Molecular Pathology of Malignant Melanoma

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Cutaneous malignant melanoma has been one of the most rapidly increasing malignancies in humans, featuring a capacity to occur in young persons and a refractoriness to therapy once metastases have occurred.[1–3] Melanocytes derive from the neural crest during embryonal development; cells from this area migrate to the skin in a pathway marked and modified by mesenchyme.[4] Epidermal melanocytes can act as sensory and regulatory cells eliciting neuroendocrine and immunomodulatory responses and operating in the context of a functional network for the maintenance of cutaneous homeostasis.[5] Epidermal melanocytes are normally mitotically inactive or can proliferate briefly after selected external signals, such as UV radiation.[2–5]

Hair follicle melanocytes express, in contrast, cyclic proliferative and melanogenic activity coupled to the growing phase of hair (anagen).[6] Because documented melanomas originating from the human follicular melanocytes are extremely rare, it seems that the local biologic clock regulating the hair cycle can act as a suppressor of malignant melanocyte transformation.[6] Cutaneous melanomas derive from epidermal melanocytes or dermal nevomelanocytes.[2,3] In addition, melanocytes or melanocyte-derived cells form one of the most common benign tumors of the body, the melanocytic nevus.[2,3] A variety of molecular markers of cutaneous nevi and malignant melanoma are presented in the following sections.

Growth Factors, Cytokines, and Their Receptors

Studies using cell culture systems have shown that normal and malignant melanocytes differ in production rate and responsiveness to growth factors and cytokines, as well as in the expression of their corresponding receptors.[7–20]

Melanomas have been shown to produce several growth factors and cytokines and to express their receptors, including keratinocyte growth factor (KGF), platelet-derived growth factors α and β, scatter factor (SCF), melanoma growth-stimulating activity/gro, interleukin (IL)-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, tumor necrosis factor (TNF) α, interferon (INF)-γ, and INF-β.[8,10–22] These factors can act as regulators of melanoma cell proliferation, differentiation, and motility. They also can stimulate angiogenesis and regulate expression of major histocompatibility antigens, cell adhesion molecules, integrins, nonintegrin matrix adhesion receptors, and extracellular matrix proteins on melanocytes and on surrounding cells. The expression of multicytokine resistance and multigrowth factor autonomy due to autocrine stimulatory mechanisms is characteristic of advanced stage melanoma.[17,21]

Basic fibroblast growth factor has a central role as growth regulator of normal melanocytes.[7,8] In situ studies show absence of basic fibroblast growth factor expression in epidermal melanocytes and increased expression during progression of melanocytic lesion.[22]

The role of insulin-like growth factors (IGFs), insulin, and their receptors in the regulation of melanoma growth has been suggested since insulin can stimulate or inhibit the growth of melanoma cells in vivo and in vitro.[23–28]

The antigenic profile of melanoma is distinctive according to stages of progression.[29] In situ analyses of the expression and localization of receptors for nerve growth factor, transferrin, and epidermal growth factor (EGF)[29–31] and of growth factors such as transforming growth factor (TGF)-α,[32] TGF-β,[33–35] and IGF-I[36] may correlate with different stages of development of melanocytic lesions. Most remarkably, increased expression of TGF-β, IGF-I, and the receptor for EGF (EGFR) seems to correlate with increased depth of invasion and higher frequency of metastases in malignant melanoma. More recent studies show higher production of TGF-α by dysplastic nevi in comparison with benign nevi,

supporting the hypothesis that the TGF-α/EGFR system has a role in the evolution of melanocytic lesions.[31,32]

The expression of β-human chorionic gonadotropin messenger RNA (mRNA) may serve as a marker for cutaneous melanoma.[37] Eighteen of 24 melanoma lines examined expressed β-human chorionic gonadotropin mRNA.[37]

Melatonin is a potent antitumor and immunostimulatory agent[38,39] available to the public without prescription. Melatonins at physiologic concentrations can inhibit cell proliferation and at pharmacologic doses, they can inhibit melanogenesis.[40,41] This hormone can be produced and degraded by mammalian skin.[42] Melatonin receptors are expressed on human melanoma cells,[39] and it has been suggested that defective melatonin receptors may be involved in the development of vitiligo.[43]

Recent evidence has documented that human skin expresses genes for corticotropin releasing hormone (CRH), CRH receptor (CRHR), and proopiomelanocortin (POMC) and can produce and secrete CRH- and POMC-derived peptides including melanocyte-stimulating hormone (MSH), corticotropin (ACTH), and β-endorphin.[16,44–50] These peptides can have an important role in skin physiology and pathology, such as regulation of the skin immune system, melanogenesis, and melanoma behavior.[16,49]

CRH, CRHR, and POMC all are components of the main adaptive response to systemic stress,[51,52] and also are expressed in human skin.[48,49,53] It is of particular interest that UV light ("melanocyte carcinogen") can stimulate production of CRH, MSH, and ACTH and expression of CRHR and MC1 receptor genes. Furthermore, POMC-derived ACTH, MSH, and β-endorphin can act as immunomodulators and also can regulate the function of normal and malignant melanocytes. Melanomas can produce MSH peptides and, consequently, autoregulate their behavior via intracrine, autocrine, and paracrine mechanisms.[16,46,49,54,55] For example, α-MSH is an immunosuppressor and stimulator of melanogenesis, and, depending on the genotype, it can regulate melanocyte proliferation.[16,56,57] It also has been reported that a mutation in the MC1 receptor may be associated with melanoma development.[58] The immunosuppressive and melanogenic functions of α-MSH[16,49,59] suggest that an activation of the MSH-MC1 receptor system could accelerate melanoma development.

