stressors (Miller and Chen, 2010).

Little is known about the effect of early life stress in patient populations, such as individuals diagnosed with cancer. In particular, there is a lack of understanding of the effects of early life stress on immune outcomes relevant to cancer control, such as NKCA and the proinflammatory cytokine, IL-6. IL-6 is notable for its pleiotropic tumor promoting activities including anti-apoptotic, pro-invasive, and immune-stimulatory effects attributable to the activation of Stat3 target genes (D'Anello et al., 2010; Hartman et al., 2011; Kishimoto, 2005; Knupfer and Preiss, 2007). There is an increasing literature in breast cancer patients indicating that high serum IL-6 is an independent negative prognostic indicator (Knupfer and Preiss, 2007). In patients with metastatic breast cancer, circulating IL-6 is associated with worse survival and at later stages of breast cancer progression IL-6 may have a net stimulatory effect on tumor growth (Knupfer and Preiss, 2007). Further, inflammation is thought to contribute to the development and progression of various cancers, including breast (Van der Auwera et al., 2004). IL-6 also up-regulates VEGF expression in several tumors via Stat3 activation of the gp130/Jak pathway and this type of up-regulation has been observed in various cancers (Goldberg and Schwertfeger, 2010; Huang et al., 2004; Steiner et al., 2004) and may be vital to the angiogenic process that occurs in tumors, maintaining and/or driving their growth.

NK cells contribute to cancer defense (Vivier et al., 2011) and previous studies show that on average women experiencing the stress associated with breast cancer diagnosis exhibit reductions in NKCA (Thornton et al., 2007; Witek-Janusek et al., 2008; Witek-Janusek et al., 2007). Also, women who report greater subjective stress after breast cancer surgery, but prior to adjuvant therapy, have lower basal and interferon augmented NKCA (Andersen et al., 1998, 2004). Yet, the extent of immune dysregulation at breast cancer diagnosis and the rate of recovery post-treatment show individual variation, which is associated with subjective stress perception. Thornton et al., demonstrated that women who showed an early decline in stress perception after their breast cancer surgery also showed the most rapid recovery of NKCA (Thornton et al., 2007). Optimal NKCA is relevant to women with breast cancer. NK cells defend against tumor initiation and tumor metastasis and breast cancer is an epithelial tumor that is susceptible to the anti-tumor effects of NK cells (Avraham and Ben-Eliyahu, 2007; Ben-Eliyahu, 2003; Dighe et al., 1994; Kagi et al., 1994; Kaplan et al., 1998; Lutgendorf et al., 2007; Seki et al., 2003; Smyth et al., 1998; Smyth et al., 1999; Stojanovic and Cerwenka, 2011; Street et al., 2001; van den Broek et al., 1996; Vivier et al., 2011). During critical times, such as after surgery and during adjuvant treatment, women are at risk for post-surgical tumor dissemination and NKCA is more effective in halting nascent tumor cell seeding when tumor burden is low (Avraham and Ben-Eliyahu, 2007; Lutgendorf et al., 2007). Thus, greater and more prolonged reduction in NKCA may jeopardize cancer control.

Given that early life adverse experiences can shape future behavioral and immunological stress reactivity, the purpose of this study was to determine whether childhood adversity influences the intensity and duration of perceived stress, depressive symptoms, fatigue, quality of life, levels of circulating IL-6, as well as NKCA of women from breast cancer diagnosis through early survivorship. Most studies evaluating the effect of early life stress on adult outcomes have employed healthy or community samples and used cross sectional designs. Few studies have evaluated longitudinal effects of early life adversity in individuals facing the threat of serious illness. Such a determination may identify a potential vulnerability factor posited to contribute to a worse behavioral and immunological profile in women during breast cancer treatment, as well as during recovery from the cancer experience. This is significant given that a substantial number of women are at risk for more intense and/or prolonged behavioral symptoms and immune dysregulation, which would not only diminish quality of life, but may also reduce effectiveness of immune-based cancer control mechanisms.

2. Methods

2.1. Participants and procedure

Women with early stage breast cancer were enrolled from the breast oncology clinics of three medical centers located in the west-suburban Chicago metropolitan area. Eligible women were identified after completion of their breast surgery and when their full surgical pathology report was available. Women were excluded if diagnosed with recurrent breast cancer or other cancers, immune-based or inflammatory disease, major psychiatric disorder or cognitive dysfunction. They were also excluded if they were substance abusers, used tobacco products, were taking corticosteriods, or if they were to receive systemic chemotherapy as part of their cancer treatment plan. Forty women were enrolled from participating breast oncology clinics; 34 were treated with breast conserving surgery followed by radiation therapy, while 6 women were treated with surgery only and no adjuvant radiation therapy. Participating women were evaluated five times, spanning a period of nine months. T1 evaluations occurred at least 2 weeks after surgery to allow dissipation of effects of anesthesia and surgical stress; on average T1 occurred 7 ± 5 weeks after surgery. With re-

spect to T1, subsequent evaluations were T2 = 5 ± 2 weeks, T3 = 9 ± 2 weeks, T4 = 15 ± 3 weeks, T5 = 34 ± 3 weeks. Participants completed instruments for perceived stress, depressive mood, fatigue and quality of life at each assessment; whereas, childhood adversity was measured once. For each assessment, venipuncture for blood sample procurement was accomplished in the morning between 6:30 and 11:30 AM. Blood specimens were used for determination of NKCA and measurement of circulating IL-6 levels. This study was approved by the Institutional Review Boards for the Protection of Human Subjects of all participating sites and informed consent was obtained from all participants.

2.2. Psychometric instruments

2.2.1. Childhood trauma questionnaire (CTQ)

To assess childhood adversity, the CTQ-Version 3 was administered. This instrument measures the nature and extent of exposure to childhood trauma/maltreatment. The CTQ assesses five domains of maltreatment (emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect) and the rate of occurrence of each item on a 5-point scale from 'Never True' to 'Very Often True'. The CTQ has demonstrated excellent test–retest reliability (Bernstein et al., 1994) and good convergent validity, as it correlated highly with interview-based rating of childhood abuse, as well as with therapists' ratings of abuse (Bernstein et al., 1994).

2.2.2. Perceived stress scale (PSS)

Perception of stress was measured using the PSS, a brief 10-item scale that assesses the degree to which life experiences are appraised as uncontrollable. It is a widely used measure of general perceived stress (Cohen et al., 1983). Reliability (stability) is 0.85 and Cronbach alphas range from 0.75 to 0.86 (Cohen and Williamson, 1988).

2.2.3. Center for epidemiologic studies, depression scale (CES-D)

Depressive symptoms were measured using the CES-D, which is a 20-item tool that assesses the frequency and duration of depressive symptoms (Radloff, 1977). It has been used widely in cancer studies. Scores range from 0 to 60 and a score of greater than 16 indicates risk for clinical depression. The CES-D has good construct validity in clinical and community samples, good test–retest reliability and an internal consistency alpha of .86 (Radloff, 1977).

2.2.4. Multidimensional fatigue scale inventory

To capture the multidimensional nature of cancer-related fatigue, fatigue was measured over the past month using the Multidimensional Fatigue Symptom Inventory Short Form (MFSI-SF) (Pickard-Holley, 1991; Stone et al., 1998). The MFSI-SF measures overall fatigue, as well as fatigue experienced in five domains (general, emotional, physical, mental, vigor). Factor analysis demonstrated good fit for the five factors. Internal consistency ranges from 0.87 to 0.96 (Stein et al., 2004).

2.2.5. Quality of life index

Quality of life was defined as "a person's sense of well-being that stems from satisfaction or dissatisfaction with the areas of life that are important to him/her" and was assessed using the Ferrans Quality of Life Index (QLI) (Ferrans, 1990). QLI measures life satisfaction in four domains: health/functioning, socioeconomic, psychological/spiritual, and family. Two parts evaluate 34 items on a 6-point scale. Part I measures satisfaction within each domain. Part II measures perceived importance of each item. Satisfaction scores are weighted by importance. QLI has been used in many breast cancer studies and QLI demonstrates good content and construct validity (Ferrans and Powers, 1985). The Cronbach alpha for breast cancer patients ranges from 0.93 to 0.96 (Ferrans, 1990; Hughes, 1993), while criterion validity is 0.80 (Ferrans, 1990) and test–retest reliability is 0.87 (Ferrans and Powers, 1985).

2.2.6. Demographic and medical history

Demographic information, including age, race, marital status, education, and employment status, was obtained using a self-report questionnaire. Cancer pathology, staging, and cancer treatment were validated by medical records. Information regarding presence of other comorbidities and use of medications was collected by self-report. A weighted index of comorbid disease was calculated (Charlson et al., 1987) and used to control for co-morbidities in the statistical analyses.

2.3. Immunological measures

2.3.1. Isolation of peripheral blood mononuclear cells

Blood was collected in sterile heparinized tubes and processed immediately. Heparinized peripheral blood was overlaid onto Ficoll/Hypaque and centrifuged at 1000*g* for 20 min. The peripheral blood mononuclear cells (PBMC) at the interface were washed twice with Hank's balanced salt solution prior to assessment of NKCA. The PBMC subsets were determined as described previously (Witek-Janusek et al., 2007) and did not change across assessment periods (i.e. T1–T5).

2.3.2. NKCA

Natural killer cell lytic activity against tumor targets was assessed using PBMC in a standard chromium release assay, as previously described (Witek-Janusek et al., 2007). K562 tumor cells were radioactively labeled with 100 uCi of [^{51}Cr] (New England Nuclear, Boston, MA). Radiolabeled K562 cells were incubated for 4 h with PBMC. Following incubation, the supernatants were removed using a Skatron harvesting press (Skatron Inc., Sterling, VA) and the associated radioactivity was determined. Effector to target ratios for NKCA was 50, 25, 12, and 6:1. Results are expressed as % cytotoxicity and calculated by the formula:

$$\%\ \text{Cytotoxicity} = \frac{(\text{experimental DPM}^{*}) - (\text{minimum DPM})}{(\text{maximum DPM}) - (\text{minimum DPM})} \times 100.$$

All experimental means were calculated from triplicate values. Lytic units (LU) were calculated by a program written by David Coggins, FCRC, Frederick, MD and represent the number of cells per 10^7 effectors required to achieve 20% lysis of the targets. *DPM = disintegrations per minute.

2.3.3. Plasma IL-6

Enzyme linked immunoabsorbent assay (ELISA) was used to measure circulating IL-6 in plasma samples (R & D Systems, Minneapolis MN). All samples were analyzed in duplicate. Sensitivity was <7 pg/ml and intra assay variability was <7%.

2.4. Statistical analysis.

Preliminary analyses were carried out using SPSS 17.0. Summary descriptive statistics for all outcome and predictor variables were calculated and all variables were examined for normality of distribution. Values for IL-6 were log transformed, as these were not normally distributed. Bivariate correlations among the scales from the Childhood Trauma Questionnaire (i.e. physical abuse, physical neglect, emotional abuse, and emotional neglect) were conducted. There was a strong positive association between the emotional abuse and the emotional neglect scales ($r = 0.90$, $p < 0001$). In order to avoid multicollinearity effects in the subsequent multilevel regression models, the scores on these two scales were combined into one index, termed as emotional neglect/abuse.

Physical abuse and physical neglect scales were moderately correlated (*r*s = 0.30–42) and were treated as separate predictor variables. The sexual abuse scale was not included in the final analysis because only three participants reported "*rarely true*" for one or more questions regarding their history of sexual abuse. Hence, three childhood adversity factors were evaluated in the final analysis: physical abuse, physical neglect, and emotional neglect/abuse.

HLM 6.08 software for computing multilevel model for change (Raudenbush and Bryk, 2002), based on full maximum likelihood estimation, was used to examine intra-individual and inter-individual differences in initial status and trajectories of change over time in depressive symptoms, perceived stress, fatigue, quality of life, plasma IL-6, and NKCA. Unlike the traditional analysis of variance for repeated measures, HLM estimates change for each individual and not merely change in group trends across time (Hedeker, 2004). Such an approach allows for individual-specific effects to be included in the model to capture the heterogeneity among participants in both the initial level and growth over time. Importantly, HLM treats time as a continuous variable letting each participant have her own data collection schedule. Another advantage of the multilevel approach is that it allows for the analysis of participants with incomplete and unbalanced data across time points, resulting in increased statistical power and less biased findings (Hedeker, 2004). HLM also estimates variance components associated with the initial level and the time trend, which is indicative of the sample's heterogeneity.

With HLM of longitudinal data, the outcome variables (i.e. depressive symptoms, fatigue, perceived stress, quality of life, IL-6, and NKCA) are conceptualized to be nested within individuals and the growth modeling of change in these variables has two levels. At Level 1, the outcome variable is a function of person-specific parameters (i.e. initial level and time trend) plus error. At Level 2, the subject's intercept and slope are modeled as a function of the predictor variables that vary between participants (i.e. childhood trauma questionnaire scales), plus an error associated with each individual for each parameter. Combination of these two levels results in a mixed model with fixed and random effects (Raudenbush and Bryk, 2002).

The HLM analysis for each of the outcome variables was performed in three stages. First, in order to investigate intra-individual variability over time, an unconditional model (i.e. model with no covariates) was fit to the data. This stage examined how depressive symptoms, perceived stress, fatigue, quality of life, and NKCA changed from the time of the first assessment to approximately nine months later. Importantly, the variance components were estimated to evaluate individual variation around the sample-wide model estimates. Time was measured in weeks from T1, which was coded as zero. The slope coefficients are interpreted as change per each additional week from T1. Both linear and quadratic trends were examined and goodness-of-fit tests of the deviance between linear and quadratic models were used to assess the most appropriate fit.

