There is no doubt that the early diagnosis of tumors is the most effective way to reduce the mortality caused by neoplastic diseases. Pre-symptomatic tumor diagnostics may be considered as secondary prevention by effective screening to identify cancer at a localized and curable stage. In this book methods, possibilities and perspectives of pre-symptomatic tumor diagnostics will be discussed. Because of the heterogeneity of tumors in genes and protein expression as well as in the biological behaviour one approach alone is insufficient for a highly specific, sensitive and cost-effective screening. In four chapters the requirements for an effective screening, the value of conventional "screening" methods (tumor markers, anatomical imaging modalities), and the search for novel biomarkers (tumor associated genes, antigens and autoantibodies) in terms of non-imaging screening as well as novel developments in imaging technologies and endoscopical approaches will be described.

Pre-symptomatic tumor diagnostics is a complex field that needs interdisciplinary research. The societies Gesellschaft für Immundiagnostik e.V. (www.gfid.de) and Präsymptomatische Tumordiagnostik e.V. (www.tumornetzwerk.de) co-operate to provide a communication platform - "Innovation Forum Pre-symptomatic Tumor Diagnostics" - that supports complex research projects between clinicians, researchers, and industry to develop and validate new diagnostic technologies and products. This national platform will be turned into an international institution in the future.

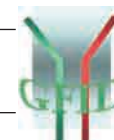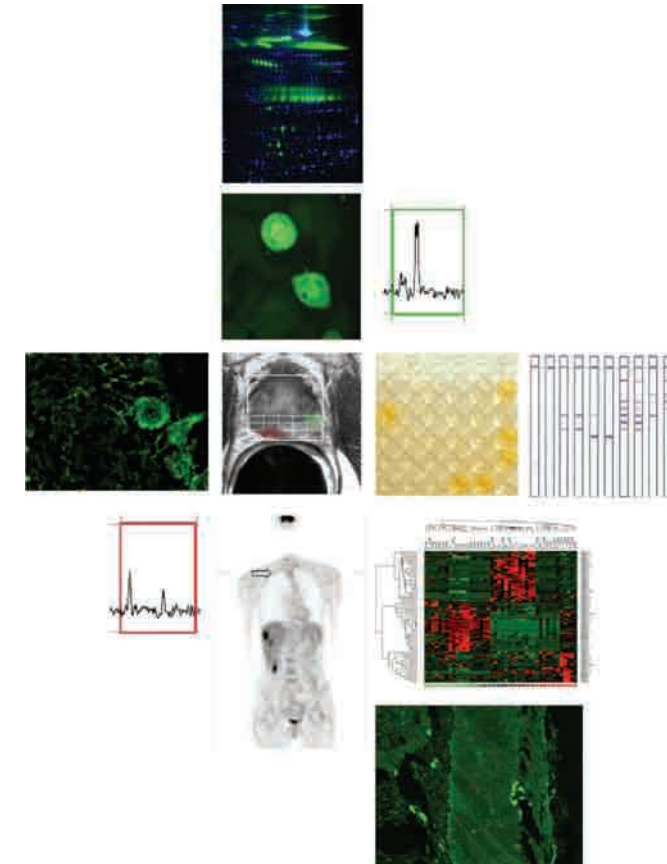Präsymptomatische
Tumordiagnostik e.V.

Methods, Possibilities and Perspectives of Pre-symptomatic Tumor Diagnostics
K. Conrad, M. Bachmann, W. Lehmann, U. Sack (Eds.)

K. Conrad, M. Bachmann, W. Lehmann, U. Sack (Eds.)

Methods, Possibilities and Perspectives of Pre-symptomatic Tumor Diagnostics

**Pre-symptomatic Tumor Diagnostics
Vol. 1**

PABST

Content

12

PREFACE

There is no doubt that the early (pre-symptomatic) diagnosis of tumors is the most effective way to reduce the mortality caused by neoplastic diseases. The search in the field of pre-symptomatic tumor diagnostics is very complex and interdisciplinary and should result in highly specific and sensitive as well as cost-effective routine diagnostic programs to identify cancer at a localized and curable stage. Many specialists working in different fields such as immunology, clinical chemistry, molecular biology, genetics, pathology, radiology, and clinical medicine are involved in the research for improving the early diagnosis of tumors. The new book series "Pre-symptomatic Tumor Diagnostics" is designed to provide clinicians and scientists of all fields in oncological research and clinical medicine with reviews and new results on all topics that are relevant for the early diagnosis of cancer. In the 1st volume, possibilities and perspectives of pre-symptomatic tumor diagnostics will be described and discussed. Because of the heterogeneity of tumors in genes and protein expression as well as in the biological behaviour one approach alone is insufficient for a highly specific, sensitive and cost-effective screening. In four chapters the requirements for an effective screening, the value of conventional "screening" methods (tumor markers, anatomical imaging modalities), and the search for novel biomarkers (tumor associated genes, antigens and autoantibodies) in terms of non-imaging screening as well as novel developments in imaging technologies and endoscopical approaches are described. The topics reviewed and discussed in this volume do not represent all fields involved in pre-symptomatic tumor diagnostics. Missing aspects as well as the novel developments will be the focus of the following volumes.

The editors

Michael Bachmann
Karsten Conrad
Werner Lehmann
Ulrich Sack

Chapter 1

TUMOR AND AUTOIMMUNITY

Tumor specific autoimmune responses - Why and when do they develop?

Jian-Ying Zhang[1] and Edward K.L. Chan[2]

[1]Department of Biological Sciences, University of Texas, El Paso, Texas, USA;
[2]Department of Oral Biology, University of Florida, Gainesville, Florida, USA.

The development of cancer is attributed to many factors such as environmental and genetic contributions leading to gene mutations and dysregulations in the expression of many cellular genes. Overexpressed or abruptly expressed self proteins are released from the continuous turnover of tumor cells and are presented to the immune system of the host. The continuing elevated exposure of these proteins and/or macromolecular complexes, especially with fetal oncoproteins that are not normally expressed in adult tissues, initiates immune response that is manifested as autoantibodies. This scenario predicts that the autoimmune response to be initiated early on as the growing tumor mass is turning over sufficient antigens. The autoimmune response is likely to continue if the presentation of these self antigens persists. This hypothesis provides an explanation how autoantigens in cancer may represent reporters of aberrant or overexpressed proteins participating in the tumorigenesis process. Potential benefits to the host from these autoimmune responses have not been adequately examined. It is possible and likely that a sustained autoimmune response in cancer patients represent a failed response in tumor rejection. More work is needed to address the overall effect of these autoimmune responses to the tumor. This brief overview outlines supporting data from our laboratories.

Identification of tumor-associated antigens (TAAs)

Many studies demonstrated that cancer sera contain antibodies which react with autologous cellular antigens generally known as TAAs [1-3]. In our laboratories, the approach used in the identification of TAAs has involved initially examining the sera of cancer patients using extracts of tissue culture cells as source of antigens in Western blotting and by indirect immunofluorescence on whole cells. With these two techniques, we have identified sera which have high-titered fluorescent staining or strong signals to cell extracts on Western blotting and subsequently use these antibodies to immunoscreen cDNA expression libraries and isolate cDNA clones encoding targeted antigens. In this manner, several novel TAAs including HCC1 [4], p62 [5], p90 [6] and others have been identified. Immunoscreening of cDNA libraries with serum antibod-

ies for identifications of autoantigens is a well-established method and has been used not only to identify TAAs but also antigens in autoimmune diseases [7]. This methodology was the basis of the methods described in SEREX (serological analysis of recombination cDNA expression libraries) with the difference that cDNA expression libraries constructed from autologous patient tumor were used as substrate in immunoscreening [8]. Subsequent reports using the SEREX technique have shown that the TAAs identified are no different from standard methods using cDNA expression libraries from cell lines derived from different sources, so that there did not appear to be any advantage to using cDNA libraries from autologous patients.

Overexpression of TAA p62 and p90 in cancer

Recent data from our laboratory suggest a mechanistic process may be involved in humoral immune responses in certain cancers such as hepatocellular carcinoma (HCC). HCC is unique in that one can follow a cohort of patients with chronic liver disease who will likely progress to develop malignancy over a period of 10 or more years. It has been observed that during transition from chronic liver disease to HCC, novel autoantibodies can appear which are not detected prior to pre-malignant conditions [9,10]. These novel antibody responses may be stimulated by cellular proteins which are involved in carcinogenesis. By immunoscreening an expression library, a RNA-binding protein autoantigen p62 has been identified in HCC and autoantibodies to p62 were found in 21% of a cohort of HCC patients [5]. p62 is a cytoplasmic protein which binds to mRNA encoding insulin-like growth factor II (IGF-II), a growth factor which is known to be overexpressed in HCC and is tumorigenic in transgenic animals. We showed that p62 was aberrantly expressed in 30% of unselected HCC [11] suggesting that p62 could play a role in HCC and other tumors by upregulating expression of growth factor IGF-II in the milieu of other oncogenic factors. A "companion" antigen p90, autoantibodies to which were found associated with anti-p62 responses in the same HCC patient group, was also identified by cDNA expression cloning [6]. Indirect immunofluorescence showed that, like p62, p90 localized to the cytoplasm in cultured cells and mouse fetal, but not adult liver. Among 11 human gastric cancer tissues examined, p90 was overexpressed in 6 (55%). Our data support the working hypothesis that autoantibody production in cancer may be directly linked to aberrant autoantigen expression of p62 and p90 autoantigens.

TAAs are often tightly linked to tumorigenesis?

The types of cellular proteins which induce autoantibody responses are quite varied and include oncogene products such as HER-2/neu and ras [12,13], cellular proteins which shield mRNAs from natural physiological degradation

such as p62 [5] and CRD-BP [14], onconeural antigens in the paraneoplastic disorder syndromes [15], differentiation-antigens such as tyrosinase and the cancer/testis antigens [16]. Factors leading to the production of such autoantibodies are not completely understood but the available data show that many of the target antigens are cellular proteins whose aberrant regulation could lead to tumorigenesis, such as p53, HER-2/neu, and ras, or are proteins whose dysregulation could have tumorigenic potential including mRNA binding proteins such as p62 and cell-cycle control proteins such as cyclin B1 [17,18]. One of the most extensively studied TAAs is p53. Autoantibodies to p53 in cancer were first reported in 1982 [19] and since then there have been numerous reports confirming and extending this finding (reviewed in [20]). A highly informative study showed that lung tumors contained several types of p53 gene mutations including missense, stop codon and frameshift mutations, but it was the missense mutations with overexpression of p53 protein which altered function and increased stability that correlated with antibody production [21]. In the case of p62 which is a fetal protein absent in adult tissues, immunogenicity appears to be related to abnormal expression of p62 in tumor cells [11]. With the onconeural antigens in paraneoplastic neurological disorders, antibody responses are thought to be related to ectopic expression of neuron-restricted cellular proteins in tumor cells [15]. The immune system in certain cancer patients appears to have the capability of sensing these abnormalities and it was proposed that autoantibodies might be regarded as reporters identifying aberrant cellular mechanisms in tumorigenesis [1]. As the detection of antibody immunity to tumor antigens becomes more routine, investigations have evolved to begin to address specific clinical questions such as the role of antibody immunity as a marker for patients exposed to cancer, as a tool to monitor therapy, or as an indicator of disease prognosis [22].