The second stage of HLM analysis examined the potential effects of the following variables: demographic (age, race, education, marital status and BMI for IL-6), co-morbidities, categories of medication usage (statin agents, beta blockers, agents directed at the renninangiotensin system, anti-depressants, and oral hypoglycemic agents), and cancer treatment variables (cancer stage, type of surgery, radiation therapy, time since surgery, and hormonal treatment for cancer, i.e. tamoxifen and aromatase inhibitors). In addition, for women receiving radiation therapy, initial values of outcomes were evaluated with respect to number of radiation treatments received.

Lastly, the effect of the percentage of NK cells ($CD56^+$) was examined with respect to NKCA. Only variables that had a significant effect (*t*-value above 2.00) were included in the third stage of analysis, and only those that retained their significance in combination with other predictor variables were left in the final model. Lastly, during the third stage, potential moderating effects of the childhood adversity factors (physical abuse, physical neglect, emotional neglect/abuse) were examined. Childhood adversity factors were time-invariant predictors (assessed at T1 only) and were entered into the models simultaneously as continuous variables.

3. Results

3.1. Descriptive characteristics of participants

Forty women were enrolled and evaluated longitudinally at five distinct times (T1–T5). Missing data was minimal. For psychological data only one subject did not complete the CES-D and the QLI at T3. For NKCA, 40 subjects provided adequate blood samples for evaluation of NKCA at T1, while 37, 38, 37, and 37 subjects had NKCA evaluations at T2 through T5, respectively. For circulating IL-6, plasma samples from 40 subjects were evaluated at T1, while 38, 38, 36, and 38 samples were evaluated at T2 through T5, respectively. Reasons for missing immune data were inadequate specimen blood volume, inability to perform venipuncture, or frank refusal of venipuncture.

Demographic, disease, and treatment characteristics of the participants are summarized in Table 1. Women were predominantly Caucasian (84%), married (71%), and well educated (*M* = 15.4 years, SD = 2.8). The majority of women had Stage 0 or Stage I breast cancer (78%). All women underwent surgery for removal of their cancer (31 had breast conserving surgery and 9 women had a mastectomy). Thirty-four women received radiation therapy after their surgery and 10 of these women were also placed on hormonal therapy (tamoxifen or an aromatase inhibitor), as part of their cancer treatment. The remaining 6 women were treated with surgery only. See Table 1. The most prevalent medications used by women in this sample were statin agents (*N* = 9), followed by beta blockers (*N* = 6) and agents directed at the rennin-angiotensin system (*N* = 6). Two women used oral hypoglycemic agents and three women used anti-depressants.

Table 1
Demographic characteristics (*N* = 40).

Age (years) mean ± SD	55. 6 ± 9.4
Education (years) mean ± SD	15.4 ± 2.8
	Frequency
Race	
Caucasian	33
African American	4
Hispanic	1
PI/Asian	2
Marital Status	
Married	29
Divorced/separated	6
Single	5
Income	
$10,000–29,000	6
$30,000–59,000	6
$60,000 and higher	28
Stage of cancer	
Stage 0	12
Stage I	21
Stage IIA	7
Treatment[a]	
Surgery only	6
Surgery + radiation therapy	24
Surgery + radiation + hormonal therapy	10
Surgery Type	
Breast conserving	31
Mastectomy	9

SD = standard deviation.
[a] Some women received multiple forms of therapy.

Descriptive data (means and standard deviations) for all behavioral and immune measures for the five evaluation periods, as well as the summary statistics for the CTQ subscales are provided in Table 2. For women in our sample, the means and standard deviations for the CTQ scales are similar to that reported for a large validation sample of healthy US adult women (Scher et al., 2001). Overall, average values for depressive symptoms, perceived stress and fatigue declined from T1 to T5, while quality of life increased throughout the same time period. NKCA reached a nadir at T2 and showed a continual increase from T3–T5. NK cell percentages remained stable over time. Average values of NKCA at T5 are similar to normative values for healthy women, as previously reported from our laboratory (Witek-Janusek et al., 2008, 2007). Average levels of circulating IL-6 essentially remained the same throughout the assessment period. Of note, no group differences were found ($t(38) = 0.98$, $p = 0.33$) in the initial level of IL-6 and NKCA between women who received radiation therapy and women who did not. Also, no association was observed between the time since surgery and the baseline levels of IL-6 ($r = -0.26$, $p = 0.15$) and NKCA ($r = 0.04$, $p = 0.78$).

3.2. Unconditional effects of time: intra-individual variation

Estimates of fixed and random effects of the unconditional (i.e. no covariates) model are presented in Table 3. The unconditional effects of time for all outcome measures are illustrated in Fig. 1. The mean scores for the outcome variables depicted in the figures are estimated or predicted by the HLM results.

3.2.1. Depressive symptoms (CES-D)

Goodness-of-fit tests of the deviance indicated that a linear model fit the data better than did a quadratic model ($p < 0.01$). Estimates of fixed and random effects of the unconditional (i.e. no covariates) model for CES-D scores are presented in Table 3. The sample as a whole demonstrated a significant decrease in the level of depressive mood over the 9 months of the study ($b = -0.07$, $SE = 0.03$, $p = 0.04$). The average initial estimated level of depressive symptoms at diagnosis was 12.5 (SE = 1.25), which is below the established score of 16 indicative of elevated risk for clinical depression (Radloff, 1977). However, random effects indicated that women exhibited significant heterogeneity in the extent of depressive mood at diagnosis ($p < 0.0001$), but not in their trajectories over time. See Fig. 1a and Table 3.

3.2.2. Perceived stress (PSS)

Assessed by the goodness-of-fit test of the deviance scores, a quadratic trend was found to more adequately capture the change in the level of perceived stress ($p < 0.05$). As shown in Table 3, the average initial level of perceived stress was estimated to be 17.5 ($SE = 0.98$). This level is elevated as compared to a community sample of US adults (PSS mean = 13.0, SD = 6.1; (Cohen and Williamson, 1998), indicating that women were experiencing high perceived stress at the time of their enrollment. Both the estimated linear ($b = -0.29$, $SE = 0.10$, $p < 0.001$) and quadratic rate of change per week ($b = 0.006$, $SE = 0.002$, $p < 0.01$) were significant. The linear slope represents instantaneous rate of change at a specific moment and the quadratic slope defines the curvature of the slope (Singer and Willett, 2003). The overall trajectory is defined by the weighted combination of linear and quadratic growth factors (see Fig. 1b). The heterogeneity in individual change parameters, estimated by the variance components, indicated that there was significant variation in the initial level as well as the trajectories of perceived stress between women (p values <0.0001).

3.2.3. Fatigue (MFSI)

A quadratic model fit the data significantly better ($p > 0.05$). As shown in Table 3, on average women were estimated to have elevated levels of fatigue (Stein et al., 1998) at time of the first evaluation, 14.3 ($SE = 2.5$). A significant linear ($b = -0.6$, $SE = 0.23$, $p = 0.01$) and quadratic ($b = 0.014$, $SE = 0.007$, $p = 0.04$) change in fatigue were also observed, with average levels of fatigue decreasing most precipitously from initial levels through T4 (Fig. 1c). The variance components of the model indicated that there was a significant variation in the reports of fatigue at diagnosis and trajectories over the nine months (p values <0.001).

3.2.4. Quality of life (QLI)

A linear trend fit the data significantly better ($p < 0.001$). As shown in Table 3, initial levels of quality of life were estimated at 21.5 ($SE = 0.70$). As illustrated in Fig. 1d, on average, quality of life was estimated to improve over the nine months of the study ($b = 0.07$, $SE = 0.025$, $p = 0.01$). Similar to previous models, there was heterogeneity in the reports at diagnosis, as well as in the change over time, indicated by the variance components of the model (p values <0.001).

Table 2
Descriptive data for psychological and immune variables.

	T1		T2		T3		T4		T5	
	M	SD	M	SD	M	SD	M	SD	M	SD
CESD	12.9	10.3	12.6	10.8	10.9	8.7	10.9	9.9	10.5	9.3
PSS	16.9	7.2	16.1	7.3	15.1	6.4	15.1	7.2	15.0	7.1
MFSI	12.7	10.1	12.2	23.6	9.9	20.9	7.9	19.9	11.3	25.5
QLI	21.1	4.6	21.1	4.7	22.0	4.7	22.4	4.7	23.1	5.9
NKCA	70.5	46.9	57.2	43.8	63.7	48.4	75.8	56.9	90.1	66.7
NK cells	13.9	7.6	13.7	8.6	13.7	6.4	12.0	6.29	14.1	7.1
IL-6 (pg/ml)	1.2	0.8	0.8	0.4	0.9	0.6	1.0	0.5	1.0	0.8
IL-6 (log)	−0.02	0.3	−0.16	0.2	−0.09	0.3	−0.06	0.2	−0.08	0.3
CTQ scales										
Physical neglect					6.6				2.6	
Emotional neglect					9.2				4.9	
Emotional abuse					8.9				4.9	
Physical abuse					6.9				4.3	
Sexual abuse					6.8				3.4	
Emotional neglect/abuse					9.1				4.8	

Values are mean (M) and standard deviation (SD). CESD = Center for Epidemiologic Studies Depression Scale, PSS = Perceived Stress Scale; MFSI = Multidimensional Fatigue Symptom Inventory, QLI = Quality of Life Index, CTQ = Childhood Trauma Questionnaire, NKCA = Natural Killer Cell Activity (lytic units 20%), NK cells = percentage of $NK56^+$ cells in peripheral blood, IL-6 is pg/ml of plasma. The initial evaluation (T1) occurred 7 ± 5 weeks post surgery, and with respect to T1, subsequent evaluations were T2 = 5 ± 2 weeks, T3 = 9 ± 2 weeks, T4 = 15 ± 3 weeks, T5 = 34 ± 3 weeks.

Table 3
Unconditional and final hierarchical linear models for psychological and immune outcomes.

	CESD	PSS	MFSI	QLI	NKCA	IL-6 (log)
Unstandardized coefficients (SE)						
Fixed effects: intercept	12.55 (1.25)[b]	17.48 (.98) [b]	14.26 (2.5) [b]	21.51 (.70) [b]	70.51 (7.18) [b]	−.082 (.035) [c]
Time[a] (linear)	−.07 (.03)	−.29 (.10) [c]	−.60 (.23) [c]	.07 (.025) [c]	.55 (.41)	−.00007 (.0012)
Time[2] (quadratic)	–	.006 (.002) [d]	.014 (.007) [d]	–	–	–
Random effects: intercept	79.06[b]	48.25[b]	311.91[b]	18.47[b]	1531.94[b]	.037
Time (linear)	.012	.37[b]	1.17[b]	.018[b]	3.95[b]	.0008[d]
Time[2] (quadratic)	–	.0002[b]	.0011[b]	–	–	–
Fixed effects: baseline						
Intercept	12.16 (1.25)	16.87 (.92)	13.32 (2.62)	21.48 (.52)	70.28 (5.67)	−.082 (.03)
Age	–	−.22 (.06) [d]	−1.04 (.31) [c]	–	–	–
BMI	–	–	–	–	–	.019 (.004) [b]
Radiation start[e]	6.24(2.89) [d]	–	–	–	–	–
Physical abuse	−1.57 (.51) [c]	−1.01 (.72)	−1.09 (1.16)	.66 (.21)	.14 (1.83)	.013 (.01)
Physical neglect	.33 (.67)	−.71 (.49)	1.07 (1.40)	−.24 (.21)	4.17 (3.21)	−.013 (.012)
Emotional neg/abuse	1.21 (.38) [c]	1.20 (.28) [b]	1.61 (.79) [d]	−.69 (.16) [b]	−2.68 (1.20) [c]	−.01 (.007)
Time slope (linear)						
Intercept	−.06 (.04)	−.26 (.11)	−.75 (.25)	.07 (.02)	.73 (.35)	−.00003 (.0011)
NK cells	–	–	–	–	3.55 (.84) [b]	–
Physical abuse	.006 (.018)	−.04 (.04)	−.03 (.10)	.004 (.008)	−.12 (.13)	−.0009 (.0007)
Physical neglect	.011 (.021)	.23 (.06) [b]	.15 (.14)	−.014 (.007) [d]	.15 (.16)	.0009 (.0003) [d]
Emotional neg/abuse	−.022 (.06)	−.06 (.04)	.017 (.07)	.006 (.004)	−.04 (.09)	.0004 (.0003)
Time slope (quadratic)						
Intercept	–	.006 (.003)	.016 (.008)	–	–	–
Physical abuse	–	.001 (.001)	.002 (.003)	–	–	
Physical neglect	–	−.005 (.001) [c]	−.003 (.004)	–	–	
Emotional neg/abuse	–	.001 (.0009)	−.001 (.002)	–	–	
Random effects: intercept	48.24[b]	26.51[b]	236.80[b]	11.17[b]	809.30[c]	.0243[b]
Slope (linear)	.013	.21[d]	.91[d]	.016[b]	4.74[b]	.00001
Slope (quadratic)	–	.0001[d]	.0009[d]	–	–	–
NK cells	–	–	–	–	9.56	–

Abbreviations: CESD = Center for Epidemiologic Studies Depression Scale, MFSI = Multidimensional Fatigue Scale Index, PSS = Perceived Stress Scale, QLI = Quality of Life Index, NKCA = Natural Killer Cell Activity Lytic Units 20%, NK cells = percentage of $CD56^+$ cells in peripheral blood, BMI = body mass index; SE = Standard Error of the coefficient; IL-6 is pg/ml of plasma.

[a] Time was coded 0 at the first assessment visit.
[b] $P < 0.001$.
[c] $P < .01$.
[d] $P \leqslant .05$.
[e] Procurement of T1 data with respect to radiation start date.
[2] Refers to the quadratic rate of change.

3.2.5. NKCA

Overall NKCA demonstrated a linear increase in trajectory from the initial evaluation, which was estimated at 70.5 lytic units ($SE = 7.18$). On average, NKCA was estimated to improve over the course of the study, with the rate coefficient approaching significance ($b = 0.55$, $SE = 0.29$, $p = 0.06$). The variance components indicated significant inter-individual differences among women in NKCA at initial assessment and in change over the course of the study ($p < 0.0001$). See Table 3 and Fig. 1e.

3.2.6. Plasma IL-6

A linear model provided a significantly better fit for the data ($p < 0.05$). Although, the level of IL-6 did not change significantly over time for the sample as a whole ($b = -0.00007$, $SE = 0.0012$, $p = 0.89$), a significant amount of heterogeneity was observed for the initial status ($p < 0.001$) and slope ($p = 0.05$). See Table 3 and Fig. 1f.

3.3. Effects of childhood adversity: inter-individual variation

Childhood adversity factors (i.e. physical neglect, physical abuse, emotional neglect/abuse) and demographic characteristics were entered into the models to understand how these factors affect the pattern of change in depressive mood, perceived stress, fatigue, quality of life, and immune function (NKCA and IL-6) from diagnosis through early survivorship. In order to aid the interpretation of the fixed effects, predictor variables were grand-mean centered (i.e. the variable's sample mean was subtracted from each observation). As previously described, demographic and treatment variables were entered into the model separately. To achieve a parsimonious model, only those variables that retained their significance in combination with other co-variables and childhood adversity factors remained in the final model.

Results revealed that there was no effect of treatment variables (radiation therapy, type of surgery, time since surgery, and use of hormonal treatment for cancer therapy) on any study outcome. As well there was no effect of co-morbidities or medication usage, based on major categories of drugs used by women in this sample (i.e. statins, beta blockers, rennin-angiotensin blockers, antidepressants, or oral hypoglycemics). At initial assessment (T1), the timing of radiation significantly affected depressive symptoms but did not affect any other outcome measures. The effect of radiation timing on depression retained its significance when entered in combination with the childhood adversity factors; thus, timing of radiation was subsequently controlled for in the final model.

No effect on study outcomes was found for demographic variables (education, race, marital status, and income) and these variables were omitted from the final models. Age was found to be significantly associated with stress and fatigue intercept parameters and retained significance when entered in combination with the childhood adversity factors; thus age was controlled in the final models. BMI was significantly associated with the plasma IL-6 intercept parameter. NKCA cell percentage was associated with NKCA. Thus, both BMI and NK cell percentage were controlled in the final models (see Table 3).

NK cell percentage is a time-variant variable and was entered into the Level 1 model. Childhood adversity variables were entered

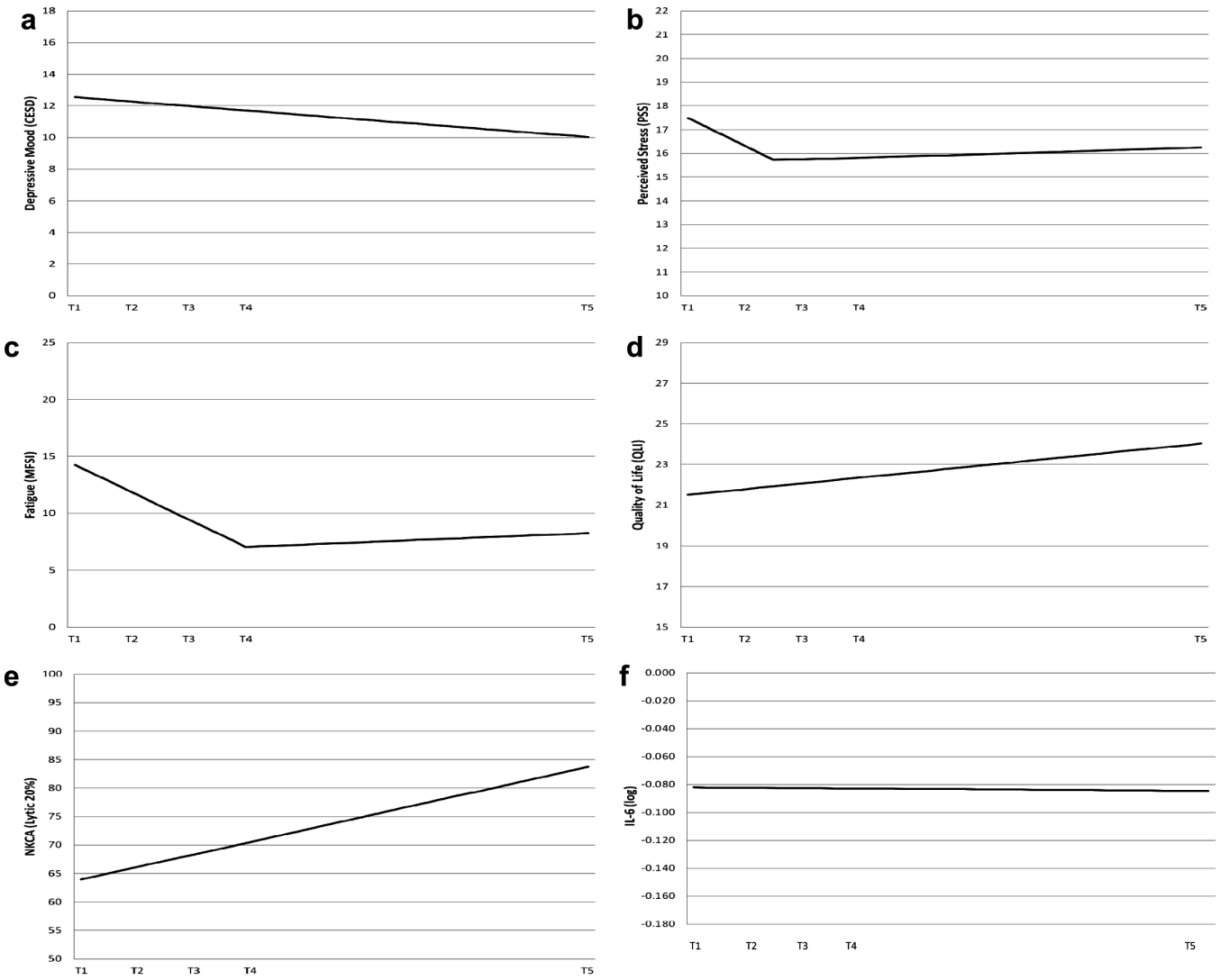

Fig. 1. Unconditional model estimates of the growth trajectories for (a) depressive symptoms, (b) perceives stress, (c) fatigue, (d) quality of life, (e) NKCA, and (f) plasma IL-6. Graphical representation of the unconditional effect of time on depressive mood (CESD scores), perceived stress (PSS scores), fatigue (MFSI scores), quality of life (QLI scores), natural killer cell activity (NKCA; lytic units 20%), and circulating level of IL-6. The initial evaluation (T1) occurred 7 ± 5 weeks post surgery, and with respect to T1, subsequent evaluations were T2 = 5 ± 2 weeks, T3 = 9 ± 2 weeks, T4 = 15 ± 3 weeks, T5 = 34 ± 3 weeks.

simultaneously into the HLMs. Figs. 2 and 3 illustrate the effects of these predictors on the trajectories of behavioral and immune outcome measures. The change curves for psychological and immune measures were based on differences in exposure to childhood emotional neglect/abuse (Fig. 2) and exposure to childhood physical neglect (Fig. 3). This was calculated as the average upper (i.e. high neglect/abuse) and the average lower quartiles (i.e. low neglect/abuse), and is illustrated in this manner.

3.3.1. Depressive symptoms

As shown in Table 3 and Fig. 2a, childhood emotional neglect/abuse ($b = 1.21$, $SE = 0.35$, $p = 0.003$) was a significant predictor of the initial level of depressive symptoms. Women who reported greater levels of childhood emotional neglect/abuse were estimated to have greater levels of depressive symptoms, which remained greater over the time evaluated. Childhood physical abuse was negatively related to the initial levels of depressive symptoms ($b = -1.57$, $SE = 0.39$, $p = 0.001$). None of the childhood adversity factors were associated with a change in the linear slope for depressive symptoms. See Table 3.

3.3.2. Perceived stress

Similarly, childhood emotional neglect/abuse was significantly associated with the initial level of perceived stress ($b = 1.20$, $SE = 0.28$, $p < 0.001$). See Table 3. Women who reported greater levels of childhood emotional neglect/abuse were estimated to have higher perceived stress at initial assessment, and these levels remained higher over the time evaluated (Fig. 2b). Age was also a significant predictor of the intercept, such that younger women were estimated to have lower initial levels of perceived stress ($b = -0.22$, $SE = 0.06$, $p = 0.02$). Further, as shown in Table 3, childhood physical neglect was a significant predictor of the estimated linear ($b = 0.23$, $SE = 0.06$, $p < 0.001$) and quadratic ($b = -0.005$, $SE = 0.001$, $p = 0.004$) trends, but was not associated with the initial level of perceived stress ($p = 0.15$). As illustrated in Fig. 3a, although all women initially reported similar elevations in the level of perceived stress, for those women who reported higher levels of childhood physical neglect, perceived stress continued to remain elevated over time. In contrast, for women with lower levels of childhood physical neglect, perceived stress declined over the first 16 weeks and slightly increased thereafter. No significant association between

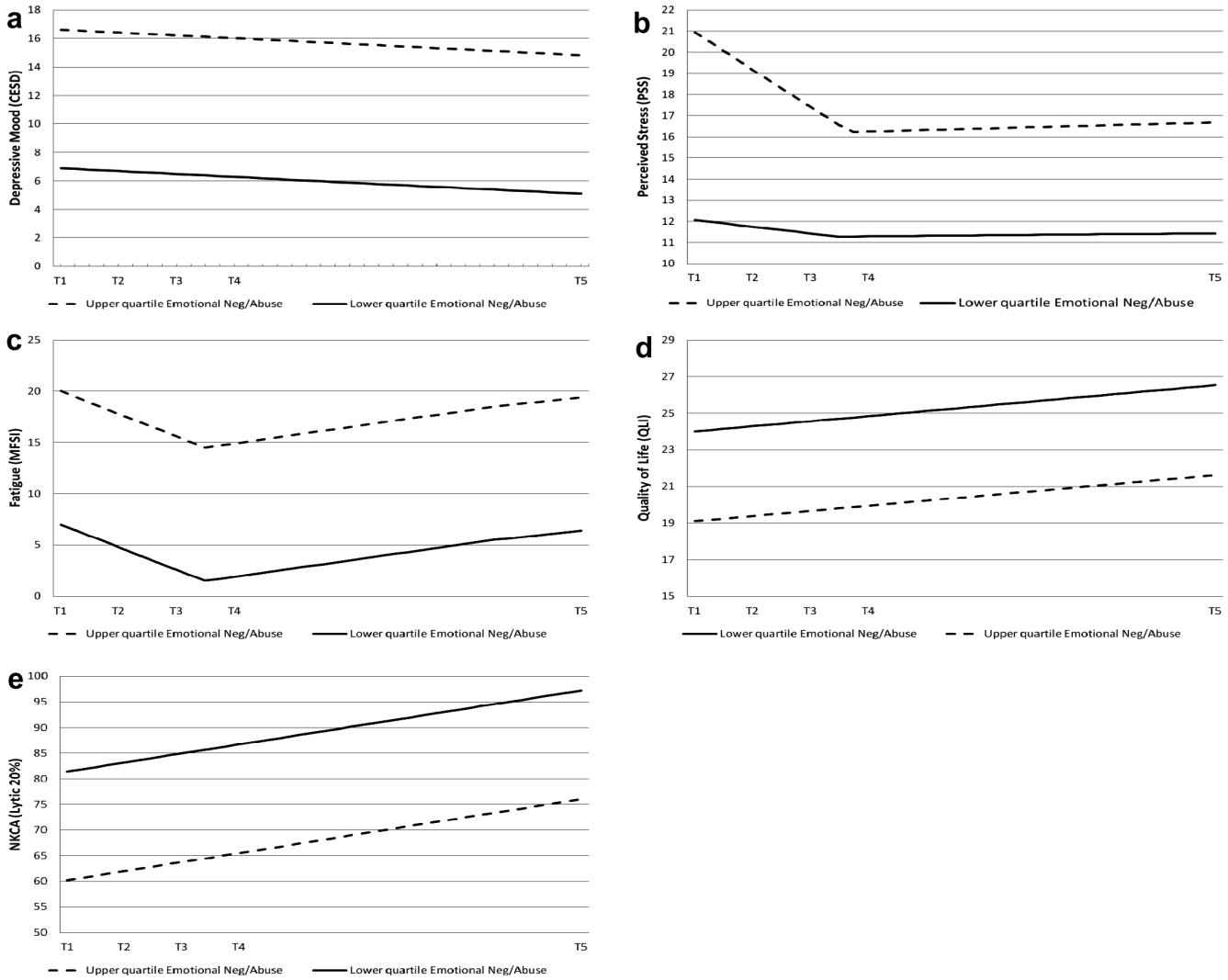

Fig. 2. Effect of emotional neglect/abuse on (a) depressive mood, (b) perceived stress, (c) fatigue, (d) quality of life, (e) NKCA. Graphical representation of the relationship between childhood emotional neglect/abuse (calculated as average upper/lower quartiles) and depressive mood (CESD scores), perceived stress (PSS scores), fatigue (MFSI scores), quality of life (QLI scores), and natural killer cell activity (NKCA; lytic units 20%). Graphs are estimated by the hierarchical linear models from the time of the initial assessment (T1) through an approximate 9-month period (T5). Women who reported greater levels of childhood neglect/abuse were estimated to have more depressive mood (b = 1.29, $p = .002$), greater perceived stress (b = 1.20, $p < .001$), more fatigue (b = .66, $p = .04$), poorer quality of life (b = -.69, $p < .001$), lower NKCA (b = -3.12, $p = .01$) at the initial assessment and through the 9-month study period. The initial evaluation (T1) occurred 7 ± 5 weeks post surgery, and with respect to T1, subsequent evaluations were T2 = 5 ± 2 weeks, T3 = 9 ± 2 weeks, T4 = 15 ± 3 weeks, T5 = 34 ± 3 weeks.

childhood physical abuse and perceived stress was observed for either the intercept or linear/quadratic slopes.