Using a mini-array of multiple TAAs to enhance antibody detection in cancer.

A feature of HCC is that antecedent liver cirrhosis and chronic hepatitis are common precursor conditions and during transition to malignancy some patients develop autoantibodies which were not present during the preceding chronic liver disease phase [9,10,23,24]. A hypothesis which has been proposed is that these antibody responses may be stimulated by cellular proteins which are involved in carcinogenesis. Many investigators have been interested in the use of autoantibodies as serological markers for cancer diagnosis, especially because of the general absence of these autoantibodies in normal individuals and in non-cancer conditions. Enthusiasm for this approach has been tempered by low sensitivity. One of our recent studies showed that detection of autoantibodies in cancer can be enhanced by using mini-array of seven TAAs as target antigens [25-27]. As shown in table 1, the mini-array comprised seven TAAs: c-myc [28], p53 [20], cyclin B1 [17,18], p62 [5,29], Koc [29,30], IMP1 [31] and survivin [32,33]. Antibody frequency to any indi-

vidual TAA was variable, and ranges from 10.8 to 24.6 percent in HCC. With the successive addition of TAAs to a final total of 7 antigens, there was step-wise increase of positive antibody reactions up to 56.9 percent in HCC. Compared to HCC, frequency of antibody to any one of these same 7 antigens was 67.9 percent in lung cancer. The fact is that antibodies to any individual antigen such as anti-p53, anti-p62 or anti-c-Myc do not reach levels of sensitivity which could become routinely useful in diagnosis [27]. These data in one way indicate that the combination of antibodies might acquire higher sensitivity for diagnosis of cancer. On the other hand, the data also suggest that in the selection of different antigen-antibody systems, some of the antigens may turn out to be more specific for a certain type of cancer while others may be not. It is conceivable that autoantibody profiles involving different panels or arrays of TAAs might be developed and the results could be useful for diagnosis of certain types of cancer. More recently, we added three other TAAs (p90, cyclin D1 and cyclin A) into our previously used mini-array system for detection of antibodies in prostate cancer. The results showed that the cumulative positive reactions in prostate cancer sera reached 92.5%, significantly higher than in benign prostatic hyperplasia (BPH) and other controls [34].

Although there have been a steadily increasing number of studies describing and characterizing autoantibodies to "cancer-related antigens" in recent years, many of these antigen-antibody systems are not found to be useful in differentiating cancer and normal. For example, we clearly demonstrate that antibodies to three of so-called "cancer antigens" α-enolase, B23 and elongation factor-1γ (EF-1-γ) are not unique to HCC, and suggest that its detection in HCC might be attributed to antibody being already present in the precursor liver diseases such as chronic hepatitis, liver cirrhosis or primary biliary cirrhosis [35]. This study also emphasizes the importance of a comprehensive analysis of antibody responses to putative cancer antigens in different pre-cancer conditions such as chronic hepatitis and liver cirrhosis before conclusions can be made regarding their contribution to HCC. Some of the identified antibody-antigen systems may be unique to cancer while others may not. A comprehensive analysis and evaluation of various combinations of selected antibody-antigen systems will be useful for the development of autoantibody profiles involving different panels or arrays of TAAs, and the results could be useful for the detection of certain types of cancer such as lung cancer, HCC and so on. Our strategy to actively eliminate false-positive TAAs from our mini-array assay is qualitatively different from other approaches published to date.

Table 1. Frequency of antibodies to seven tumor-associated antigens in cancer patients.

Number and Percent of Autoantibodies to

Cancer	No. Tested	c-myc	p53	Cyclin B1	p62	Koc	IMP1	Survivin	Any of 7 Antigens
HCC	65	16(24.6)**	7(10.8)*	8(12.3)*	8(12.3)**	9(13.8)**	10(15.4)**	7(10.8)*	37(56.9)**
Gastric	91	14(15.4)**	12(13.2)**	14(15.4)**	8(8.8)*	17(18.7)**	15(16.5)**	9(9.9)*	48(52.7)**
Colorectal	45	2(4.4)	8(17.8)**	7(15.6)**	5(11.1)*	4(8.9)*	6(13.3)*	2(4.4)	23(51.1)**
Breast	64	12(18.8)**	5(7.8)	3(4.7)	5(7.8)	9(14.1)**	5(7.8)	5(7.8)	28(43.8)**
Lung	56	6(10.7)**	9(16.1)**	15(26.8)**	12(21.4)**	5(8.9)*	4(7.1)	6(10.7)*	38(67.9)**
Total	*321*	*50(15.6)***	*41(12.8)***	*47(14.6)***	*38(11.8)***	*44(13.7)***	*40(12.5)***	*29(9.0)***	*174(54.2)***
NHS	82	0(0.0)	2(2.4)	2(2.4)	1(1.2)	1(1.2)	2(2.4)	2(2.4)	9(11.0)

Cutoff value: Mean + 3 S.D. of NHS. $*p<0.05$; $**p<0.01$. (simplified from reference [27])

References

(1) Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. J Clin Invest 2001;108:1411-5.

(2) Houghton AN. Cancer antigens: immune recognition of self and altered self. J Exp Med 1994;180:1-4.

(3) Old LJ, Chen YT. New paths in human cancer serology. J Exp Med 1998;187:1163-7.

(4) Imai H, Chan EKL, Kiyosawa K, Fu X-D, Tan EM. Novel nuclear autoantigen with splicing factor motifs identified with antibody from hepatocellular carcinoma. J Clin Invest 1993;92:2419-26.

(5) Zhang JY, Chan EKL, Peng XX, Tan EM. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. J Exp Med 1999;189:1101-10.

(6) Soo Hoo L, Zhang JY, Chan EKL. Cloning and characterization of a novel 90 kDa 'companion' auto-antigen of p62 overexpressed in cancer. Oncogene 2002;21:5006-15.

(7) Chambers JC, Keene JD. Isolation and analysis of cDNA clones expressing human lupus La antigen. Proc Natl Acad Sci USA 1985;82:2115-9.

(8) Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA 1995;92:11810-3.

(9) Imai H, Nakano Y, Kiyosawa K, Tan EM. Increasing titers and changing specificities of antinuclear antibodies in patients with chronic liver disease who develop hepatocellular carcinoma. Cancer 1993;71:26-35.

(10) Zhang JY, Zhu W, Imai H, Kiyosawa K, Chan EKL, Tan EM. De-novo humoral immune responses to cancer-associated autoantigens during transition from chronic liver disease to hepatocellular carcinoma. Clin Exp Immunol 2001;125:3-9.

(11) Lu M, Nakamura RM, Dent ED, Zhang JY, Nielsen FC, Christiansen J, Chan EKL, Tan EM. Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. Am J Pathol 2001;159:945-53.

(12) Cheever MA, Disis ML, Bernhard H, Gralow JR, Hand SL, Huseby ES, Qin HL, Takahashi M, Chen W. Immunity to oncogenic proteins. Immunol Rev 1995;145:33-59.

(13) Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA. High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. J Clin Oncol 1997;15:3363-7.

(14) Doyle GA, Bourdeau-Heller JM, Coulthard S, Meisner LF, Ross J. Amplification in human breast cancer of a gene encoding a c-myc mRNA-binding protein. Cancer Res 2000;60:2756-9.

(15) Keene JD. Why is Hu where? Shuttling of early-response-gene messenger RNA subsets. Proc Natl Acad Sci U S A 1999;96:5-7.

(16) Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 1998;187:1349-54.

(17) Covini G, Chan EKL, Nishioka M, Morshed SA, Reed SI, Tan EM. Immune response to cyclin B1 in hepatocellular carcinoma. Hepatology 1997;25:75-80.

(18) Kao H, Marto JA, Hoffmann TK, Shabanowitz J, Finkelstein SD, Whiteside TL, Hunt DF, Finn OJ. Identification of cyclin B1 as a shared human epithelial tumor-associated antigen recognized by T cells. J Exp Med 2001;194:1313-23.

(19) Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int J Cancer 1982;30:403-8.

(20) Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Res 2000;60:1777-88.

(21) Winter SF, Minna JD, Johnson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. Cancer Res 1992;52:4168-74.

(22) Disis ML, Montgomery RB, Goodell V, dela Rosa C, Salazar LG. Antibody immunity to cancer-associated proteins. Education Book of the 96th Annual AACR Meeting. 2005. p.166-9.

(23) Covini G, von Mühlen CA, Pacchetti S, Colombo M, Chan EKL, Tan EM. Diversity of antinuclear antibody responses in hepatocellular carcinoma. J Hepatol 1997;26:1255-65.

(24) Zhang JY, Wang X, Peng XX, Chan EKL. Autoantibody responses in Chinese hepatocellular carcinoma. J Clin Immunol 2002;22:98-105.

(25) Zhang JY. Tumor-associated antigen arrays to enhance antibody detection for cancer diagnosis. Cancer Detect Prev 2004;28:114-8.

(26) Koziol JA, Zhang JY, Casiano CA, Peng XX, Shi FD, Feng AC, Chan EKL, Tan EM. Recursive partitioning as an approach to selection of immune markers for tumor diagnosis. Clin Cancer Res 2003;9:5120-6.

(27) Zhang JY, Casiano CA, Peng XX, Koziol JA, Chan EKL, Tan EM. Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epidemiol Biomarkers Prev 2003;12:136-43.

(28) Yamamoto A, Shimizu E, Takeuchi E, Houchi H, Doi H, Bando H, Ogura T, Sone S. Infrequent presence of anti-c-Myc antibodies and absence of c-Myc oncoprotein in sera from lung cancer patients. Oncology 1999;56:129-33.

(29) Zhang JY, Chan EKL, Peng XX, Lu M, Wang X, Mueller F, Tan EM. Autoimmune responses to mRNA binding proteins p62 and Koc in diverse malignancies. Clin Immunol 2001;100:149-56.

(30) Mueller-Pillasch F, Lacher U, Wallrapp C, Micha A, Zimmerhackl F, Hameister H, Varga G, Friess H, Buchler M, Beger HG, Vila MR, Adler G, Gress TM. Cloning of a gene highly overexpressed in cancer coding for a novel KH- domain containing protein. Oncogene 1997;14:2729-33.

(31) Nielsen J, Christiansen J, Lykke-Andersen J, Johnsen AH, Wewer UM, Nielsen FC. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 1999;19:1262-70.

(32) Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997;3:917-21.