3.3.3. Fatigue

Childhood emotional neglect/abuse was a significant predictor of the initial level of fatigue ($b = 1.61$, $SE = 0.79$, $p = 0.04$) but was not associated with linear or quadratic change in fatigue over time. As illustrated, women who reported greater childhood emotional neglect/abuse were estimated to have higher levels of fatigue at initial assessment and their fatigue levels remained higher over time (see Fig. 2c). Age was also associated with the initial level of fatigue, with younger women reporting greater levels of fatigue ($b = -1.04$, $SE = 0.31$, $p = 0.004$). None of the childhood adversity factors were associated with the pattern of change (i.e. trajectory) in fatigue (Table 3).

3.3.4. Quality of life

Childhood emotional neglect/abuse was a significant predictor of the initial level of quality of life ($b = -0.69$, $SE = 0.16$, $p < 0.001$), but was not associated with the linear slope ($p = 0.20$). Women who reported higher levels of childhood emotional neglect/abuse were estimated to report lower quality of life and this pattern remained over the course of the 9 month period (Fig. 2d). Physical neglect or physical abuse during childhood was not associated with the initial level of quality of life. However, an association between the linear change in quality of life and childhood physical neglect approached significance ($b = -0.014$, $SE = 0.007$, $p = 0.06$). This is illustrated, in Fig. 3b, which shows that women who reported lower levels of physical neglect exhibited a faster rate of improvement in quality of life compared to women who had greater physical neglect. See Table 3 and Fig. 3b.

3.3.5. NKCA

The initial level of NKCA was significantly associated with childhood emotional neglect/abuse ($b = -2.68$, $SE = 1.20$, $p < 0.05$), in that women who reported greater levels of childhood emotional neglect/abuse were estimated to have lower NKCA at their first assessment. See Table 3 and Fig. 2e. NKCA increased over time

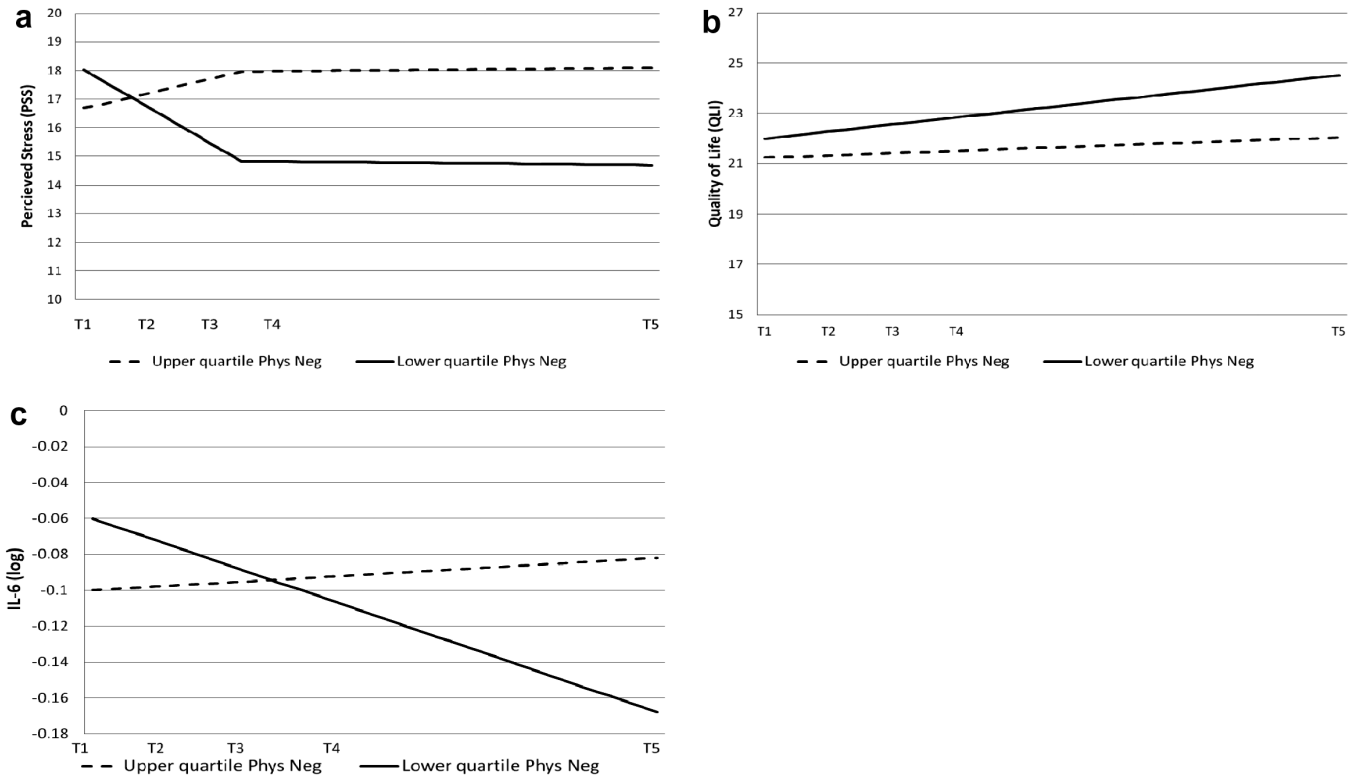

Fig. 3. Effect of physical neglect on (a) perceived stress and (b) quality of life, and (c) plasma IL-6. Graphical representation of the relationship between childhood physical neglect (calculated as average upper/lower quartiles) and perceived stress (PSS scores), quality of life (QLI scores), and circulating IL-6 level. Graphs are estimated by the hierarchical linear models from the time of the initial assessment (T1) through an approximate 9-month period (T5). At the initial assessment, there were no significant differences in perceived stress, quality of life, or plasma IL-6 between the women. However, those women who reported greater level of childhood physical neglect were estimated to have an increase in perceived stress (b = .23, $p < .001$; b quadratic = -.005, $p = .004$) over the first 16 weeks that remained elevated thereafter. Lower level of physical neglect was associated with faster improvement rate in quality of life (b = -.014, $p = .06$) and a greater decline in circulating IL-6 (b = .0009, $p = .03$). The initial evaluation (T1) occurred 7 ± 5 weeks post surgery, and with respect to T1, subsequent evaluations were T2 = 5 ± 2 weeks, T3 = 9 ± 2 weeks, T4 = 15 ± 3 weeks, T5 = 34 ± 3 weeks.

but the linear slope was not affected by childhood emotional neglect/abuse ($p > 0.05$). As a result of the lower initial levels of NKCA and the lack of the interaction between rate of change and childhood emotional neglect/abuse, women with greater childhood emotional neglect/abuse had lower NKCA for the duration of the study. For other childhood adversity factors (physical abuse and neglect), no associations with NKCA were observed for either the initial level or the linear slope (p values >0.05).

3.3.6. Plasma IL-6

Childhood physical neglect was a significant predictor of the linear slope ($b = .0009$, $SE = .0003$, $p = .03$), but not the initial level ($p = .31$) of plasma IL-6. These results indicate that although all women as a group initially have similar levels of circulating IL-6, those women who reported lower levels of physical neglect during their childhood exhibited a more precipitous decrease of IL-6 during the course of the study (Fig. 3c). In contrast, circulating IL-6 levels increased slightly, for women who reported greater childhood physical neglect. Other childhood adversity factors (physical abuse and emotional neglect/abuse) were not associated with IL-6 (Table 3).

4. Discussion

The results of this study demonstrate childhood emotional neglect/abuse to predict greater perceived stress, fatigue, depressive symptoms, and poorer quality of life, as well as lower NKCA in women evaluated after their breast cancer surgery (i.e. initial assessment in this study). It is possible that these worse outcomes are related to enduring effects of childhood emotional neglect/abuse on stress response systems, which heighten reactivity to the stress-associated with breast cancer diagnosis and treatment. Yet because these women could not be evaluated prior to their breast cancer diagnosis, it is just as likely that they had pre-existing behavioral traits, which may have contributed to or accounted for the findings observed at initial assessment. However, the present results also show childhood physical neglect to predict worse trajectories for perceived stress, circulating IL-6 and quality of life in women with breast cancer. Those findings support the notion that these women were more susceptible to the challenges associated with breast cancer diagnosis and treatment. As such, childhood adversity emerges as a salient vulnerability factor for women with breast cancer, which may affect their recovery.

Although previous studies have described individual differences in the psychological adjustment and immune recovery after breast cancer (Dhruva et al., 2010; Dunn et al., 2011; Henselmans et al., 2010; Thornton et al., 2007), to our knowledge this is the first investigation to demonstrate early adverse experiences during childhood to influence behavioral and immune function in women with breast cancer. The occurrence of childhood adversity is not uncommon for women. Nearly 30% of the women in a community sample reported some form of childhood maltreatment (Scher et al., 2004). Women in our study reported means and standard deviations for the CTQ scales similar to that reported for a large validation sample of healthy US adult women (Scher et al., 2001). Even though women in our sample were largely well-educated, middle income women, childhood adversity still emerged as an important pre-existing risk factor for psychological morbidity

and dysregulated immune function. It is likely that more profound effects would be observed in women raised in an environment of social disadvantage (Carroll et al., 2011), emphasizing the need for health care providers to recognize this potential risk factor in cancer patients.

For most women breast cancer diagnosis and associated cancer treatment are accompanied by psychological distress. Over time most women successfully adapt and recover (Bárez et al., 2007; Stanton et al., 2005). Yet there is considerable individual difference in the trajectory of psychological distress following breast cancer diagnosis. A recent report identified four distinct trajectories of distress in the first year after breast cancer diagnosis: women with no distress (36%); women with distress during active treatment only, which dissipated by two months post-treatment (33%); women with distress that emerged during early survivorship (15%); and women with chronic distress (16%) (Henselmans et al., 2010). The present study showed that childhood emotional neglect/abuse predicted greater levels of perceived stress at initial assessment; whereas, physical neglect predicted a sustained trajectory of increasing perceived stress. Finding differential effects for childhood emotional versus physical neglect is not unexpected. Evidence from numerous studies Illustrate that the type of adversity (emotional, physical or sexual) shapes the nature of adult vulnerability, as well as relative risk for future psychopathology (Ford, 2010; Teicher et al., 2006).

Symptoms of depression can persist in approximately one-third of women in the first year after breast cancer diagnosis (Bower, 2008; Burgess et al., 2005; Miller et al., 2008), indicating individual susceptibility for depressive symptoms. Consistent with this, longitudinal studies identify unique trajectories of depressive symptoms in women with breast cancer (Deshields et al., 2006; Dunn et al., 2011). Deshields et al. identified five distinct subgroups of depression (using the CES-D) in women undergoing radiation therapy: Never depressed, recover, become depressed, stay depressed, and vacillate (Deshields et al., 2006). More recently, Dunn et al. characterized four distinct profiles of depressive symptoms, based on CES-D scores obtained prior to surgery and up to 6 months after surgery. The trajectory of these profiles was described as Low Decelerating (38.9%), Intermediate (45.2%), Late Accelerating (11.3%), and Parabolic (4.5%). In that study over 60% of women met criteria for clinically relevant depressive symptoms, with younger women being more susceptible (Dunn et al., 2011). Collectively, this literature emphasizes the magnitude of depression risk among women with breast cancer, as well as the individual nature of timing and duration of symptom expression. Our results add to this literature by identifying childhood emotional neglect/abuse as a pre-existing risk factor contributing to individual variation in the manifestation of depressive symptoms. This is clinically relevant, as depressive symptoms heighten risk for poor cancer treatment adherence (Lawrence et al., 2004).

Fatigue is commonly experienced by women with breast cancer, and, like depression, fatigue can persist beyond treatment (Bower et al., 2000). Cancer-related fatigue is more intense and enduring than fatigue resulting from inadequate sleep or physical exertion (Poulson, 2001) and leads to more profound impairments in quality of life (Bower, 2008; Curt, 2000). Thus, for women in this study, who were exposed to childhood adversity, the co-occurrence of depressive symptoms and fatigue likely synergize and compromise recovery of quality of life after breast cancer treatment. On average women in our sample report high levels of fatigue after surgery and during treatment, with MFSI scores approaching values established for clinically relevant fatigue (scores >5) (Liu et al., 2009; Stein et al., 1998). Over time we observed a decreasing pattern of fatigue with levels normalizing approximately 2–3 months after completion of radiation therapy. Childhood emotional neglect/abuse did not influence the pattern of change in fatigue; but predicted higher levels of fatigue at the initial assessment that continued to be higher over the time evaluated. Other longitudinal studies report individual variations in the experience of fatigue among women with breast cancer, and identify demographic, trait, and disease/treatment variables to influence levels of fatigue. Dhruva et al. measured morning and evening fatigue in women with breast cancer before, during and up to 2-months post radiation therapy. Their results showed that being younger, having greater sleep disturbance, and having higher trait anxiety predicted greater morning fatigue at baseline; whereas, advanced disease stage and more medical co-morbidities predicted worse trajectories of fatigue (Dhruva et al., 2010). In contrast, our findings show no effect of disease stage or cancer treatment on initial fatigue levels or the trajectory of fatigue. This is likely due to the more homogenous sample of women evaluated in our study, as none of the women received chemotherapy, while 55% of the women in the study by Dhruva et al. received chemotherapy, in addition to radiation therapy (Dhruva et al., 2010). Consistent with others (Dhruva et al., 2010; Knobf and Sun, 2005), we observed that younger women had higher initial levels of fatigue that persisted over time.

There is a growing literature demonstrating that adults who experienced childhood abuse or neglect exhibit heightened emotional responsiveness to future stress exposure, increasing their risk for mood and anxiety disorders (Green et al., 2010; McLaughlin et al., 2010b). Such enhanced emotional responsiveness to stress has been linked to greater autonomic nervous system and hypothalamic–pituitary–adrenocortical (HPA) axis reactivity to stress (Heim et al., 2008). Considerable evidence from animal models demonstrates that early life stress leads to persistent hyperactivity of brain corticotrophin releasing hormone (CRH) peptidergic systems, which is posited to sensitize the HPA axis to subsequent stress (Meaney, 2001). Consistent with these findings in animals, women exposed to childhood abuse, with or without depression, were found to produce a greater adrenocorticotropic hormone (ACTH) response to stress challenge, compared to control women and to depressed women without such abuse (Heim et al., 2001, 2000b). Moreover, a history of child abuse predicted elevations in cerebral spinal fluid levels of CRH (Heim et al., 2008). It is theorized that childhood adversity induces a neuro-biological vulnerability that predisposes for heightened stress reactivity. For women in our study, this may contribute to greater depressive symptoms and fatigue, two common co-occurring symptoms in women with breast cancer. This supposition is supported by recent findings that identify stress-induced neuroendocrine hormone elevations to serve as a common biological mechanism for the co-occurrence of depressive symptoms and fatigue in women with breast cancer (Thornton et al., 2010).

Childhood abuse or maltreatment can engender a proinflammatory phenotype that persists over the life span (Brydon et al., 2004; Carpenter et al., 2010; Danese et al., 2007; Pace et al., 2006). A large birth cohort study demonstrated maltreated children to exhibit a significant and graded increased risk for low grade inflammation, years later in adulthood (Danese et al., 2007). In contrast, greater maternal emotional warmth was shown to reduce production of the proinflammatory cytokine, IL-6 (Chen et al., 2010a). When subjected to acute laboratory-induced social evaluative stress, healthy adults who experienced childhood maltreatment were shown to mount a greater proinflammatory response than adults without early maltreatment (Carpenter et al., 2010). In our sample of women, mean levels of plasma IL-6 did not change over time; yet, we observed substantial heterogeneity in IL-6 levels between the women. This heterogeneity can be attributed in part to childhood adversity, as women in our study who reported greater childhood physical neglect, exhibited sustained elevations in IL-6, with a slightly increasing trajectory. This contrasted with the decreasing IL-6 trajectory for women with little or no childhood physical ne-

glect. Sustained elevations in IL-6 may contribute to greater risk for more prolonged inflammation-related behavioral symptoms (Bower, 2008; Miller et al., 2008). Substantial evidence demonstrates that circulating proinflammatory cytokines can signal the brain and generate behavioral symptoms (Dantzer and Kelley, 2007; Miller et al., 2008, 2009a; Raison et al., 2006). Upon accessing the brain, peripheral proinflammatory cytokines affect the availability of neurotransmitters relevant to behavior, as well as increase brain CRH production, a key regulator of the stress response that is also implicated in depressive and anxiety like behaviors (Owens and Nemeroff, 1991). Moreover, proinflammatory cytokines can directly affect brain activity in regions involved in mood and anxiety disorders (Capuron et al., 2005, 2007). Previous studies demonstrated that women diagnosed with breast cancer have increased production of proinflammatory cytokines (Bower, 2008; Witek-Janusek et al., 2008, 2007), with concomitant fatigue and depressive symptoms (Bower, 2008; Jehn et al., 2006; Miller et al., 2008; Schubert et al., 2007). It is possible that a proinflammatory phenotype, rooted in childhood adversity, contributes to more fatigue and depressive symptoms in women with breast cancer. Furthermore, sustained elevations in IL-6 may negatively impact cancer prognosis, as others show that circulating IL-6 is associated with worse survival (Knupfer and Preiss, 2007).

The psychological stress associated with breast cancer is accompanied by a reduction in NKCA toward tumor cells (Thornton et al., 2007; Varker et al., 2007; Witek-Janusek et al., 2008, 2007). However, considerable individual differences in the reduction in NKCA during diagnosis and treatment, as well as in the restoration of NKCA following cancer treatment are observed (Thornton et al., 2007). Our results show that group means for NKCA exhibit an increasing trajectory of NKCA with time. However NKCA does not recover to levels consistent with women without breast cancer until months after completion of cancer treatment (Witek-Janusek et al., 2007, 2008). We also observe considerable heterogeneity in individual levels of NKCA and show that a portion of this heterogeneity is related to childhood adversity. Childhood emotional neglect/abuse predicted lower NKCA at initial assessment, and although the trajectory in NKCA was not influenced by childhood adversity, NKCA remained lower for months after completion of cancer treatment for women with such adversity. NK cells are relevant to cancer control, protecting against tumor initiation, growth and metastasis in a variety of ways (Vivier et al., 2011). Higher levels of NKCA in cancer patients correlate with a better prognosis (Gonzales et al., 1998; Koda et al., 1997; Liljefors et al., 2003; Nakamura et al., 2000; Seo and Tokura, 1999; Taketomi et al., 1998); while impaired NKCA correlates with increased tumor invasiveness (Ishigami et al., 2000; Konjević et al., 2001; Levy et al., 1984; Takeuchi et al., 2001; Villegas et al., 2002). NK cells are especially important during critical times marked by risk for tumor dissemination, such as after surgery and during the early recovery phase after completion of adjuvant therapy (i.e. the period of time when women were evaluated in this study) (Avraham and Ben-Eliyahu, 2007; Ben-Eliyahu, 2003; Lutgendorf et al., 2007; Stojanovic and Cerwenka, 2011). Consequently, women with a history of childhood emotional neglect and abuse may be at greater risk for poor health outcomes resultant from reduced NKCA.

As noted, childhood adversity intensifies the inflammatory response to acute laboratory stress during adulthood (Brydon et al., 2004; Carpenter et al., 2010; Pace et al., 2006) and is associated with greater IL-6 and TNF-alpha levels in older adults experiencing chronic stress related to caregiving (Kiecolt-Glaser et al., 2010). However, to our knowledge the findings reported here are the first to demonstrate that early life adversity in humans not only results in a more persistent elevation of circulating IL-6, but also can predispose to lower levels of NKCA during the time period after surgery (i.e. initial assessment). These findings were observed in women experiencing a naturalistic stressor (i.e. breast cancer diagnosis) with clear onset, as opposed to an acute laboratory stressor (Carpenter et al., 2010) or a stressor of long duration, with a slower onset (i.e. caregiving) (Kiecolt-Glaser et al., 2010). Recently, a similar effect was observed in rodents. That study showed early life stress (maternal separation) to not only depress NKCA but to also increase tumor colonization in adult offspring subjected to chronic restraint stress (Nakamura et al., 2011).

The biological mechanism(s) linking childhood emotional neglect/abuse to reduced NKCA in women diagnosed with breast cancer is not clear. It is possible that women with such a history have higher cortisol levels, placing them at greater risk for cortisol-mediated reduction in NKCA (Webster Marketon and Glaser, 2008). Exposure to early life stress alters responsiveness of the HPA axis (Heim et al., 2000a,b; LaPrairie et al., 2010). More recently, early life stress was shown to result in epigenetic modification of stress response systems, especially the HPA axis, as reviewed (Mathews and Janusek, 2011). Evaluations of early life stress in rodents found that adult offspring of low nurturing mothers display anxiety-like behaviors and heightened HPA stress reactivity (Liu et al., 1997; Zhang and Meaney, 2010). This has been linked to reduced glucocorticoid feedback consequent to epigenetic modification of the proximal promoter of the glucocorticoid receptor within the hypothalamus (Francis et al., 1999; Weaver et al., 2004). A similar epigenetic modification to the glucocorticoid receptor gene promoter was observed in human suicide victims with a history of child abuse (McGowan et al., 2009). Further, we show epigenetic (histone) modifications to be associated with the functional activity of PBMC of women diagnosed with breast cancer. These epigenetic modifications were exhibited only during periods of increased psychosocial stress and were reversed when the stress dissipated (Mathews et al., 2011). Whether epigenetic modification, consequent to childhood adversity, underlies the profile of behavioral and immune dysregulation we observed in women with breast cancer requires further investigation.

It is important to understand the influence of childhood adversity on behavioral and immune outcomes in individuals with a serious disease, like cancer. Few studies have done so. A limitation inherent to such studies, including this one, is that it is not realistic to obtain baseline (pre-diagnosis) data. It is conceivable that women exposed to childhood adversity have greater behavioral symptoms and immune dysregulation prior to breast cancer diagnosis, contributing to our observations at initial assessment. Yet our findings are consistent with the literature demonstrating that childhood adversity exerts long-lasting effects resulting in increased stress responsivity during adulthood (Heim et al., 2008; McLaughlin et al., 2010b); thus, the possibility that women are responding more intensely to the stress associated with breast cancer remains plausible. Moreover, this possibility is strengthened in that we observe worse trajectories in perceived stress, quality of life, and circulating IL-6 (i.e. beyond initial assessment) for women who have experienced childhood physical neglect. These results suggest that childhood physical abuse results in slower recovery from the breast cancer experience. Findings from this study were derived from a relatively limited number of women and this can potentially compromise the reliability of the model estimates. However, estimated reliabilities for the initial status and growth rates ranged between 0.62 and 0.85, indicating that the variability in the presented models are likely due to systematic relations between growth estimates and child adversity factors. The longitudinal design and the application of growth curve modeling adds considerable strength to the design, as this approach provides valuable insight regarding intra-individual and inter-individual differences in behavioral and immune outcomes of women across the breast cancer trajectory.

With more women surviving breast cancer, it is increasingly important to understand those factors that can predict worse adjustment and predispose to protracted psychological and immunological recovery from this stressful experience. This is not only essential from the standpoint of quality of life, but also important in that restoration of NKCA after cancer treatment allows for optimal defense against nascent tumor cells that may remain after completion of surgery and adjuvant cancer treatment (Avraham and Ben-Eliyahu, 2007; Ben-Eliyahu, 2003; Lutgendorf et al., 2007).

Conflict of Interest

The authors of this manuscript have nothing to declare.

Acknowledgments

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Diurnal cortisol rhythm as a predictor of lung cancer survival

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ABSTRACT

Background: Poorly coordinated diurnal cortisol and circadian rest-activity rhythms predict earlier mortality in metastatic breast and colorectal cancer, respectively. We examined the prognostic value of the diurnal cortisol rhythm in lung cancer.
Methods: Lung cancer patients ($n = 62$, 34 female) were within 5 years of diagnosis and had primarily non small-cell lung cancer, with disease stage ranging from early to advanced. Saliva collected over two days allowed calculation of the diurnal cortisol slope and the cortisol awakening response (CAR). Lymphocyte numbers and subsets were measured by flow cytometry. Survival data were obtained for 57 patients. Cox Proportional Hazards analyses were used to test the prognostic value of the diurnal cortisol rhythm on survival calculated both from study entry and from initial diagnosis.
Results: The diurnal cortisol slope predicted subsequent survival over three years. Early mortality occurred among patients with relatively "flat" rhythms indicating lack of normal diurnal variation (Cox Proportional Hazards $p = .009$). Cortisol slope also predicted survival time from initial diagnosis ($p = .012$). Flattened profiles were linked with male gender ($t = 2.04$, $df = 59$, $p = .046$) and low total and cytotoxic T cell lymphocyte counts ($r = -.39$ and $-.30$, $p = .004$ and .035, respectively). After adjustment for possible confounding factors, diurnal slope remained a significant, independent predictor of survival.
Conclusions: Flattening of the diurnal cortisol rhythm predicts early lung cancer death. Data contribute to growing evidence that circadian disruption accelerates tumor progression.

1. Introduction

Among patients with advancing cancer, dysregulation of circadian physiology is often conspicuous and extensive. Disrupted circadian cycles are evident in endocrine, immune, metabolic, and cellular systems (Mazzoccoli et al., 2010; Hrushesky, 1985; Mormont et al., 2000). Dysregulation of circadian hypothalamic–pituitary-adrenal (HPA) rhythms has been linked specifically with advancing cancer and accelerated tumor growth rates (Mormont and Lévi, 1997; Touitou et al., 1995; Hrushesky et al., 1998; Sephton and Spiegel 2003; Eismann et al., 2010).