(33) Rohayem J, Diestelkoetter P, Weigle B, Oehmichen A, Schmitz M, Mehlhorn J, Conrad K, Rieber EP. Antibody response to the tumor-associated inhibitor of apoptosis protein survivin in cancer patients. Cancer Res 2000;60:1815-7.

(34) Shi FD, Zhang JY, Liu D, Rearden A, Elliot M, Nachtsheim D, Daniels T, Casiano CA, Heeb MJ, Chan EKL, Tan EM. Preferential humoral immune response in prostate cancer to cellular proteins p90 and p62 in a panel of tumor associated antigens. Prostate 2005;63:252-8.

(35) Tan EM, Zhang JY, Chan EKL. Autoantibodies to insulin-like growth factor II mRNA-binding proteins in hepatocellular carcinoma. Immunology and Liver. Lancaster, UK: Kluwer Academic Publishers; 2000:8-15.

54 Jian-Ying Zhang and Edward K.L. Chan

Autoantibodies as indicators of tumor development

Karsten Conrad, Dirk Roggenbuck[1], Michael Bachmann

Institute of Immunology, Medical Faculty of the Technical University of Dresden, and [1]Generic Assays GmbH, Dahlewitz, Germany

Autoantibodies (AAB) against various types of tumor associated antigens (TAA) with varying sensitivities and specificities for malignancy have been described. The immunogenicity of TAA mainly depends on the level and kind of expression. Therefore, tumor associated AAB (TA-AAB) can be regarded as reporters identifying aberrant cellular mechanisms in tumorigenesis and may be used as early indicators of tumor development. However, most of the TA-AAB lack sufficient sensitivity and specifitiy for use as biomarkers in the clinical practice. An optimal combination of highly specific TA-AAB in multiparametric assays as well as the standardization of the AAB analysis is necessary to realise the potential of TA-AAB in the early (pre-symptomatic) diagnosis and monitoring of malignancies.

Introduction

The malignant transformation of cells is a result of altered expression of genes involved in cell growth control and differentiation. Mutations, inherited through the germline or, more commonly, arising in somatic tissues later in life, as well as epigenetic changes that influence the expression and/or function of oncogenes and tumor-suppressor genes drive the development of tumors from the earliest to the last stage [1-3]. Fortunately, the tumor development can be checked by different tumor surveillance mechanisms acting on the level of DNA (repair of damaged DNA sequences), cell proliferation control (programmed cell death) and immune defence [4]. The loss or impairment of such control mechanisms also influence the development and progression of tumors. Therefore, tumor growth may depend on the balance between tumor promoting and tumor controlling factors/mechanisms.

The early detection of cancer that allows an effective and probably curative therapeutic management is most important in clinical oncology. However, current biomarkers often lack specificity and sensitivity for an efficient and cost-effective screening, leading to false results and late detection of malignancies. In theory, all changes in expression, structure and/or function of genes/proteins involved in the tumorigenesis may be markers for pre-symptomatic tumor diagnosis. Different approaches are used to find parameters/biomarkers (single or in combination) for a specific and sensitive diagno-

sis of early cancer stages (see Chapter 2 and 3). Here we describe the potential of tumor associated autoantibodies (TA-AAB) as indicators of cancer development and hence early (pre-symptomatic) tumor diagnosis.

Anti-tumor immune responses

It is well known that tumors in humans and animal models may trigger anti-tumor immune responses. Those responses may be differentiated in (1) immune defence mechanisms according to the "immune surveillance" hypothesis, (2) polyclonal activation of naturally occuring autoantibodies / activation of natural defence mechanisms [5], and (3) immune responses against overexpressed or aberrantly expressed self-antigens independent of immune defence mechanisms.

The so-called immune surveillance reflects the immune defence mechanisms that control tumor development mainly at the level of precancerous cells. By this way the immune system protects the organism from the growth of potentially neoplastic cells that are initiated by viruses (e.g., human papilloma viruses, Epstein–Barr and other human Herpes viruses) and perhaps other mechanisms. Besides effects of specific induced immune responses, tumor growth may be modulated by naturally occuring autoantibodies (NOA) that bind tumor cells or components of the immune system [6,7]. NOA are germline-encoded polyreactive antibodies probably involved in the "first line defence", in the clearance of senescent products and in immune regulatory mechanisms. Among the first functions described for NOA was the reactivity with tumor cells [8]. This reactivity is most probably a manifestation of polyreactivity rather than an interaction between a tumor-specific antigen and a corresponding antibody [9]. NOA may have different biological effects ranging from enhancement to surveillance of tumor development [6,7].

Immune reactivity against tumor cells does not always represent an anti-tumor defence mechanism but simply may be a result of an antigen-driven immune response. Regardless of their biological role, autoantibodies (AAB) that specifically recognize antigens of the tumor may have a great potential for the detection of early tumor development. However, only a subset of patients with a tumor type develops a humoral response to a particular antigen, for example p53 [10]. The immunogenicity of a tumor depends on several factors which may be variable among tumors of a similar type. Regarding the (tumor-associated) antigens, the level of expression, post-translational modification, or variations in protein processing, are of great importance (table 1). Furthermore, the specific immune response to a defined antigen depends on the structure of the highly polymorphous MHC molecules.

Table 1. Causes and mechanisms of anti-tumor immune responses

Mechanisms underlying immunogenicity	Cause(s)	Genes/proteins involved	Examples	References
Overexpression of autoantigens in malignant tissues	Gene amplification and other mechanisms of enhancement of gene expression (e.g. promoter hypomethylation)	Oncogenes and other antigens involved in cancer development (cell-cycle/mitosis associated proteins, helicases)	p185$^{HER-2/neu}$ c-myc L-myc eIF-4γ p160^{ROCK} Fibulin-1 WT1 CAGE	14,16,20, 28,110-112
Expression of aberrant autoantigens	Point mutations	Oncogenes, tumor suppressor proteins	p53 p21^{ras}	15,113
	Truncation		B23 (nucleophosmin) CRT32 (truncated form of calreticulin)	82,114
Modified expression in tumors (underglycosylation, sialysation)	Aberrant post-translational modifications	Carbohydrate membrane antigens	PEM/MUC1 Gangliosides (GM1, GM2, GD2)	115
Protein modification	Apoptosis-associated proteolytic cleavage		BARD-1	31
Generation of cross-reactive epitopes	Aberrant expression of an unrelated autoantigen		Calreticulin isotypes?	116
Expression of chimeric proteins	Chromosomal/gene translocations	SSX-2 gene c-abl proto-oncogene (2;5) translocation > hybrid oncogenic tyrosine kinase	HOM-MEL-40 p210$^{bcr-abl}$ NPM-ALK	13,117,118
	Gene activation or derepression	Cancer-testis antigens (CTA)	NY-ESO1 MAGE-1 MAGE-3	46
Ectopic expression of autoantigens	Gene activation or derepression or post-transcriptional	Onconeural antigens (ONA)	Hu, Ri, Yo Amphiphysin VGCC	49
	regulatory mechanisms (?)		PGP 9.5 Annexin-I, -II	76,80
Autoantigen secretion by tumor cells and/or release of autoantigens by tumor necrosis and cell death		Many intracellular antigens	RS/DJ-1	83

Target antigens of tumor associated autoantibodies

There is growing evidence that antigens that become aberrantly or overexpressed during the transition to malignancy can be targets of the cellular and/or humoral immune response (table 1) under special conditions (e.g. sequence of MHC molecules, pro-inflammatory stimuli). Those antigens may be involved in or the consequence of the tumorigenesis and therefore, immune responses (e.g. autoantibodies) against them can be regarded as tumor associated or even tumor specific. Alterations in at least three groups of genes are responsible for the tumorigenesis: oncogenes, tumor-suppressor genes and stability genes (reviewed in [11]). Examples for autoantibodies against products of those genes are shown in table 2.

Oncogene products. The cellular oncogenes are derived from their normal counterparts, the protooncogenes, by activating mutations or transcriptional activations [12]. Their products include a broad range of factors that promote cancer (e.g., growth factors, growth factor receptors, downstream mediators and modulators of cellular signal transduction pathways, transcriptional regulators). An important example of an anti-receptor immune response is the AAB against the HER-2/neu product, a 185 kDa transmembrane protein with extensive homology to the epidermal growth factor receptor. Anti-HER-2 AAB are found in breast cancer patients in up to 55% [13,14]. The AAB response correlates with HER-2/neu protein overexpression in the patient´s primary tumor, but were also found in women with HER-2/neu negative breast cancer, suggesting an active immunoselection for HER-2/neu negative variants [14]. This possibility is also underlined by the higher frequency and the higher titers of p185$^{HER-2/neu}$ AAB in the early stage of disease [14]. AAB against the p21 ras protein, a member of the GTPase complex whose transforming activity evolves by point mutations, has been found in 32% of patients with colon cancer [15]. Although p21^{ras} is activated by point mutations, most AAB detect epitopes near the carboxyl terminus of the wild-type protein [15]. In addition to these AAB directed against growth factor receptors (p185$^{HER-2/neu}$) or GTP binding proteins (p21^{ras}), AAB to another group of oncoproteins have been described in patients with solid tumors (colorectal, breast, ovary, lung cancer) and patients with leukemias/lymphomas. These AAB are directed against nuclear regulatory proteins such as myb and myc [17-21]. However, with the exception of AAB to the L-myc protein, these antibodies have been shown to be relatively unspecific for tumors in some studies. Furthermore, the frequencies of c-myc AAB in healthy volunteers and SLE patients varied greatly in the different studies [16-18]. In general, different methods of AAB determinations and differences in the populations studied may account for varying results. The source and purification of autoantigens and the assays used for AAB determination may influence results dramatically.