Human cortisol peaks 30–45 min after first awakening and drops to a nadir during sleep (Clow et al., 2010). From 30% to 70% of patients with advanced cancer display idiosyncratic rhythm abnormalities including unsynchronized peaks and troughs, consistently high or low levels and erratic circadian fluctuations. Viewed in aggregate, the diurnal cortisol profiles of advanced cancer patients often appear "flattened" (Mormont and Lévi, 1997; Sephton et al., 2000). The diurnal cortisol slope captures multiple types of rhythm deviation and is measurable in saliva (Turner-Cobb et al., 2000; Kraemer et al., 2006; Hellhammer et al., 2009). Elevated diurnal cortisol slope predicts early metastatic breast cancer mortality, independent of other prognostic factors (Sephton et al., 2000). Flattened slopes were associated with low natural killer cell counts and cytotoxicity, sleep disruption, and marital dissolution; none of which explained the cortisol-survival relationship (Sephton et al., 2000). Circadian rest/activity rhythms are also irregular in patients with advancing cancer (Mormont and Lévi,

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1997), and are measurable by wrist-worn actigraphy (Ancoli-Israel et al., 2003). Poor circadian coordination of rest/activity rhythm predicts early mortality over four years in metastatic colorectal cancer patients (Mormont et al., 2000), a finding recently replicated in large multi-site study (Innominato et al., 2009). Among cancer patients, circadian disruption may be a preexisting cause, a correlate of distress and physiological burden, a direct effect of cancer on the brain, and/or a mediator of psychosocial effects on tumor progression (Sephton and Spiegel 2003; Eismann et al., 2010; Chida et al., 2008). A few clinical prospective studies have provided a fascinating demonstration of prognostic value for circadian rhythms in human cancers. These require replication and testing in other cancer types.

As compared with the comorbidities of other cancers, lung cancer-related distress, depression, fatigue, and sleep disruption are extreme (Parker et al., 2008). All these symptoms have been linked with circadian disruption in lung cancer, suggesting that this disease may convey uniquely high circadian disruption-mediated risk (Hrushesky et al., 2009). Lung cancer patients demonstrate markedly poor integration of circadian neuroendocrine-immune function evidenced by high evening cortisol, suppressed melatonin, and changes in the circadian patterns of distribution of peripheral lymphocytes (Mazzoccoli et al., 2003, 2005). Non-small cell lung cancer generally has a better prognosis than small-cell disease; however, five-year survival rates for both are poor (49–75% for early stage, and < 1% for advanced disease).

We examined the prognostic value of the diurnal salivary cortisol rhythm in lung cancer. Secondary analyses explored associations with potential explanatory and/or confounding factors including traditional prognostic indicators, depression, fatigue, and sleep quality. Additional biological factors were chosen based on their relevance in cancer studies of the effects of the stress, sleep, and circadian rhythms (Cole and Sood, 2012; Antoni et al., 2006; Eismann et al., 2010; Mazzoccoli et al., 2003, 2005). These included serum cortisol, sympathetic activation measured by overnight urinary catecholamines, lymphocyte counts and lymphocyte subsets.

2. Methods

2.1. Subjects and procedure

Eligible patients were at least 18 years old and had been diagnosed with Stage I-IV non-small cell lung cancer or with limited to extensive small cell lung cancer during the last five years. Exclusions were made for history of psychiatric hospitalization, alcohol abuse or dependence within the past two months, use of prednisone or concurrent medical conditions likely to influence short-term (six month) survival. A total of 223 patients were referred or approached for this study, 145 (65%) were eligible and of those eligible, 62 (42.7%) patients were enrolled after informed consent. Physician's diagnosis of lung cancer was confirmed. Reasons reported for refusal included feeling too ill, being too busy, or lack of interest.

Participants were scheduled for a one hour interview with a research assistant during which they provided informed consent, demographic, and medical information including smoking history and performance status. A blood sample was drawn and they were given instructions and materials for home-based saliva collection as well as self-report questionnaires. Home-based questionnaires assessed depressive symptoms, fatigue, and sleep difficulties. Eight saliva samples were collected over two consecutive days for measurement of diurnal salivary cortisol profiles. Fifteen-hour overnight urine collection provided estimates of catecholamine hormone release. Blood samples were drawn for flow cytometry. Participants were provided with $100 compensation when home-based data collection materials were returned. Medical data, including cancer stage at diagnosis, was collected both from medical chart review and from the Kentucky Cancer Registry.

2.1.1. Sample characteristics

Study participants ranged in age from 41 to 84 years with a mean age of 64 years (SD = 9.12). Thirty-four (55%) of those enrolled were female and 58 (94%) had non-small cell lung cancer. Eighty-five percent of the sample was Caucasian and 55% had twelve years of education or less. Forty-six percent of the sample was married and 25% were employed. Participants were a mean age of 62 years at the time of lung cancer diagnoses (SD = 8.9). The median time since lung cancer diagnosis was 21 months, with a range of 1 month to 5 years. Six participants (10.7%) scored $\geqslant$ 19 on the BDI, which is consistent with clinically significant depressive symptoms. Table 1 displays the frequency and percentage of participants according to disease status. The sample included similar proportions of early (stage 1, $n = 29$) and more advanced (stage 2–4, $n = 33$) disease. Table 2 presents descriptive information for psychological and physiological variables.

Approximately two years after enrollment closed, data tracking for the study was closed and the investigators contacted the Kentucky Cancer Registry and consulted medical records for survival data. Registry and record review yielded documentation of death for 23 patients. Among patients who had died, median time from study entry to death was 317 days (range: 34 days to 2.7 years). Median time from diagnosis to death was 2.7 years (range: 7 months to 6 years). Causes of death included malignant neoplasms of the bronchus or lung ($n = 11$, 47.8%), chronic obstructive pulmonary disease ($n = 1$, 4.3%), and acute myocardial infarction ($n = 1$, 4.3%). Information on cause of death was unavailable for nine of those who died (39.1%). Among the entire sample, median follow up time from study entry was 2.2 years (range 34 days to 3.4 years). Median follow up from the date of lung cancer diagnosis was 4 years (range 7 months to 8.9 years).

2.2. Physiological measures

2.2.1. Diurnal salivary cortisol

At the initial interview subjects received a saliva collection kit and were given instructions for collection. Kits included 8 pre-labeled collection tubes, or "salivette" devices (Walter Sarstedt Inc., Newton, North Carolina) with instructions on how and when to collect saliva samples and a questionnaire covering factors likely to introduce variation in cortisol levels (e.g., stressors, exercise). Subjects were instructed to sample saliva on two consecutive days. Saliva was collected at waking, 45 min after waking (45+), 1600, and 2100 h on each day. This enabled measurement of the diurnal cortisol slope, diurnal mean levels, the cortisol response to awakening (CAR), diurnal mean, area under the curve (AUC), and waking and evening levels. Subjects stored cortisol kits in their refrigerator until they were returned. Samples were centrifuged, aliquoted, and

Table 1
Numbers of participants with each cancer type and stage.

	Frequency	Percent (%)
Non-small cell lung cancer	58	94
Stage I	27	44
II	7	11
III	17	27
IV	7	11
Small cell lung cancer[a]	4	6

[a] Among the four patients with small-cell lung cancer, two had limited, and two had extensive stage cancer.

Table 2
Mean and standard deviation (SD) values for psychological and physiological variables.

	N	Mean	SD
Diurnal cortisol slope log(μg/dL)/Hr	61	−0.114	0.062
CAR log(μg/dL)/Hr	58	0.705	1.426
BDI	61	10.5	8.2
PSQI	62	13.8	7.7
Serum cortisol μg/dL	55	14.5	8.0
Urinary Epinephrine, ng/mL/Hr	57	1.80	1.53
Urinary Norepinephrine, ng/mL/Hr	62	62.60	45.00
White blood cell count $\times 10^3$/μL	54	6.748	2.389
Absolute lymphocyte count $\times 10^3$/μL	54	1.494	0.662
CTL count $\times 10^3$/μL	52	0.344	0.224
NK cell count $\times 10^3$/μL	52	0.233	0.179

stored at −80 °C until assay. Cortisol levels were assessed using an enzyme immunoassay developed for use in saliva (Salimetrics, Inc., State College, PA). Assay sensitivity was 0.007 μg/dL. The inter-assay coefficient of variation (*CV*) was 9.55% using the low control and 4.75% using the high control. Intra-assay *CV* average was 3.75% using the low control and 3.06% using the high control.

The diurnal cortisol slope was calculated by regressing log-transformed cortisol on sample collection time with 45 + samples excluded: these were used to calculate the cortisol awakening response (CAR; Kraemer et al., 2006). Since the distribution of raw cortisol values is typically skewed and the normal diurnal profile may be approximated by an exponential curve, raw values were log transformed. The regression of six of the values (waking, 1600, and 2100 collections over two days) upon the hour of sample collection was calculated. The unstandardized beta (slope of the regression) was used as a measure of the diurnal salivary cortisol rhythm (Kraemer et al., 2006). Mean log-transformed cortisol values were calculated. In accordance with recommendations that meaningful CAR measurement should include both the first waking sample plus a measure of change (Kraemer et al., 2006; Clow et al., 2010), the CAR was calculated by regressing log-transformed values of the first two samples (waking and 45 + over two days) upon collection time and using the unstandardized beta (slope of the regression) as a measure of CAR. Recommendations are based on the notion that diurnal slope and CAR are driven by somewhat different regulatory mechanisms. Both calculations involved the waking cortisol value and thereby share some variance; but post-waking samples were used only in the CAR calculation, allowing greater statistical distinction between these two outcomes and reflecting their different biological underpinnings.

2.2.2. Serum cortisol

A blood sample totaling less than 30 ml was obtained during the interview. Aliquots of 1 ml serum were frozen at −80 °C until enzyme-linked immunosorbent assays (ELISA) were conducted to determine levels of serum cortisol (ALPCO Diagnostics, Salem, NH). The sensitivity for the assay of serum cortisol was 0.3 μg/dL. The inter-assay *CV* for serum cortisol was 14.5%. The intra-assay *CV* was 11.2%.

2.2.3. Sympathetic activation

Fifteen-hour urinary epinephrine and norepinephrine levels were measured as an indicator of systemic sympathetic activation. Participants were given urine collection containers and asked to complete a 15-h overnight urine collection from 5:00 pm to 8:00 am the next morning. Samples were kept in coolers until return, at which time urine volume was recorded. Four aliquots were frozen at −80 °C until ELISA assays for epinephrine and norepinephrine were performed (ALPCO Diagnostics, Windham, NC). The sensitivity, inter-assay *CV*, and intra-assay *CV* for urinary epinephrine were 0.33 ng/mL, 15.5%, and 11.6%, respectively. The sensitivity, inter-assay *CV*, and intra-assay *CV* for urinary norepinephrine were 1.33 ng/mL, 10.3% and 8.5%, respectively. Concentrations are expressed per hour of collection.

2.2.4. Lymphocyte counts

Whole blood samples were subjected to flow cytometry for measurement of white blood cell count, total lymphocyte count, CD3/CD8 cytotoxic T lymphocyte (CTL) count, and CD3-/CD56 natural killer (NK) cell count.

2.3. Psychosocial measures

2.3.1. Beck depression inventory (BDI)

The BDI is a 21-item questionnaire that is routinely used to assess affective, cognitive, motivational, behavioral, and biological symptoms of depression and has acceptable psychometric properties (Beck et al., 1988). Internal consistency for the BDI ranges from .73 to .92 with a mean of .86 (Beck et al., 1988). The BDI demonstrates alpha coefficients of .86 and .81 for psychiatric and non-psychiatric populations, respectively and has a split-half reliability coefficient of .93 (Beck et al., 1988). The summary score was used in this study.

2.3.2. Fatigue symptom inventory (FSI)

The FSI is a 13-item self-report measure designed to assess the intensity and duration of fatigue and its interference with quality of life (Hann et al., 1998). This instrument has demonstrated construct and convergent validity as well as high internal consistency (Hann et al., 1998, 2000). The FSI is established as a valid and reliable measure of fatigue in cancer patients (Hann et al., 1998, 2000). The fatigue interference with quality of life score was used in analyses.

2.3.3. Pittsburgh sleep quality index (PSQI)

The PSQI is a measure of sleep quality and disturbances over a 1-month time period (Buysse et al., 1989). Psychometric evaluation supports the internal consistency, reliability and construct validity of the PSQI and it has proven utility in cancer patient populations (Beck et al., 2004; Carpenter and Andrykowski, 1998). The global PSQI score was used in analyses, a higher score indicates poorer sleep quality.

2.4. Statistical methods

Survival information was available for 57 patients who remained in Kentucky. We received sufficient salivary data to calculate the diurnal cortisol slope for all but one of these participants. Survival was calculated both from each participant's date of study entry and date of initial diagnosis.

The primary hypothesis was tested by analyzing the relationship between baseline diurnal cortisol slope and subsequent survival time. Separate regressions were conducted on survival time measured from the date of study entry and date of initial diagnosis, using the Cox Proportional Hazards Model in two-tailed tests. These analyses were repeated after excluding the minority of subjects with small cell lung cancer.