Table 2. Autoantibodies against targets that are involved in tumorigenesis

	Autoantibodies against	Tumor entities	References
Products of oncogenes	p185$^{HER-2/neu}$	Breast cancer (11-55%)	13-16, 19, 21, 118-121
	p21^{Ras}	Colon cancer (32%)	
	c-myc	Colorectal (57%) and lung (13.2%) cancer	
	L-myc	Lung cancer (10%)	
	c-myb	Breast (43%), colon (40%) and ovary cancer (33%)	
	p145^{Abl}, p210$^{Bcr-Abl}$	Chronic myelogenous leukemia (50-70%)	
	csk	Colon (20%), bladder (19%), lung (17%), ovary (10%) and breast (5%) cancer	
	ALK	Anaplastic large-cell lymphoma (ALCL) (90.1%)	
Products of tumor-suppressor genes	p53	Most cancer (3-65%)	10, 28, pp. 92-107
	WT1	Leukemias (AML: 81.3%, ALL: 45.5%, CML: 61.5%)	
Products of stability genes	Rad51	Pancreatic cancer (7%)	30,32
	hMSH2	Pancreatic cancer (13.5%)	
	hPMS2	Pancreatic cancer (8.1%)	
Inhibitor of apoptosis proteins	Survivin	Lung (21.6-58,1%), colorectal (8.2%), head and neck (46%), breast (6,3%), and prostate (3%) cancer	34-39
	Livin	Lung (41.3%) and gastrointestinal (47%) cancer	

For example, c-myc AAB were determined with ELISAs using 31-mer c-myc peptides [17] or human prokaryotically expressed recombinant c-myc protein [18], or with immunoblotting using the recombinant protein [16]. Furthermore, variations in the definition of standards of detectability led to different frequencies, as has been shown for p185[HER-2/neu] AAB in breast cancer patients (11-21%) and in healthy volunteers (0-1%) [14]. But ethnic differences and different influences of endogeneous and exogenous factors in the populations studied may also be relevant for the variation in results. For example, the frequency of p185[HER-2/neu] AAB is highest in women with premenopausal breast cancer because there is also highest frequency of HER-2/neu protein overexpression [13]. In conclusion, there is a further need for studies of the clinical and biological nature concerning humoral autoimmune responses to oncoproteins such as (a) the evaluation of diagnostic relevance (diagnostic sensitivity and specificity) and the prognostic significance (correlation with the stage of the disease and survival) in defined patient groups using optimized and standardized methods, (b) the search for associations of antibody titers with disease progression or relapse and therapeutic effects, (c) the search for possible mechanisms of AAB induction (correlation with protein overexpression, mutations or presence of oncoproteins in the circulation) and (d) the search for possible effects of AAB on tumor cells.

Tumor suppressor proteins. Alterations in tumor suppressor genes that lead to the loss or disturbance of the function(s) of their products are of major importance in tumorigenesis. Mutations may lead to impaired function and cellular accumulation of the protein by different mechanisms such as a prolonged half-life [22] with the potential of the induction of immune responses. The p53 AAB is the most extensively studied humoral autoimmune response in human cancer patients (reviewed in [10,23], see also Montenarh (pp. 92-107). Mutations of the p53 tumor suppressor gene are the most frequently reported gene alteration in human cancers [24,25]. In 1982 it was shown for the first time that p53 may become immunogenic in cancer patients [26]. In the years that followed p53 AAB were detected in sera from a variety of cancer patients in frequencies between 3 and 65 % depending on tumor type and method of antibody detection, whereas the prevalence of such AAB in normal populations was low [10,23,27]. Recently, another humoral anti-tumor suppressor immune response has been described in patients with hematopoietic malignancies, the response against Wilms tumor gene product gene WT1 [28]. IgG anti-WT1 antibodies were found in 27.2-50% of leukemia patients and in 69.6% of patients with myelodysplastic syndromes (MDS) but only in 4.7% of healthy volunteers. The simultaneous production of IgM and IgG antibodies was limited to the patients.

Products of stability genes. Stability genes or caretakers encode products that keep genetic alterations to a minimum. Inactivation of those genes leads to a higher mutation rate and, thus, a higher risk of tumor development. The best known examples of cancer genes of this group are the breast cancer associated BRCA genes (see Jandrig, pp. 237-248). AAB against BRCA prod-

ucts have not been described so far. Interestingly, AAB have been found against the recombination factor Rad51 and the BRCA associated ring domain BARD1 proteins that are co-localized with BRCA products to areas of damages DNA supporting a role in DNA repair [29]. Rad51 is highly expressed in human pancreatic adenocarcinoma and AAB were found in 7% of patients but not in healthy volunteers [30]. Anti-BARD1 AAB have been induced during treatment of rats with peritoneal colon carcinomatosis by injections of apoptotic bodies derived from tumor cells and interleukin 2 [31]. It was demonstrated that the humoral immune response was directed against a cleaved form of BARD1 present in apoptotic bodies derived from rat and human colon and mammary carcinoma cell lines. Recently, another autoimmune response to stability genes, the DNA mismatch repair enzymes hMSH2 and hPMS1, has been described in patients with pancreatic cancer [32]. These enzymes are also overexpressed in pancreatic ductal adenocarcinomas.

Inhibitors of apoptosis proteins (IAP). An important mechanism involved in cancer formation is the inhibition of apoptosis, which, by extending the lifespan of cells, favors the accumulation of transforming mutations [33]. Apoptosis pathways are effectively blocked by proteins belonging to the IAP family that directly inhibits caspase and pro-caspase molecules. It has been shown recently that at least two of the known IAPs, survivin and livin, are targets of an autoimmune response in human cancer [34-39]. Survivin is abundantly expressed in fetal tissue and in a variety of human tumors including lung, colon, breast, prostate, pancreatic, and gastric cancer as well as in high-grade lymphomas and neuroblastomas [40-42]. Anti-survivin AAB are described so far in lung, colorectal, head and neck, breast and prostate cancer (table 2). Livin is also highly expressed in cancer, but shows little or no expression in normal tissues [43,44]. Therefore, it was no surprise to find AAB in cancer patients. Contrary to survivin AAB, which were found in all clinical stages, anti-livin AAB were found only in patients with advanced stages [36,37]. It has been speculated that larger expression of livin is needed for induction of AAB responses to livin compared with survivin.

Cancer/testis class of tumor antigens (CTA) are expressed in a variable proportion of a wide range of human tumors, but are silent in most normal tissues except the testis. They were initially identified as targets for cytotoxic T cells (MAGE, GAGE, BAGE) and, later on, uncovered by SEREX (serological analysis of recombinant cDNA expression libraries of human tumors with autologous serum) analysis (reviewed in [45,46]). CTA identified by SEREX elicited an AAB response in tumor patients. Therefore, this methodology leads not only to the detection of new tumor antigens but also to the identification of specific humoral responses which may be used for diagnostic purposes. Stockert et al. were the first who tested a great number of tumor sera for humoral immune response to SEREX-identified tumor antigens, including several CTA, by ELISA with recombinant proteins [47]. They showed that 9.4% of melanoma patients, 12.5% of ovarian cancer patients, 4.2% of patients with lung cancer and 7.7% of patients with breast cancer have AAB against NY-

ESO-1. No AAB were found in 47 patients with NY-ESO-1 negative melanomas, but were present in 53 % patients with NY-ESO-1 positive melanomas, suggesting an autoantigen driven response. No AAB against CTA were found in 70 blood donors [47]. The current determined tumor associated humoral anti-CTA responses are summarized in table 3. With few exceptions those responses are relatively infrequent. NY-ESO-1 AAB seem to be the most frequently observed anti-CTA response. Jäger et al. showed that both NY-ESO-1 autoantibodies and cytotoxic T cells (CTL) against NY-ESO-1 peptides can be present in the same patient [48]. This suggests that the screening for an AAB response may be a simple and effective way to identify concomitantly CTL reactivity.

Onconeural antigens (ONA) are normally restricted to the nervous system but are aberrantly expressed in a number of tumors possibly by gene activation or derepression or post-transcriptional regulatory mechanisms. They may then be recognized by the immune system as "foreign" and elicit an autoimmune response causing paraneoplastic syndromes affecting the nervous system. AAB against ONA may detect neuronal nuclear antigens (= anti-neuronal nuclear antibodies ANNA), cytoplasmic antigens of Purkinje cells (= anti-Purkinje cell antibodies APCA), synaptic or retinal proteins (see table 4). In most cases of paraneoplastic syndromes the detection of specific AAB can strongly suggest the presence of a tumor (for review see [49-54]). Anti-Hu positive patients with paraneoplastic encephalomyelopathies (PEM) or subacute sensory neuronopathy (SSN) most often have small cell lung cancer (SCLC) as underlying disease. Similarly, anti-Yo positive patients with paraneoplastic cerebellar degeneration (PCD) often harbor gynaecological neoplasms. Furthermore, Hu, Yo and Ri AAB can be found in lower frequency and at lower titers in SCLC or ovarian cancer patients without neurological diseases [55,56]. Neurological syndromes associated with AAB to ion channel proteins have a lower frequency of tumors as the syndromes associated with Hu, Yo and Ri antibodies. Patients with anti-VGKC positive acquired neuromyotonia have SCLC or thymoma in 20% and only 10-15% of the AchR antibody positive Myasthenia gravis (MG) cases are associated with thymoma. In thymoma associated MG other autoantigens such as ryanodine receptor and titin may also play a pathogenic role [57,58]. Recently, AAB against neuroectodermal antigens, SOX group B and zinc-finger gene of the cerebellum (ZIC)2 proteins, have been found in high frequency of SCLC patients without the presence of paraneoplastic neurologic syndroms [59]. The presence of a strong humoral autoimmune response to SOX1 and ZIC2 proteins without concomitant development of autoimmune neurologic disease and the correlation of anti-SOX1/ZIC2 AAB with prognosis suggest that these antigens could be targets for cancer vaccine strategies [59].

Table 3. Autoantibodies against cancer/testis class of tumor antigens (CTA)

CTA family	Autoantibodies against	Tumor entities	References
MAGE-A	MAGE-A1	Lung (4%), colon (12.8%) and ovarian (3.1%) cancer, melanoma (1-36%)	47,122
	MAGE-A3	Colon cancer (8.1%)	123
CAGE	CAGE	Colon cancer (12%), endometrial cancer (26.7%), melanoma (10%)	124
XAGE	XAGE-1b	Lung cancer (25%, all adenocarcinoma), prostate cancer (1.6%)	125,126
SCP	SCP-1 (HOM-TES-14)	Breast (50%) and renal (3.2%) cancer	127
ESO	NY-ESO-1	Lung (4-20%), colon (6.8%), prostate (8.2-20%), ovarian (12.5%), and breast cancer (8%), melanoma (9.4%)	47,48,123, 128-130
cTAGE	cTAGE-1	Colon cancer (25%), cutaneous T-cell lymphoma (33%: only stage D2)	131,132
GAGE-A	Truncated GAGE	Colon cancer (12.5%)	131
SSX2	SSX2	Colon cancer (2.7%), melanoma (18%)	122,123
LAGE	LAGE-1	Prostate cancer (15%)	129

Proliferation associated antigens other than oncoproteins. Proteins involved in cell cycle regulation / progression and mitosis, but also other proteins involved in cellular processes that might be increased in unregulated cell growth, may drive autoimmune responses in tumor patients. AAB against nuclear and cytoplasmic cell cycle regulated or regulating proteins and proteins involved in splicing processes and ribosome biosynthesis could be detected in tumor patients. This supports previous observations that AAB responses against intracellular antigens are often directed at molecules involved in cellular biosynthetic or proliferative functions [60].

Cyclins and cyclin-dependent kinases (CDK) are a group of cell cycle regulating proteins acting at different points of the cell cycle progression. They are amplified and overexpressed in many tumors [61-64]. Covini et al. showed that AAB to cyclin B1, cyclin A and CDK2 are present in sera of patients with hepatocellular carcinomas (HCC) in 15%, 1% and 1%, respectively [65]. Furthermore, anti-cyclin B1 antibodies could be found in patients with a higher risk of HCC development, e.g. in patients with chronic hepatitis (in 1 of 70 cases) and cirrhosis (in 3 of 70 cases), suggesting a predictive relevance of these AAB. Recently, AAB against cyclin B1 have been found in frequencies higher than 10% in patients with lung, colorectal, gastric and protate cancer [38,66]. In prostate cancer also anti-cyclin D1 and anti-cyclin A AAB have been described [38].

Many other AAB against proliferation associated antigens with more or less specificity are present in sera of cancer patients, such as AAB against (1) SG2 nuclear antigen, a member of a novel family of calmodulin binding proteins associated with the serine/threonine phosphatase PPA2 [67,68], (2) the centromere protein F [69], (3) DNA topoisomerase II [70], (4) a novel nuclear autoantigen with splicing factor motifs, provisionally designated HCC1 [71], (5) insulin-like growth factor II (IGF-II) mRNA-binding proteins IMP-1, IMP2 (p62), and IMP-3 (Koc) that regulate the expression of IGF-II [72,73], (6) DEAD-box protein 48 (DDX48), also known as eukaryotic initiation factor 4A-like NUK-34 [74], (7) 32 kDa subunit of replication protein A (RPA32) [75], (8) annexins I, II, and XI-A [76,77], AIS gene product p40 [78], only to name a few. The list of cancer autoantigens is growing rapidly: several hundreds have been found via recognition by AAB in cancer patients sera using SEREX [45,46,79; see Koslowski, pp. 78-91 or proteomics-based technologies [74,81-83; see also Klein-Scory, p. 141]. How can we use this potential on humoral autoimmune responses in research and clinical practice?

Table 4. Autoantibodies against onconeural (neuroectodermal) antigens and associated diseases

Autoantibodies against	Paraneoplastic syndromes	Associated tumors	References
Hu antigens (HuD, HuC, Hel-N1)	Paraneoplastic encephalomyelopathies Subacute sensory neuronopathy	Small-cell lung cancer	53,55
Ri antigens (NOVA-1, NOVA-2)	Opsoclonus/myoclonus syndrome	Breast cancer Small-cell lung cancer	52
Yo antigens (cdr34, cdr62-1, cdr62-2)	Paraneoplastic cerebellar degeneration	Ovarian cancer Breast cancer Small-cell lung cancer Hodgkin's lymphoma	54
Synaptic vesicle-related protein amphiphysin	Paraneoplastic stiff man syndrome Paraneoplastic encephalomyelitis	Breast cancer Small-cell lung cancer	133
Protein(s) of the P/Q, N and L type of voltage-gated calcium channels (VGCC)	Lambert-Eaton myasthenic syndrome (LEMS)	Small-cell lung cancer	51,52
Protein(s) of voltage-gated potassium channels	Acquired neuromyotonia (Isaacs' syndrome)	Small-cell lung cancer Thymoma	51
Protein(s) of the acetylcholine receptor Cross-reactive titin epitopes Ryanodine receptor	Myasthenia gravis	Thymoma or thymic carcinoma	57,58
Recoverin, a protein of photoreceptor cells	Cancer-associated retinopathy	Breast cancer Small-cell lung cancer	105
Zinc-finger protein ZIC4	Subacute cerebellar degeneration	Small-cell lung cancer	134
Zinc-finger protein ZIC2		Small-cell lung cancer	59
Collapsin response mediator protein-5 (CRMP-5)	Subacute cerebellar degeneration	Small-cell lung cancer	134
Sry-like high mobility group box (SOX) antigens SOX1 and SOX2		Small-cell lung cancer	59

Relevance of tumor associated autoantibodies

Since AAB response in tumor patients is often associated with aberrant or overexpression of TAA in tumor tissue or serum, most TA-TAA seem to be a result of antigen-driven immune responses like those suggested for autoimmune diseases [60]. Furthermore, it may be concluded that TA-AAB can be viewed as reporters from the immune system revealing the identity of antigens which might be playing roles in the tumorigenic processes [84,85]. Therefore, TA-AAB are important markers for different approaches:

Molecular probes for the identification of novel proliferation associated antigens and pathways [68,71,86-88]. Sera of cancer patients have been shown to be useful reagents for identifying new cellular proteins possibly involved in tumor development. A new cell cycle specific DNA-binding nuclear protein has been identified using autoimmune serum of a patient with bladder and metastatic lung cancer [67,89]. This serum produced a previously undescribed cell cycle-related staining pattern on HEp-2 cells. According to the cell cycle distribution the detected antigen was provisionally named SG2NA (S/G2 nuclear antigen). The centromere protein F is another novel proliferation associated and cell cycle dependent protein detected by autoimmune sera. Casiano et al. identified a centromere protein provisionally designated p330[d] (doublet polypeptide of 330 kDa), which accumulates in the nuclear matrix during S phase, reaching maximum levels during G2 phase and localized at the centromeres during prophase and metaphase and at the central spindle and midbody regions during anaphase and telophase [86]. The same protein, designated centromere protein F (CENP-F), was identified by Rattner et al. using a serum from a lung cancer patient [90].

Search of new targets for vaccine-based therapies. Most TA-AAB detect antigens that are highly expressed predominantly in tumor cells. Those tumor associated antigens are putative candidates for a tumor vaccination strategy because the B-cell response is often accompanied by a cellular immune response [48].

Biomarkers for the prediction, diagnosis and monitoring of tumors. Despite the large number of TA-AAB detected until now the practical application is limited by the following reasons: (1) The aberrant or overexpression of TAA in tumors is, with few exceptions necessary, but not sufficient for immune activation. Usually, individual TA-AAB are detected in only small numbers (<20%) of patients with cancer depending on the MHC status and other individual factors. Therefore, the diagnostic sensitivity of most TA-AAB is too low for diagnostic screening. (2) Overexpressed genes/proteins are thought to elicit an immune response by over-riding thresholds critical for the maintenance of tolerance [91]. Because proliferation associated antigens may be

also overexpressed in other hyperproliferative disorders such as autoimmune diseases [92], the AAB response is often not specific for malignancy [17,93,94]. (3) The results regarding sensitivity and specifcity of TA-AAB may differ dramatically from study to study due to different methods used for AAB determination, study design and ethnicity of tested subjects. Nevertheless, TA-AAB may have a great potential in the early (pre-symptomatic) diagnosis of cancer, because the autoimmune response does not depend on the size of the tumor.

Tumor associated autoantibodies – biomarkers for the prediction and early diagnosis of cancer?

There is still a need for parameters which are specific for tumors and are detectable in preclinical stages. The ideal tumor marker should be highly sensitive and highly specific for tumors. The tumor sensitivity should be higher than that of other diagnostic methods and the earlier diagnosis should lead to an improvement of therapy. Tumor-associated antigens present in sera of cancer patients (e.g., CEA, NSE, SCC, CA50 etc.) can be useful markers for prognosis and for monitoring cancer therapy but have a limited value for diagnosis, especially for the early diagnosis of cancer. Novel approaches and developments (see Oehr, pp. 26-43 and Chapter 2-4] may improve the diagnostic possibilities but are too expensive for a broader use or for the screening of risk groups. TA-AAB may develop (very) early with respect to tumor formation. Hints for predicitive relevance of TA-AAB are given by different observations and approaches:

TA-AAB are significantly more often detectable in risk groups for cancer development than in healthy volunteers. A higher risk of tumor development is observed in populations expressing cancer susceptibility genes (hereditary cancer syndromes, see Jandrig, pp. 237-248), populations who are exposed to carcinogenic substances (e.g., uranium miners), and in populations with preneoplastic or cancer predisposing diseases (e.g., Barretts' oesophagus, chronic liver inflammation or cirrhosis, dermatomyositis, Sjögren's syndrome). TA-AAB have been descibed in some of the known risk groups in higher frequencies than in healthy controls: Anti-p53 AAB in uranium miners [95]; anti-p53 AAB in patients with liver cirrhosis and other chronic liver diseases [96]; anti-Crt32 AAB in HBV positive chronic hepatitis [82]; anti-WT AAB in myelodysplastic syndromes [28]; anti-hMSH2 and hPMS1 AAB in patients with dermato/polymyositis [32]; anti-HMdU (5-hydroxymethyl-2'-deoxyuridine) and anti-p53 AAB in otherwise healthy women who had a family history of breast cancer [97,98]. However, the TA-AAB response in these populations does not indicate that all AAB positive subjects will develop cancer, because overexpression of the relevant autoantigens with the potential of AAB induction can be observed also in non-tumorous cells [99]. The AAB

responses rather reflect changes that might be relevant in tumorigenesis and therefore indicate a rising risk of tumor development in those subjects.

TA-AAB are significantly more often detectable in pre-malignant and early tumor stages than in healthy volunteers: Anti-RPA32 and anti-p53 AAB are detectable in ductal carcinoma in situ of the breast with early, non-palpable (3-5 mm) lesions [75,100]; Anti-YKL-40 AAB are detectable in early stage of ovarian cancer more often than the conventional tumor markers (65% vs 35% CA125 and 13% CA15-3) [101].

TA-AAB against neuroectodermal antigens in paraneoplastic syndromes. Paraneoplastic syndromes are (most often neurologic) disorders (PND), that are caused by a strong immune response against a shared antigen in the tumor and normal host tissues. Because there is a direct relationship between those TA-AAB and paraneoplastic manifestations, the PND specific AAB response is an early indicator of tumor development (table 4). It has been shown that AAB against the neuroectodermal antigens HuD, amphiphysin, recoverin, or enolase precede the diagnosis of cancer in approximately 70% of patients by up to 4 years [102-105].

Retrospective studies demonstrate how long TA-AAB are detectable before disease manifestation or the definite diagnosis with conventional methods. Lubin et al. [106] were the first who described that the humoral anti-p53 response may be an early event during tumorigenesis and can be detected before clinical manifestation of the disease. In two retrospective studies Trivers et al. showed that p53 AAb were present months to years before the manifestation of tumors: (1) angiosarcoma of the liver in workers occupationally exposed to vinyl chloride and (2) lung cancer in heavy smokers with chronic obstructive pulmonary disease [107,108]. In a retrospective study on former uranium miners, we could show that anti-p53, anti-NY-ESO-1 and anti-survivin AAB are detectable in sera from patients with lung cancer collected over a period of up to 10 years prior to disease manifestation or confirmed diagnosis [34,95,109]. Anti-RPA32 AAB was shown to be present 18 months before diagnosis of cancer in one patient [75] and in another one anti-IMP1 and anti-IMP3 (Koc) AAB were detectable approximately 8 years before diagnosis of HCC [73].