Secondary hypotheses were exploratory in nature, and were tested with conservative intent, to rule out the possibility that any relationship between the cortisol slope and survival time was explained by variance due to some other factor related to the disease or treatment process. These hypotheses evaluated the possible contribution of traditional prognostic indicators, including cancer stage, performance status, age at diagnosis, time since diagnosis, gender, socioeconomic status, smoking, prior chemotherapy and radiotherapy, and additional possible explanatory and/or con-

founding variables, including depressive symptoms, fatigue, self-reported sleep, serum cortisol, overnight urinary catecholamine levels, and lymphocyte counts and subsets. Bivariate Spearman rank correlations with one-tailed tests were used with the intent of most conservatively identifying factors associated with the cortisol slope that might explain the relationship between cortisol rhythm and survival. For factors significantly associated with the diurnal cortisol rhythm, the contributions of these variables to the cortisol rhythm-survival relationship were tested by entering each variable as a covariate with the cortisol slope in a separate Cox regression. As a check, we also entered simultaneously into one Cox regression the cortisol slope with all potential confounding variables significantly associated with diurnal cortisol slope.

Because of tight connections between the hypothalamic central clock and the CAR response, we explored the predictive value of the CAR on survival with the rationale that it is also a possible indicator of circadian disruption. We tested the predictive value of cortisol AUC to assess possible effects of overall cortisol elevation as opposed to diurnal rhythmicity. The prognostic value of total lymphocyte and CTL counts was also tested because of previous data suggesting their potential value (Sephton et al., 2000; Blake-Mortimer et al., 2007).

Based on the effect sizes we reported previously (Sephton et al., 2000), a power estimate of 76% was predicted for this sample (Faul et al., 2007).

3. Results

The diurnal cortisol slope predicted subsequent survival over a period of up to 3 years. Early mortality occurred among patients with higher slopes, or relatively "flat" rhythms indicating lack of normal diurnal variation (Cox Proportional Hazards two-tailed p(Wald) = .009; hazard ratio = 68052.8; 95% confidence interval (CI) = 15.481–299154617.4). Cortisol slope also predicted survival time from initial diagnosis (p = .019; hazard ratio = 34867.4; 95% CI = 5.488–221531615.074). Results retained significance when primary analyses were repeated after removing the four subjects with small cell lung cancer (p = .050). Neither the diurnal cortisol AUC nor the CAR were associated with subsequent survival time.

Although the survival analyses was conducted using the entire sample and cortisol slope as a continuous variable, for descriptive (not statistical) purposes, subjects were split into two equal groups based on a median split of the cortisol slope. Fig. 1 shows the mean raw cortisol levels at wake, 1600 and 2100 h for these two groups. Fig. 2 shows Kaplan–Meier survival curves of the two groups with

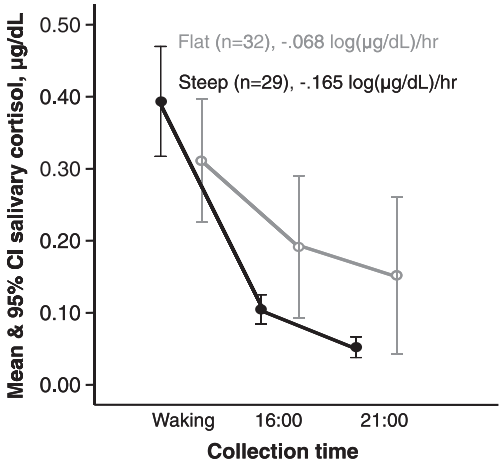

Fig. 1. For descriptive purposes, mean and 95% confidence interval raw diurnal salivary cortisol levels are shown across two days of saliva sample collection. Patients were split at the median diurnal cortisol slope, −.107 log(µg/dL)/hr.

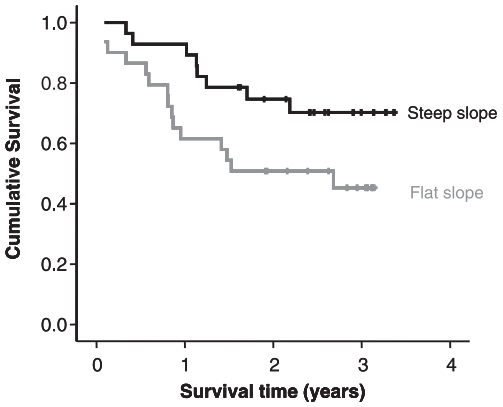

Fig. 2. Kaplan–Meier survival curves for patients split at the median diurnal cortisol slope into two equal groups at the median diurnal cortisol slope. Cox proportional hazards models demonstrated that diurnal cortisol slope was prognostic for lung cancer survival over three years.

survival measured from the time of study entry. The divergence in survival as a function of slope emerged within the first year after cortisol assessment and extended at least three years after. Comparison of the two groups of patients split at the median cortisol slope reveals that 54% of those with flattened rhythms died, but death occurred in only 27% of those with comparatively rhythmic diurnal cortisol patterns. Survival plots of the two groups diverged significantly (log-rank, p = .046, two-tailed). Table 3 presents mean and SD numbers of lymphocytes and counts of lymphocyte subsets within groups split on the median diurnal cortisol slope.

Flattened cortisol rhythms were linked with more advanced lung cancer stage (r = .35, p = .003), poor performance status (Karnofsky; r = −.29, p = .012), male gender (t = 2.04, df = 59, p = .028; Fig. 3) and low total lymphocytes and CTL r = −.39 and −.30, p = .002 and .017, respectively). After adjustment for each of these factors, diurnal slope remained a significant, independent predictor of survival. As a check, we also entered simultaneously into a Cox regression the cortisol slope in addition to stage, Karnofsky rating, gender, total lymphocyte counts, and CTL counts. The diurnal cortisol slope retained significance in this analysis (p = .02). When the sample was split by gender, the diurnal cortisol slope retained prognostic significance only among women (p = .014), while it lost significance among the small group of male cancer patients.

Analyses revealed no significant associations of diurnal cortisol slope with the CAR or with age at diagnosis, cancer type, time since radio- or chemotherapy, socioeconomic status, prior marital disruption, depressive symptoms, fatigue, or sleep difficulties. The diurnal salivary cortisol slope was not associated with serum cortisol levels, urinary catecholamine levels, or NK cell counts.

4. Discussion

Flattening of the diurnal cortisol rhythm predicted early mortality among lung cancer patients, a replication of our metastatic

Table 3
Mean and SD absolute lymphocyte counts and subsets within groups of lung cancer patients who were split at the median diurnal cortisol slope (cortisol and survival data for this grouping of participants are shown in Figs. 1 and 2).

	Diurnal cortisol slope			
	Steep		Flat	
	Mean	SD	Mean	SD
White blood cell count × 10^3/µL	6.064	1.930	7.289	2.648
Total lymphocyte count × 10^3/µL	1.604	0.632	1.361	0.661
CTL count × 10^3/µL	0.416	0.239	0.246	0.179
NK cell count × 10^3/µL	0.216	0.163	0.249	0.198

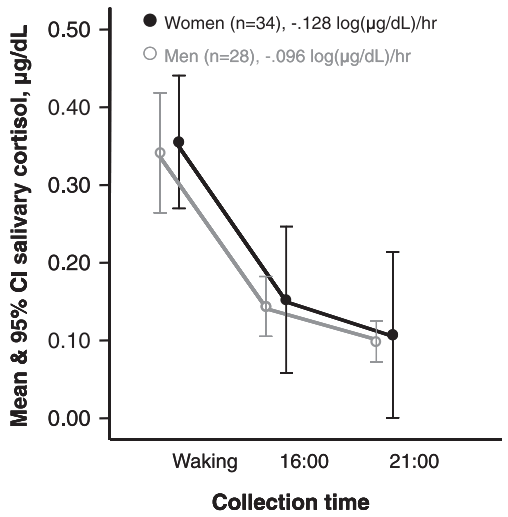

Fig. 3. For descriptive purposes, mean raw cortisol levels are shown at waking, 1600 and 2100 h for male and female patients. Cortisol rhythms examined by gender revealed that men had significantly higher (flatter) slope values than women, $t(59) = 2.039$, $p = .04$.

breast cancer finding (Sephton et al., 2000), and recently noted in patients with metastatic renal cell carcinoma (Cohen et al., 2012). As originally observed in breast cancer patients, early mortality occurred in patients with relatively flat diurnal rhythms. These results are comparable with data from two clinical prospective studies demonstrating similar prognostic value for circadian rest/activity rhythms among colorectal cancer patients (Mormont et al., 2000; Innominato et al., 2009).

Cortisol and rest/activity rhythms are both strong markers of endogenous circadian phase with potential roles as zeitgeibers: signals from the central clock that synchronize peripheral circadian rhythms (Reppert and Weaver, 2002; Mavroudis et al., 2012). The hypothalamic suprachiasmatic nucleus (SCN) coordinates rhythmic activities of peripheral cells using multiple signaling mechanisms including circadian rhythms of cortisol, behavior, and sympathetic nervous activity (Reppert and Weaver, 2002; Dickmeis and Foulkes, 2011; Lee et al., 2010; Gillette and Abbott, 2006; Balsalobre et al., 2000). Circadian signals regulate peripheral tumor suppressor and cell cycle genes that control cancer-relevant processes including cell proliferation, differentiation, and apoptosis (Lee et al., 2010). Recent data suggest the central circadian clock functions as a tumor suppressor (Fu and Lee, 2003).

4.1. Circadian disruption can promote cancer incidence and accelerate tumor progression

New data from clinical, animal, cellular, and molecular studies reveal that central circadian disruption can promote cancer incidence and accelerate tumor growth (Sephton and Spiegel 2003; Eismann et al., 2010). Increased risk for both incidence and progression of human breast cancer is conferred by variants of circadian genes Per3 and NPAS2 (Zhu et al., 2005, 2008; Yi et al., 2010). Manipulating murine light–dark cycles can increase spontaneous and radiation-induced tumor development, speed implanted tumor growth, and cause early death (Lee et al., 2010; Filipski et al., 2002). Mice with SCN lesions or central clock gene mutations suffer spontaneous tumor development and accelerated growth of implanted tumors (Fu and Lee, 2003; Filipski et al., 2006, 2009). When the central clock is disturbed, HPA rhythms are altered and peripheral cellular clocks appear to lose control over cell proliferation, resulting in amplification of tumor growth rhythms (Lee et al., 2010; Yang et al., 2009a,b). Interestingly, tumor growth is doubled under an experimental regimen that selectively disrupts circadian glucocorticoid rhythms (Filipski et al., 2002).

Human data also point to circadian effects on tumor initiation and growth. Recent epidemiological results have been so convincing that the World Health Organization now concludes shift work is probably carcinogenic (2A) (Megdal et al., 2005; Straif et al., 2007).

4.2. Cancer can initiate and exacerbate circadian disruption

Circadian–cancer relationships are bidirectional. Animal data show that cancer can cause circadian disruption. Tumors may become unresponsive to host circadian signals, disrupt the function of the central clock, and dysregulate peripheral circadian timing systems (Mormont and Lévi, 1997; Sephton and Spiegel, 2003; Eismann et al., 2010; Fu and Lee, 2003). Tumor burden-induced changes in physiological factors, including circulating cytokines, can disrupt circadian rhythms both in the brain and in the periphery. Further, human cancer and its treatments may profoundly disrupt circadian function (Mormont and Lévi, 1997; Sephton and Spiegel, 2003; Eismann et al., 2010): cancer-related anxiety and depression can induce sleep loss. Medication side effects, physical, existential, and interpersonal stressors can all exact a toll on circadian systems (Sephton and Spiegel 2003; Eismann et al., 2010; Chida and Steptoe, 2009). Given the bidirectional nature of effects, it is impossible to infer causality from the current prognostic data.

4.3. Circadian disruption: an important factor in psycho-oncology

We also replicated correlations between flattened cortisol rhythms, more advanced cancer stage, and poor performance status (Mazzoccoli et al., 2010; Sephton and Spiegel 2003; Eismann et al., 2010). Flattened diurnal cortisol rhythms have previously been noted among metastatic cancer patients with depression or emotional repression/anxiety (Jehn et al., 2006; Giese-Davis et al., 2004). Cancer patients with marked circadian disruption suffer greater fatigue, sleep disruption, anxiety, depression, and cognitive difficulties (Miller et al., 2008) as well as more intrusive thoughts and greater use of avoidant coping (Dedert et al., 2012). Concomitant alteration of cortisol secretion with elevated immune and inflammatory mediators has been observed in both cancer patients and cancer survivors (Sephton et al., 2009; Rich et al., 2005; Irwin et al., 2012; Bower et al., 2005a,b). Although these findings do not imply the direction of effects, such associations are important future considerations for psycho-oncology research (Porter, 2012).

Lung cancer prognosis is generally poorer in men than in women. The more marked cortisol disruption seen among males mirrors some, but not all data from healthy individuals (Saxbe et al., 2008; Larsson et al., 2009). Fig. 3 shows mean raw cortisol for male and female patients. Although men had significantly flatter diurnal profiles ($t(59) = 2.039$, $p = .04$), gender was not a significant predictor of survival, nor did it significantly alter the cortisol-survival relationship. The persistence of prognostic value for the cortisol rhythm among the subsample of female patients is remarkable, given the small number of subjects in this secondary analyses.

4.4. Circadian effects in cancer-relevant psychoneuroimmune and endocrine pathways

This study was guided by our theoretical model of circadian effects in cancer-relevant psychoneuroimmune and endocrine pathways (Sephton and Spiegel 2003; Eismann et al., 2010).

As hypothesized, lung cancer patients with flattened cortisol rhythms had lower total lymphocyte and CTL counts. Similar associations have previously been noted among lung and metastatic

breast cancer patients (Mazzoccoli et al., 2010; Sephton et al., 2000) as well as healthy subjects (Dimitrov et al., 2009). Low CTL counts have previously been shown to predict early cancer mortality (Blake-Mortimer et al., 2004), but this association was not observed here.

Links between hormonal and immune rhythm disturbances have been observed in numerous cancer types (Touitou et al., 1995; Touitou et al., 1996; Abercrombie et al., 2004; Mazzoccoli et al., 2003, 2010; Mormont and Lévi, 1997).

Among cancer patients with flattened cortisol rhythms; suppressed functional cellular immunity and inflammation have been reported (Miller et al., 2008; Desantis et al., 2011; Mravec et al., 2008), and this group of symptoms has been linked with poor treatment outcome (Rich et al., 2005). Glucocorticoids may dysregulate immunity via circadian-immune communication (Arjona and Sarkar, 2008) or epigenetic mechanisms (Krukowski et al., 2011). In turn, cancer-related cytokine secretion may contribute to the dysregulation of circadian HPA rhythms (Raison et al., 2006).

A large prospective study recently demonstrated prognostic significance for flattened diurnal cortisol slope in cardiovascular death (Kumari et al., 2011), possibly reflecting associations of circadian disruption with inflammation. Similar to our results, this study found no predictive value for the CAR or diurnal cortisol AUC. The distinct prognostic value of the diurnal cortisol slope may be related to specific mechanisms of regulatory control. For example, the SCN and sympathetic nervous system are crucial regulators of both circadian and awakening glucocorticoid responses (Clow et al., 2010); but circadian cortisol is specifically responsive to pineal melatonin and the peripheral adrenal circadian clock, which controls diurnal sensitivity of the axis (Dickmeis, 2009; Son et al., 2008).

In contrast with previous studies among cancer samples (Weinrib et al., 2010; Miller et al., 2008); the diurnal cortisol slope was not associated with distress, fatigue, or sleep difficulties in this sample. Within lung cancer research, associations between circadian dysregulation and health-related quality of life have primarily been noted in advanced stage patients (Levin et al., 2005). The mixed-stage composition of our sample may not have lent itself to detection of this association.

4.5. Limitations of this research

These data do not prove a causal connection between cortisol rhythm and lung cancer survival. The sample was relatively small, and this problem was compounded by the loss of some physiological data due to limited sample volumes and difficulty collecting blood from a few of the patients (Table 2).

4.6. Future directions for research

Recent research has focused on clock genes as tumor suppressors (Reddy and O'Neill, 2010), but few studies have examined circadian impacts on tumor defense mechanisms or cellular factors that may support tumor growth (e.g., VEGF; Koyanagi et al., 2003).

Circadian effects in cancer-relevant psychoneuroendocrine and immune pathways should also be considered. We need to elucidate interrelationships of circadian, neuroendocrune and immune function and discover whether circadian-immune impairments can accelerate neoplastic disease (Logan and Sarkar, 2012; Scheff et al., 2010). Our model (Sephton and Spiegel, 2003; Eismann et al., 2010) and additional recent data (Cohen et al., 2012; Lyon et al., 2008) suggest comprehensive assessment of cytokines and other immune parameters will help describe these relationships, reveal mediating mechanisms, and inform treatment strategies. Recent data highlight multiple pathways by which stress may accelerate tumor progression, and include convincing results regarding sympathetic neural pathways (Antoni et al., 2006; Cole and Sood, 2012). However, very little focus has been devoted to circadian disruption as a possible mediator of psychosocial effects on tumor progression (Chida et al., 2008; Sephton and Spiegel, 2003; Eismann et al., 2010). Cortisol rhythms are strong candidate mechanisms due to their prognostic value and potential to coordinate peripheral cell processes crucial to tumor growth (Sapolsky and Donnelly, 1985; Ben-Eliyahu et al., 1991). Poor coordination of cortisol rhythms has been associated with failure of negative feedback inhibition of the HPA axis, suggesting that central clock and downstream HPA dysregulation are interrelated in advanced cancer patients (Spiegel et al., 2006). Future studies should also consider the endogenous pulsatility of the HPA axis as an aspect of circadian regulation (Young et al., 2004).

Manipulating circadian biology may prove an important weapon in the anti-cancer arsenal (Gery and Koeffler, 2010). A number of clinical strategies are known to effectively synchronize the central clocks of normal individuals. It remains to be seen if these are effective in reversing cancer-related circadian disruption, and transmitting quality or quantity-of-life benefits (Hrushesky et al., 2009). Behavioral and/or pharmacologic interventions may productively be targeted at circadian phenomena including HPA rhythms, sleep, light exposure and melatonin, physical activity, dietary and social behavior (Porter, 2012; Eismann et al., 2010). A promising current application of circadian biology is chronomodulation of chemotherapy, in which the timing of drug delivery is modulated according to circadian rhythms of the cancer patient to increase efficacy and reduce toxicity (Hrushesky, 1985; Innominato et al., 2010). A recent meta-analysis of chronotherapy studies reported a significant survival benefit (Liao et al., 2010).

Lung cancer poses a range of challenges exemplified by elevated rates of anxiety and depression related to breathlessness and pain (Pasquini et al., 2006). This complex range of factors for lung cancer patients. Suggests a need for multifaceted interventions.

5. Conclusions

Animal, cell, and molecular data support bidirectional effects: tumors can cause circadian disruption, but circadian disruption can also accelerate tumor growth and mortality (Fu and Lee, 2003). Taken together; the prospective nature of the current data, the independence of the primary finding from influence of any other prognostic indicator, the consistency with prior work in other cancers, and the support of a growing body of evidence from animal and cellular studies allow some confidence in the conclusion that circadian dysregulation is associated with more rapid human cancer progression. Together with research on tumor-suppressive effects of the central clock and clinical studies showing benefit from chronomodulation in cancer treatment; these data point to disrupted HPA rhythms as a risk factor for cancer progression and an area for additional therapeutic opportunity.

Conflict of Interest

The authors of this manuscript have nothing to declare.

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Brain, Behavior, and Immunity 30 (2013) S171–S172

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Articles in Press

Fabricia Petronilho, Susane Raquel Périco, Francieli Vuolo, Francielle Mina, Larissa Constantino, Clarissa M. Comim, João Quevedo, Diogo Onofre Souza, Felipe Dal-Pizzol. Protective effects of guanosine against sepsis-induced damage in rat brain and cognitive impairment

Chiara Bufalino, Nilay Hepgul, Eugenio Aguglia, Carmine M. Pariante. The role of immune genes in the association between depression and inflammation: A review of recent clinical studies

Christopher P. Fagundes, Ronald Glaser, Beom Seuk Hwang, William B. Malarkey, Janice K. Kiecolt-Glaser. Depressive symptoms enhance stress-induced inflammatory responses

Marco Di Nicola, Annamaria Cattaneo, Nilay Hepgul, Marta Di Forti, Katherine J. Aitchison, Luigi Janiri, Robin M. Murray, Paola Dazzan, Carmine M. Pariante, Valeria Mondelli. Serum and gene expression profile of cytokines in first-episode psychosis

Paula A. Garay, Elaine Y. Hsiao, Paul H. Patterson, A.K. McAllister. Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development

Shawn Hayley, Jeff Scharf, Hymie Anisman. Central administration of murine interferon-α induces depressive-like behavioral, brain cytokine and neurochemical alterations in mice: A mini-review and original experiments

Francis E. Lotrich, Barry Sears, Robert K. McNamara. Elevated ratio of arachidonic acid to long-chain omega-3 fatty acids predicts depression development following interferon-alpha treatment: Relationship with interleukin-6

Angela W. Corona, Diana M. Norden, John P. Skendelas, Yan Huang, Jason C. OóConnor, Marcus Lawson, Robert Dantzer, Keith W. Kelley, Jonathan P. Godbout. Indoleamine 2,3-dioxygenase inhibition attenuates lipopolysaccharide induced persistent microglial activation and depressive-like complications in fractalkine receptor (CX_3CR1)-deficient mice

Thomas W. McDade, Morgan Hoke, Judith B. Borja, Linda S. Adair, Christopher Kuzawa. Do environments in infancy moderate the association between stress and inflammation in adulthood? Initial evidence from a birth cohort in the Philippines

Brian J. Miller, Andrew Mellor, Peter Buckley. Total and differential white blood cell counts, high-sensitivity C-reactive protein, and the metabolic syndrome in non-affective psychoses

Andrew Steptoe, Anna Wikman, Gerard J. Molloy, Nadine Messerli-Burgy, Juan-Carlos Kaski. Inflammation and symptoms of depression and anxiety in patients with acute coronary heart disease

Mirjam van Zuiden, Annemieke Kavelaars, Elbert Geuze, Miranda Olff, Cobi J. Heijnen. Predicting PTSD: Pre-existing vulnerabilities in glucocorticoid-signaling and implications for preventive interventions

Star W. Lee, Ursula Haditsch, Branden J. Cord, Raphael Guzman, Soo Jeong Kim, Chotima Boettcher, Josef Priller, Brandi K. Ormerod, Theo D. Palmer. Absence of CCL2 is sufficient to restore hippocampal neurogenesis following cranial irradiation

Nicole DellaGioia, Lesley Devine, Brian Pittman, Jonas Hannestad. Bupropion pre-treatment of endotoxin-induced depressive symptoms

Jonathan Savitz, Mark Barton Frank, Teresa Victor, Melissa Bebak, Julie H. Marino, Patrick S.F. Bellgowan, Brett A. McKinney, Jerzy Bodurka, T. Kent Teague, Wayne C. Drevets. Inflammation and neurological disease-related genes are differentially expressed in depressed patients with mood disorders and correlate with morphometric and functional imaging abnormalities

Elizabeth Breece, Brian Paciotti, Christine Wu Nordahl, Sally Ozonoff, Judy A. Van de Water, Sally J. Rogers, David Amaral, Paul Ashwood. Myeloid dendritic cells frequencies are increased in children with autism spectrum disorder and associated with amygdala volume and repetitive behaviors

Jennifer C. Felger, Li Li, Paul J. Marvar, Bobbi J. Woolwine, David G. Harrison, Charles L. Raison, Andrew H. Miller. Tyrosine metabolism during interferon-alpha administration: Association with fatigue and CSF dopamine concentrations

Vanessa A. Peters, Jennifer J. Joesting, Gregory G. Freund. IL-1 receptor 2 (IL-1R2) and its role in immune regulation

B.S. Rawdin, S.H. Mellon, F.S. Dhabhar, E.S. Epel, E. Puterman, Y. Su, H.M. Burke, V.I. Reus, R. Rosser, S.P. Hamilton, J.C. Nelson, O.M. Wolkowitz. Dysregulated relationship of inflammation and oxidative stress in major depression

Brian J. Miller, Andrew Mellor, Peter Buckley. Total and differential white blood cell counts, high-sensitivity C-reactive protein, and the metabolic syndrome in non-affective psychoses

Sherry Anders, Midori Tanaka, Dennis K. Kinney. Depression as an evolutionary strategy for defense against infection

Kimberly L. D'Anna-Hernandez. The Oxford Handbook of Psychoneuroimmunology Book Review, Kimberly L. D'Anna-Hernandez, California State University San Marcos, San Marcos, CA, USA.

Masaaki Iwata, Kristie T. Ota, Ronald S. Duman. The inflammasome: Pathways linking psychological stress, depression, and systemic illnesses

Igor Smirnov, James T. Walsh, Jonathan Kipnis. Chronic mild stress eliminates the neuroprotective effect of Copaxone after CNS injury

Marisa Möller, Jan L. Du Preez, Francois P. Viljoen, Michael Berk, Robin Emsley, Brian H. Harvey. Social isolation rearing induces mitochondrial, immunological, neurochemical and behavioural deficits in rats, and is reversed by clozapine or N-acetyl cysteine

Marta Kubera, Katarzyna Curzytek, Weronika Duda, Monika Leskiewicz, Agnieszka Basta-Kaim, Boguslawa Budziszewska, Adam Roman, Alena Zajicova, Vladimir Holan, Ewa Szczesny, Wladyslaw Lason, Michael Maes. A new animal model of (chronic) depression induced by repeated and intermittent lipopolysaccharide administration for 4 months

Nicola J. Paine, Christopher Ring, Jos A. Bosch, Mark T. Drayson, Jet J.C.S. Veldhuijzen van Zanten. The time course of the inflammatory response to the *Salmonella typhi* vaccination

Hanne Hamre, Bernward Zeller, Adriani Kanellopoulos, Ellen Ruud, Sophie D. Fossa, Jon H Loge, Pal Aukrust, Bente Halvorsen, Tom Eirik Mollnes, Cecilie E. Kiserud. Serum cytokines and chronic fatigue in adults surviving after childhood leukemia and lymphoma

Susannah S. Lewis, Mark R. Hutchinson, Yingning Zhang, Dana K. Hund, Steven F. Maier, Kenner C. Rice, Linda R. Watkins. Glucuronic acid and the ethanol metabolite ethyl-glucuronide cause toll-like receptor 4 activation and enhanced pain

Leah M. Pyter, Sean D. Kelly, Constance S. Harrell, Gretchen N. Neigh. Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats

Lauren L. Williamson, Staci D. Bilbo. Chemokines and the hippocampus: A new perspective on hippocampal plasticity and vulnerability

Natalie L. Payne, Guizhi Sun, Courtney McDonald, Leon Moussa, Ashley Emerson-Webber, Séverine Loisel-Meyer, Jeffrey A. Medin, Christopher Siatskas, Claude C.A. Bernard. Human adipose-derived mesenchymal stem cells engineered to secrete IL-10 inhibit APC function and limit CNS autoimmunity