Prospective studies. The follow-up of TA-AAB positive persons is of great importance to show the real risk of cancer development in those subjects. Up to now only one study has been published: Women, healthy at blood donation but who were diagnosed 0.5-6 years later with breast or colorectal cancer exhibited significantly increased anti-HMdU (5-hydroxymethyl-2'-deoxyuridine) AAB over the age-matched controls [97]. Hopefully, further prospective studies of TA-AAB positive persons will show whether defined AAB can be used in the screening for preneoplastic or microinvasive tumor lesions allowing an early diagnosis and an early intervention of cancer.

Conclusions

Of the several hundreds AAB specificities detected in sera of tumor patients so far, more and more have become candidates for introduction in the clinical practice. According to the spectrum of reactivity, two classes of TA-AAK can be differentiated: those associated with specific types of cancer (e.g., anti-HCC1) and those common to a wide spectrum of cancer (e.g., anti-p53, anti-survivin, and anti-RPA32 AAB). For further use TA-AAB should be selected for their specificity regarding malignancies and for their potential clinical application (screening of risk groups, early diagnosis, disease monitoring). If selected for high specificity, for the screening of risk groups, the sensitivities of most TA-AAB are too low. Even the highest frequencies of about 30 to 50%

found in lung, colorectal, head and neck cancer (anti-p53, -p21^{ras}) and breast cancer (anti-HER-2/neu) with concomitant high specificity are not sufficient for a screening programme. A combined determination of two or more tumor specific AAB may overcome this problem. Therefore, a further evaluation of the relevance of known AAB specificities as well as the search for novel diagnostically relevant TA-AAB is necessary.

Taken together, the screening for an AAB response in tumor patients may lead to new diagnostic tumor markers and may be a simple and effective way to identify concomitantly CTL reactivity. Furthermore, as "reporters from the immune system" such AAB could be used to elucidate the nature of autoantigens which drive the immune response.

References

(1) Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.

(2) Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415-28.

(3) Herman JG. Hypermethylation of tumor suppressor genes in cancer. Semin Cancer Biol 1999;9:359-67.

(4) Klein G. Cancer, apoptosis and nonimmune surveillance. Cell Death Differ 2004;11:13–17.

(5) Reipert BM, Tanneberger S, Pannetta A, Bedosti M, Poell M, Zimmermann K, Stellamor MT. Increase in autoantibodies against Fas (CD95) during carcinogenesis in the human colon: a hope for immunoprevention of cancer? Cancer Immunol Immunother 2005;54:1038-1042.

(6) Cahalon L, Korem S, Gonen B, Puri J, Smorodinsky NI, Witz IP. Autoantibody-mediated regulation of tumor growth. Ann NY Acad Sci 1992;621:393-408.

(7) Manson LA. Does antibody-dependent epitope masking permit progressive tumour growth in the face of cell-mediated cytotoxicity? Immunol Today 1991;12:352-355.

(8) Ehrlich R, Witz IP. Natural killer cells and naturally occurring antibodies as representative of natural tumor immunity. Pathobiol Annu 1982;12:85-113.

(9) Witz IP, Agassy-Cahalon L. Do naturally occuring antibodies play a role in progression and proliferation of tumor cells? Int Rev Immunol 1988;3:133-145.

(10) Soussi T. p53 antibodies in the sera of patients with various types of cancer: a review. Cancer Res 2000;60:1777–1788.

(11) Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nature Med 2004;10:789-799.

(12) Bishop JM. The molecular genetics of cancer. Science 1987;235:305-311.

(13) Cheever MA, Disis ML, Bernhard H, Gralow JR, Hand SL, Huseby ES, Qin HL, Takahashi M, Chen W. Immunity to oncogenic proteins. Immunol Rev 1995;145:33-59.

(14) Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA. High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. J Clin Oncol 1997;15:3363-3367.

(15) Takahashi M, Chen W., Burd DR, Disis ML, Huseby ES, Qin H, McCahill L, Nelson H, Shimada H, Okuno K, Yasutomi M, Peace DJ, Cheevers MA. Antibody to ras proteins in patients with colon cancer. Clin Cancer Res 1995;1:1071-1077.

(16) Ben-Mahrez K, Sorokine I, Thierry D, Kawasumi T, Ishii S, Salmon R, Kohiyama M. Circulating antibodies against c-myc oncogene product in sera of colorectal cancer patients. Int J Cancer 1990;46:35-38.

(17) Deguchi Y, Negoro S, Kishimoto S. Autoantibody to human c-myc oncogene product in autoimmune patients' sera. Int Arch Allergy Appl Immunol 1988;87:313-316.

(18) LaFond RE, Eaton RB, Watt RA, Villee CA, Actor JK, Schur PH. Autoantibodies to c-myc protein: elevated levels in patients with African Burkitt's lymphoma and normal Ghanians. Autoimmunity 1992;13:215-224.

(19) Yamamoto A, Shimizu E, Ogura T, Sone S. Detection of auto-antibodies against L-myc oncogene products in sera from lung cancer patients. Int J Cancer 1996;69:283-289.

(20) Yamamoto A, Shimizu E, Sumitomo K, Shinohara A, Namikawa O, Uehara H, Sone S. L-myc overexpression and detection of auto-antibodies against L-myc in both the serum and pleural effusion from a patient with non-small cell lung cancer. Intern Med 1997;36:724-727.

(21) Sorokine I, Ben-Mahrez K, Bracone A, Thierry D, Ishii S, Imamoto F, Kohiyama M. Presence of circulating anti-c-myb oncogene product antibodies in human sera. Int J Cancer 1991;47:665-669.

(22) Davidoff AM, Humphrey PA, Iglehart JD, Marks JR. Genetic basis for p53 over-expression in human breast cancer. Proc Natl Acad Sci USA 1991;88:5006-5010.

(23) Montenarh M. Humoral immune response against p53 in human malignancies. In: Conrad K, Humbel RL, Meurer M, Shoenfeld Y, Tan EM, editors. Pathogenic and diagnostic relevance of autoantibodies. Lengerich, Berlin: Pabst Science Publishers, 1998; 377-394.

(24) Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hosteter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P. Mutation in the p53 gene occur in diverse human tumour types. Nature 1989;342:705-708.

(25) Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancer. Science 1991;253:49-53.

(26) Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. Int J Cancer 1982;30:403-408.

(27) Lubin R, Schlichtholz B, Teillaud JL, Garay E, Bussel A, Wild CP. p53 antibodies in patients with various types of cancer: Assay, identification, and characterization. Clin Cancer Res 1995;1:1463-1469.

(28) Elisseeva O, Oka Y, Tsuboi K, Ogata K Wu F, Kim EH, Soma T, Tamaki H, Kawakami M, Oji Y, Hosen N, Kubota T, Nakagawa M, Yamagami T, Hiraoka A, Tsukaguchi M, Udaka K, Ogawa H, Kishimoto T, Nomura T, Sugiyama H. Humoral immune responses against Wilms tumor gene WT1 product in patients with hematopoietic malignancies. Blood 2002;99:3272-3279.

(29) Scully R, Chen J, Ochs R, Keegan K, Hoeckstra M, Feunteun J, Livingston DM. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 1997;90:425-435.

(30) Maacke H, Hundertmark C, Miska S, Voss M, Kalthoff H, Stürzbecher HW. Autoantibodies in sera of pancreatic cancer patients identify recombination factor Rad51 as a tumor-associated antigen. J Cancer Res Clin Oncol 2002; 128:219-22.

(31) Gautier F, Irmgard I, Gregoire M, Meflah K, Harb J. Identification of an apoptotic cleavage product of BARD1 as an autoantigen: a potential factor in the antitumoral response mediated by apoptotic bodies, Cancer Res 2000;12:6895–6900.

(32) Okada T, Noji S, Goto Y, Iwata T, Fujita T, Okada T, Matsuzaki Y, Kuwana M, Hirakata M, Horii A, Matsuno S, Sunamura M, Kawakami Y. Immune responses to DNA mismatch repair enzymes hMSH2 and hPMS1 in patients with pancreatic cancer, dermatomyositis and polymyositis. Int J Cancer 2005;925-933.

(33) LaCasse EC, Baird S, Korneluk RG, McKenzie AE. The inhibitors of apoptosis (IAP) and their emerging role in cancer. Oncogene 1998;17:3247–3259.

(34) Rohayem J, Diestelkoetter P, Weigle B, Oehmischen A, Schmitz M, Mehlhorn J, Conrad K, Rieber EP: Antibody response to the tumor-associated inhibitor of apoptosis protein Survivin in cancer patients. Cancer Res 2000; 60:1815-1817.

(35) Yagihashi A, Asanuma K, Nakamura M, Araya J, ManoY, Torigoe T, Kobayashi D, Watanabe N. Detection of anti-survivin antibody in gastrointestinal cancer patients. Clin Chem 2001;47:1729-1731.

(36) Yagihashi A, Asanuma K, Tsuji N, Torigoe T, Sato N, Hirata K, Watanabe N. Detection of anti-livin antibody in gastrointestinal cancer patients. Clin Chem 2003;49:1206-1208.

(37) Yagihashi A, Asanuma K, Kobyashi D, Tsuji N, Shijubo Y, Abe S, Hirohashi Y, Torigoe T, Sato N, Watanabe N. Detection of autoantibodies to livin and survivin in sera from lung cancer patients. Lung Cancer 2005;48:217-221.

(38) Shi FD, Zhang JY, Liu D, Rearden A, Elliot M, Nachtsheim D, Daniels T, Casiana CA, Heeb MJ, Chan EKL, Tan EM. Preferential humoral immune responses in prostate cancer to cellular proteins p90 and p62 in a panel of tumor.associated antigens. Prostate 2005;63:252-258.

(39) Chang JT, Wong FH, Liao CT, Chen ICH, Wang HM, Cheng AJ: Enzyme immunoassay for serum autoantibodies to survivin and ist finding in head-and-neck cancer patients. Clin Chem 2004; 50:1261-1264.

(40) Ambrosini G, Adida C, Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3:917-921.

(41) Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J, Altieri DC. Developmental regulated expression of the novel cancer antiapoptosis gene survivin in human and mouse differentiation. Am J Pathol 1998;152:43-49

(42) Lu CD, Altieri DC, Tanigawa N. Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. Cancer Res 1998;58:1808-1812.

(43) Gazzaniga P, Gradilone A, Giuliani O, Silvestri I, Nofroni I, Frati L, Agliano AM. Expression and prognostic significance of livin, survivin and other apoptosis-related genes in the progression of superficial bladder cancer. Ann Oncol 2003;14:85-90.

(44) Tanabe H, Yagihashi A, Tsuji N, Shijubo Y, Abe S, Watanabe N. Expression of survivin mRNA and livin mRNA in non-smallcell lung cancer. Lung Cancer 2004;46:299-304.

(45) Sahin U, Türeci Ö, Pfreundschuh M. Serological identification of human tumor antigens. Curr Opin Immunol 1997;9:709-716.

(46) Old LJ, Chen YT. New paths in human cancer serology. J Exp Med 1998;187:1163-1167.

(47) Stockert E, Jäger E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LL. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 1998;187:1349-1354.

(48) Jäger E, Chen YT, Drijfhout JW, Karbach J, Ringhoffer M, Jäger D, Arand M, Wada H, Noguchi Y, Stockert E, Old LJ, Knuth A: Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: Definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med 1998; 187:265-270.

(49) Dropcho EJ. Update on paraneolastic syndromes. Curr Opin Neurol 2005;18:331-336.

(50) Posner JB, Dalmau J. Paraneoplastic syndromes. Curr Opin Immunol 1997;9:723-729.

(51) Lang B, Vincent A. Autoantibodies to ion channels at the neuromuscular junction. Autoimmun Rev 2003;2:94-100.

(52) Lennon VA. Calcium channel and related paraneoplastic disease autoantibodies. In: Peter JB, Shoenfeld Y (Eds.). Autoantibodies. Amsterdam: Elsevier, 1996;139-146.

(53) Furneaux HM. Neuronal nuclear autoantibodies, Type 1 (Hu). In: Peter JB, Shoenfeld Y, editors. Autoantibodies. Amsterdam: Elsevier, 1996;551-554.

(54) Dalmau JO, Posner JB. Purkinje cell autoantibodies, type 1 (Yo). In: Peter JB, Shoenfeld Y, editors. Autoantibodies. Amsterdam :Elsevier, 1996;655-659.

(55) Graus F, Dalmau J, Rene R, Tora M, Malats N, Verschuuren JJ, Cardenal F, Vinolas N, del Muro G, Vadell C, Mason WP, Rosell R, Posner JB, Real FX. Anti-Hu antibodies in patients with small-cell lung cancer: association with complete response to therapy and improved survivial. J Clin Oncol 1997;15:2866-2872.

(56) Drlicek M, Bianchi G, Bogliun G, Casati B, Grisold W, Kolig C, Liszka-Setinek U, Marzorati L, Wondrusch E, Cavaletti G. Antibodies of the anti-Yo and anti-Ri type in the absence of paraneoplastic neurological syndromes: a long-term survey of ovarian cancer patients. J Neurol 1997;244:85-89.

(57) Voltz RD, Albrich WC, Nägele A, Schumm F, Wick M, Freiburg A, Gautel M, Thaler HT, Aarli J, Kirchner T, Hohlfeld R. Paraneoplastic myasthenia gravis: Detection of anti-MGT30 (titin) antibodies predict thymic epithelial tumor. Neurol 1997;49:1454-1457.

(58) Mygland A, Aarli JA, Matre G, Gilhus NE. Ryanodine receptor (RyR) antibodies are detected in about 50% of patients with myasthenia gravis who have a thymoma. J Neurol Neurosurg Psychiatry 1994;57:843-846.

(59) Vural B, Chen LC, Saip P, Chen YT, Ustuner Z, Gonen M, Simpson AJG, Old LJ, Ozbek U, Gure AO. Frequency of SOX group B (SOX1, 2, 3) and ZIC2 antibodies in Turkish patients with small cell lung carcinoma and their correlation with clinical parameters. Cancer 2005;103:2575-2583

(60) Tan EM. Autoantibodies in pathology and cell biology. Cell 1991;67;841-842.

(61) Hunter T, Pines J. Aberrant expression of cyclins may be intimately associated with the neoplastic process. Cell 1991;66:1071-1074.

(62) Gong J, Ardett B, Traganos F, Darzynkiewicz Z. Unscheduled expression ofcyclin B1 and cyclin E in several leukemic and solid tumor cell lines. Cancer Res 1994;54:4285-4288.

(63) Buckley MF, Sweeney KJ, Hamilton JA, Sini RL, Manning DL, Nicholson RI, de Fazio A, Watts CK, Musgrove EA, Sutherland RL. Expression and amplification of cyclin genes in human breast cancer. Oncogene 1993;8:2117-2133.

(64) Wang A, Yoshimi N, Ino N, Tanaka T, Mori H. Overexpression of cyclin B1 in human colorectal cancers. J Cancer Res Clin Oncol 1997;123:124-127.

(65) Covini G, Chan EKL, Nishioka M, Morshed SA, Reed SI, Tan EM. Immune response to cyclin B1 in hepatocellular carcinoma. Hepatol 1997;25:75-80.

(66) Zhang JY, Casiano C, Peng XX, Koziol JA, Chan EKL, Tan EM: Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. Cancer Epid Biomarkers Prevent 2003;12:136-143.

(67) Muro Y, Chan EKL, Landberg G, Tan EM. A cell-cycle nuclear autoantigen containing WD-40 motifs expressed mainly in S and G_2 phase cells. Biochem Biophys Res Comm 1995;207:1029-1037

(68) Moreno CS, Park S, Nelson K, Ashby D, Hubalek F, Lane WS, Pallas DC. WD40 repeat proteins striatin and S/G(2) nuclear autoantigen are members of a novel family of calmodulin-binding proteins that associate with protein phosphatase 2A. J Biol Chem 2000;275:5237-5263

(69) Rattner JB, Rees J, Whitehead CM, Casiano CA, Tan EM, Humbel RL, Conrad K, Fritzler M. High frequency of neoplasia in patients with autoantibodies to centromere protein CENP-F. Clin Invest Med 1997;20:308-319.

(70) Imai H, Furuta K, Landberg G, Kiyosawa K, Liu LF, Tan EM. Autoantibodies to DNA topoisomerase II in primary liver cancer. Clin Cancer Res 1995;1:417-422.

(71) Imai H, Chan EKL, Kiyosawa K, Fu XD, Tan EM. Novel nuclear autoantigen with splicing factor motifs identified with antibody from hepatocellular carcinoma. J Clin Invest 1993;92:2419-2426.

(72) Zhang JY, Chan EKL. Autoantibodies to IGF-II mRNA binding protein p62 and overexpression of p62 in human hepatocellular carcinoma. Autoimm Rev 2002;1:146-153.

(73) Himoto T, Kuriyama S, Zhang JY, Chan EKL, Nishioka M, Tan EM. Significance of autoantibodies against insulin-like growth factor II mRNA-binding proteins in patients with hepatocellular carcinoma. Int J Oncol 205;26:311-317.

(74) Xia Q, Kong XT, Zhang GA, Hou XJ, Qiang H, Zhong RQ. Proteomic-based identification of DEAD-box protein 48 as a novel autoantigen, a prospective serum marker for pancreatic cancer. Biochem Biophys Res Comm 2005;330:526-532

(75) Tomkiel JE, Alansari H, Tang N, Virgin JB, Yang X, VandeVord P, Karvonen RL, Granda JL, Kraut MJ, Ensley JF, Fernández-Madrid F. Autoimmunity to the Mr 32,000 subunit of replication protein A in breast cancer. Clin Cancer Res 2002; 8:752-758.

(76) Brichory FM, Misek D, Yim A, Krause M, Giordano T, Beer D, Hanash S. An immune response manifested by common occurrence of annexins I and II autoantibodies and high circulating levels of interleukin 6 in lung cancer. Proc Natl Acad Sci USA 2001;98:9824–9829.

(77) Fernandez-Madrid F, Tang N, Alansari H, Granda JL, Tait L, Amirikia KC, Moroianu M, Wang X, Karvonen RL. Autoantibodies to Annexin XI-A and other autoantigens in the diagnosis of breast cancer. Cancer Res 2004;64:5089–5096.

(78) Yamaguchi K, Patturajan M, Trink B, Usadel H, Koch W, Jen J, Sidransky D. Circulatiing antibodies to p40[AIS] in the sera of respiratory tract cancer patients. Int J Cancer 2000;89:524-528.

(79) Türeci Ö, Sahin U, Pfreundschuh M: Serological analysis of human tumor antigens: molecular definition and implications. Mol Med Today 1997;3:342-349.

(80) Brichory F, Beer D, Le Naour F, Giordano T, Hanash S. Proteomics-based identification of protein gene product 9.5 as a tumor antigen that induces a humoral response in lung cancer. Cancer Res 2001;61:7908-7912

(81) Canelle L, Bousquet J, Pionneau C, Deneux L, Imam-Sghiouar N, Caron M, Joubert-Caron R. An efficient proteomics-based approach for the screening of autoantibodies. J Immunol Meth 2005;299:77-89.

(82) Le Naour F, Brichory F, Misek DE, Brechot C, Hanash SM, Beretta L. A distinct repertoire of autoantibodies in hepatocellular carcinoma identified by proteomic analysis. Mol Cell Proteomics 2002;1:197-203.

(83) Le Naour F, Misek DE, Krause MC, Deneux L, Giordano TJ, Scholl S, Hanash SM. Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer. Clin Cancer Res 2001;7:3328-3335.

(84) Tan EM. Autoantibodies in diagnosis and in identifying autoantigens. Immunologist 1999; 7:85-92.

(85) Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanism in tumorigenesis. J Clin Invest 2001;108:1411-1415.

(86) Casiano CA, Landberg G, Ochs R, Tan EM. Autoantibodies to a novel cell cycle-regulated protein that accumulates in the nuclear matrix during S phase and is localized in the kinetochores and spindle midzone during mitosis. J Cell Sci 1993;106;1045-1056.

(87) Zhang JY, Chan EK, Peng XX, Tan EM. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. J Exp Med 1999; 189:1101-1110.

(88) Mueller-Pillasch F, Lacher U, Wallrapp C, Micha A, Zimmerhackl F, Hameister H, Varga G, Friess H, Büchler M, Beger HG, Vila MR, Adler G, Gress TM. Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. Oncogene 1997;14:2729-2733.

(89) Landberg G, Tan EM. Characterization of a DNA-binding nuclear autoantigen mainly associated with S phase and G2 cells. Exp Cell Res 1994;212;255-261.

(90) Rattner JB, Rao A, Fritzler MJ, Valencia DW, Yen TJ. CENP-F is a ca. 400 kDa kinochore protein that exhibits a cell-cycle dependent localization. Cell Motil Cytoskeleton 1993;26;214-226.

(91) Viola A, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds. Science 1996;273:104-106.

(92) Deguchi Y, Hara H, Negoro S, Kakunaga T, Kishimoto S: Protooncogene expression in peripheral blood mononuclear cells from patients with systemic lupus erythematosus as an indicator of the disease activity. Clin Immunol Immunopathol 1987;45:424-439

(93) Husby G, Williams RC, Watt RA, West SG, Tung KSK. Tissue c-myc expression and immune responses in systemic lupus erythematosus. Clin exp Immunol 1987;69:493-500.

(94) Yamauchi T, Naoe T, Kurosawa Y, Shiku H, Yamada K: Autoantibodies to c-myc nuclear protein products in autoimmune disease. Immunology 1990;69:117-120.

(95) Conrad K. Autoantibodies in cancer patients and in persons with a higher risk of cancer development. In: Shoenfeld, Y.; Gershwin M.E. (Eds.): Cancer and Autoimmunity. Amsterdam: Elsevier Science B.V., 2000, S. 159-173.

(96) Raedle J, Oremek G, Truschnowitsch M,Lorenz M, Roth WK, Caspary WF, Zeuzem S. Clinical evaluation of autoantibodies to p53 protein in patients with chronic liver disease and hepatocellular carcinoma. Eur J Cancer 1998;34:1198-1203 .

(97) Frenkel K, Karkoszka J, Glassman T, Dubin N, Toniolo P, Taioli E, Mooney LA, Kato I: Serum autoantibodies recognizing 5-hydroxymethyl-2'-deoxyuridine, an oxidized DNA base, as biomarkers of cancer risk in women. Cancer Epidemiol Biomarkers Prev 1998; 7:49-57.

(98) Green JA, Mudenda B, Jenkins J, Leinster SJ, Tarunina M, Green B, Robertson L. Serum p53 auto-antibodies: incidence in familial breast cancer. Eur J Cancer 1994;30A;580-584.

(99) Livni N, Eid A, Llan Y, Rivkind A, Rosenmann E, Blendis LM, Shouval D, Galun E. p53 expression in patients of cirrhosis with and without hepatocellular carcinoma. Cancer 1995;75:2420-2426.

(100) Regele S, Kohlberger P, Vogl FD, Böhm W, Kreienberg R, Runnebaum IB. Serum p53 autoantibodies in patients with minimal lesions of ductal carcinoma in situ of the breast. Br J Cancer 1999; 81:702-704.

(101) Dupont J, Tanwar MK, Thaler HAT, Fleisher M, Kauff N, Hensley ML, Sabbatini P, Anderson S, Aghajanian C, Holland EC, Spriggs DR. Early detection and prognosis of ovarian cancer using serum YKL-40. J Clin Oncol 004;22:3330-3339.

(102) Graus F, Keime-Guibert F, Rene R, Benyahia B, Ribalta T, Ascaso C, Escaramis G, Delattre JY. Anti-Hu-associated paraneoplastic encephalomyelitis: analysis of 200 patients. Brain 2001;124:1138–1148.

(103) Saiz A, Dalmau J, Butler MH, Chen O, Delattre JY, De Camilli P, Grauss F. Anti-amphiphysin I antibodies in patients with paraneoplastic neurological disorders associated with small cell lung carcinoma. J Neurol Neurosurg Psychiatry 1999;66:214–217.

(104) Lucchinetti CF, Kimmel DW, Lennon VA. Paraneoplastic and oncologic profiles of patients seropositive for type 1 antineuronal nuclear autoantibodies. Neurology 1998;50:652–657.

(105) Adamus G, Ren G, Weleber RG. Autoantibodies against retinal proteins in paraneoplastic and autoimmune retinopathy. BMC Ophthalmol 2004;4:5.

(106) Lubin R, Zalcman G, Bouchet L, Tredanel J, Legros Y, Cazals D, Hirsch A, Soussi T. Serum p53 antibodies as early markers of lung cancer. Nature Med 1995;1:701-702.

(107) Trivers GE, Cawley HL, De Benedetti VMG, Hollstein M, Marion MJ, Bennett WP, Hoover ML, Prives C, Tamburro C, Harris CC. Anti-p53 antibodies in sera of workers occupationally exposed to vinyl chloride. J Natl Cancer Inst 1995;87:1400-1407.

(108) Trivers GE, De Benedetti VMG, Cawley HL, Caron G, Harrington AM, Bennett WP, Jett JR, Colby TV, Tazelaar H, Pairolero P, Miller RD, Harris CC. Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. Clin Cancer Res 1996;2:1767-1775.

(109) Conrad K, Krause P, Türeci O, Heine W, Luxemburger U, Mehlhorn J, Sahin U: NY-ESO1 autoantibody – A novel candidate for early diagnosis of lung cancer. In: Conrad, K.; Fritzler, M.; Meurer M.; Sack, U.; Shoenfeld Y. (Eds.): From Proteomics to Molecular Epidemiology: Relevance of Autoantibodies. Lengerich: Pabst Science Publishers, 2002;130

(110) Brass N, Rácz A, Bauer C, Heckel D, Sybrecht G, Meese E. Role of amplified genes in the production of autoantibodies. Blood 1999;93:2158-2166

(111) Forti S, Scanlan MJ, Invernizzi AM, Castiglioni F, Pupa S, Agresti R, Fontanelli R, Morelli D, Old LJ, Pupa SM, Ménard S. Identification of breast cancer-restricted antigens by antibody screening of SKBR3 cDNA library using a preselected patient's serum. Breast Cancer Res Treatment 2002;73:245-256

(112) Cho B, Lee H, Jeong S, Bang YJ, Lee HJ, Hwang KS, Kim HY, Lee YS, Kang GH, Jeoung DI. Promoter hypomethylation of a novel cancer/testis antigen gene CAGE is correlated with its aberrant expression and is seen in premalignant stage of gastric carcinoma. Biochem Biophys Res Commun 2003;307:52-63.

(113) Mitsudomi T, Suzuki S, Yatabe Y, Nishio M, Kuwabara M, Gotoh K, Hatooka S, Shinoda M, Suyama M, Ogawa M, Takahashi T, Ariyoshi Y, Takahashi T. Clinical implications of p53 autoantibodies in the sera of patients with non-small-cell lung cancer. J Natl Cancer Inst 1998;90:1563-1568.

(114) Ulanet DB, Torbenson M, Dang CV, Casciola-Rosen L, Rosen A. Unique conformation of cancer autoantigen B23 in hepatoma: A mechanism for specificity in the autoimmune response. Proc Natl Acad Sci USA 2003;100:12361-12366

(115) Rughetti A, Turchi V, Ghetti CA, Scambia G, Panici PB, Roncucci G, Manusco S, Frati L, Nuti M. Human B-cell immune response to polymorphic epithelial mucin. Cancer Res 1993;53:2457-2459.

(116) Hong SH, Misek DE, Wang H, Puravs E, Giordano TJ, Greenson JK, Brenner DE, Simeone DM, Logsdon CD, Hanash SM. An autoantibody-mediated immune response to calreticulin isoforms in pancreatic cancer. Cancer Res 2004;64:5504-5510.

(117) Tureci O, Sahin U, Schobert I, Koslowski M, Schmitt H, Schild HJ, Stenner F, Seitz G, Rammensee HG, Pfreundschuh M. The SSX-2 gene, which is involved in the t(X;18) translocation of synovial sarcomas, codes for the human tumor antigen HOM-MEL-40. Cancer Res 1996;53:4766-4772.

(118) Pulford K, Falini B, Bantham AH, Codrington D, Roberton H, Hatton C, Mason DY. Immune response to the ALK oncogenic tyrosine kinase in patients with anaplastic large-cell lymphoma. Blood 2000;96:1605-1607.

(119) Yamamoto, A., Shimizu, E., Takeuchi, E., Houchi, H., Doi, H., Bando, H., Ogura, T., and Sone, S. Infrequent presence of anti-c-Myc antibodies and absence of c-Myc oncoprotein in sera from lung cancer patients. Oncology 1999;56:129-133.

(120) Talpaz M, Qiu X, Cheng K, Cortes JE, Kantarjian H, Kurzrock R. Autoantibodies to abl and bcr proteins. Leukemia 2000;14:1661-1666.

(121) Bénistant C, Bourgaux JF, Chapuis H, Mottet N, Roche S, Bali JP. The COOH-terminal Scr kinase Csk is a tumor antigen in human carcinoma. Cancer Res 2001;61:1415-1420.

(122) Sahin U, Türeci Ö, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA 1995;92:11810-11813.

(123) Scanlan MJ, Welt S, Gordon CM, et al. Cancer-related serological recognition of human colon cancer: identification of potential diagnostic and immunotherapeutic targets. Cancer Res 2002;62:4041–7.

(124) Iwata T, Fujita T, Hirao N, Matsuzaki Y, Okada T, Mochimaru H, Susumu N, Matsumoto, Sugano K, Yamashita N, Nozawa S, Kawakami Y. Frequent immune responses to a cancer/testis antigen, CAGE, in patients with microsatellite instability-positive endometrial cancer. Clin Cancer Res 2005;11:3949-3957.

(125) Ali Eldib AM, Ono T, Shimono M, Kanako M, Tanaka R, Noguchi Y, Nakayama E. Immunoscreening of a cDNA library from a lung cancer cell line using autologous patient serum: identification of XAGE-1b as a dominant antigen and its immunogenicity in lung adenocarcinoma. Int J Cancer 2004;108:558-563.

(126) Koizumi F, Noguchi Y, Saika T, Nakagawa K, Sato S, Ali Eldib AM, Nasu Y, Kumon H, Nakayama E. XAGE-1 mRNA expression in prostate cancer and antibody response in patients. Microbiol Immunol 2005;49:471-476 .

(127) Tureci O, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M. Identifica-

tion of a meiosis-specific protein as a member of the class of cancer/testis antigens. Proc Natl Acad Sci USA 1998;95:5211-5216.

(128) Tureci O, Mack U, Luxemburger U, Heinen H, Krummenauer F, Sester M, Sester U, Sybrecht GW, Sahin U. Humoral immune responses of lung cancer patients against tumor antigen NY-ESO-1. Cancer Lett 2005;in press.

(129) Fossa A, Alsoe L, Crameri R, Funderud S, Gaudernack G, Smeland EB; Serological cloning of cancer/testis antigens expressed in prostate cancer using cDNA phage surface display. Cancer Immunol Immunother 2004; 53:431-438.

(130) Nakada T, Noguchi Y, Satoh S, Ono T, Saika T, Kurashige T, Gnjatic S, Ritter G, Chen YT, Stockert E, Nasu Y, Tsushima T, Kumon H, Old LJ, Nakayama E. NY-ESO-1 mRNA expression and immunogenicity in advanced prostate cancer. Cancer Immun 2000;3:10.

(131) Gerhardt A, Usener D, Keese M, Sturm J, Schadendorf D, Eichmüller S. Tissue expression and sero-reactivity of tumor-specific antigens in colorectal cancer. Cancer Lett 2004;28:197-206.

(132) Eichmuller S, Usener D, Dummer R, Stein A, Thiel D, Schadendorf D. Serological detection of cutaneous T-cell lymphoma-associated antigens. Proc Natl Acad Sci U S A 2001; 98:629-634.

(133) Dropcho EJ. Antiamphiphysin antibodies with small-cell lung carcinoma and paraneoplastic encephalomyelitis. Ann Neurol 1996;39;659-667.

(134) Bataller L, Wade DF, Graus F, Stacey BS, Rosenfeld MR, Dalmau J. Antibodies to Zic4 in paraneoplastic neurologic disorders and small-cell lung cancer. Neurology 2004;62;773-782.