DNA Analysis and Cytogenetics

An observed relationship between melanoma progression and DNA content provided the support for the hypothesis that DNA ploidy may be of prognostic value for the course of malignant melanoma.[60–62] Melanocytic lesions with little or no cytologic atypia tend to be euploid, while those with high-grade atypia are most commonly aneuploid,[60] and metastases also express significantly higher aneuploidy than does the primary tumor.[61] When measured by tissue section image analysis, DNA aneuploidy has correlated with disease progression in intermediate-thickness level III melanomas, while diploid tumors have followed a benign course similar to thin, early stage cases.[62] DNA flow cytometry can be used to monitor response to chemotherapy in melanomas with metastases.[63]

In general, benign nevi have demonstrated a normal karyotype, whereas a high incidence of chromosomal abnormalities has been detected in dysplastic nevi or in atypical nevi from patients with familial atypical multiple mole melanoma syndrome.[2,3,10,64,65] Malignant melanomas are characterized by highly aneuploid karyotype with multiple chromosomal aberrations. Nonrandom karyotypic abnormalities of chromosomes 1, 6, 7, 9, 10, and 11 seem to be associated with increasing aggressiveness of melanoma.[3,10,12,65–70] Structural rearrangements of chromosome 1 have included isochromosome formation for the long arm, translocations, deletions, and duplications. Examples of these abnormalities are del(1)(p22), del(1)(p13), t(1;6)(q11;q11), t(1;19)(q12;p13), and t(1;14)(q21;q32). Studies using restriction fragment length polymorphism show significant loss of heterozygosity in 1p.[10,71] Chromosome 6 abnormalities are frequent in melanoma and have not been described in benign or dysplastic nevi.[72] The most common changes were nonreciprocal translocations or deletions involving the 6q11–6q24.[10,65,69,72] Molecular studies have shown an increased frequency of loss of heterozygosity in the middle of the 6q.[73–75] Studies with chromosome 6 transfer into melanoma cells have suggested that this chromosome may contain a tumor suppressor gene for malignant melanoma.[76,77] Studies of the loss of heterozygosity and molecular and genetic familial linkage have strongly supported a defect in chromosome 9p21 as having an important role in melanoma development,[10,64,66,67,78–82] possibly via a tumor suppressor gene or other genes located in this region.[10,66,79–81,83–88]

By using the techniques of fluorescence in situ hybridization[89] and polymerase chain reaction amplification protocols after tissue microdissection, it has been shown that loss of chromosomes 1pterp33, 16, 17, and 22 occurs in radial growth phase (RGP) and vertical growth phase (VGP), suggesting that VGP cells may be derived from the RGP.[90]

Tumor Suppressor Genes and Oncogenes

Although the *p53* tumor suppressor gene is the most commonly mutated gene identified in human cancer, significant associations between *p53* gene mutations or altered expression of p53 protein and progression of melanocytic lesions have not been significant.[91] However, p53 could act on downstream effector genes, including MDM2, GADD45,

and CIP1/WAF1 in human melanoma,[92] and modulation of p53 expression may change the response of melanoma cells to chemotherapeutic agents.[93]

The tumor suppressor gene $p16^{INK4}$, initially called MTS1/CKD41/CDKN2, maps to chromosome 9p13–22 containing putative locus of familial melanoma.[78,80,83,84] $P16^{INK4}$ binds to the catalytic subunit of CDK4 or CDK6, inhibiting the kinase activity of CDK4–cyclin D and CDK6–cyclin D kinase activity and acts as a negative regulator of cell cycle progression.[78] Human melanomas frequently carry nonsense, missense, frame shift, or $p16^{INK4}$ deletion mutations.[78,80,83–85] However, mutations at the $p16^{INK4}$ locus also have been detected in normal melanocytes and in benign compound nevi without signs of clinical and histologic atypia,[86] indicating that the loss of the $p16^{INK4}$ gene may not represent an initiating event in human melanocytic transformation and progression. Studies of the 9p21-linked familial melanoma suggest that germline $p16^{INK4}$ mutations explain most, if not all cases, whereas the genetic basis in the non–9p21-linked melanoma kindreds remains to be identified.[94] Recent immunocytochemical studies on expression of the $p16^{INK4}$ and CDK4 proteins showed that overexpression of CDK4 was characteristic for malignant melanoma,[95] while the expression of $p16^{INK4}$ in malignant melanoma was lower than that in benign nevi.[95] Other genes coding proteins regulating the cell cycle and potentially involved in melanocyte transformation include $p15^{INK4B}$, p18, p19, and p21.[66,78,79,81,85] The $p15^{INK4B}$ is located on chromosome 9p21, and the recent finding of homozygous deletion of $p15^{INK4B}$ in some melanomas, despite the retention and expression of $p16^{INK4}$,[81] emphasizes its possible role in melanoma progression.

Genes that may inhibit the metastatic potential of melanoma are potential candidates for tumor suppressors and include *nm23*, *nmb*, and *nma*.[10,12,96,97] The *nma* gene is located on chromosome 10, of which loss of heterozygosity has been described in malignant melanoma.[98]

The expression of the H-, K-, and N-*ras* proto-oncogenes in melanoma cells has been reported.[10,12] The mutated *ras* genes were detected in approximately 5% to 6% of noncultured melanoma specimens.[10] They were also found in benign nevi and in metastatic melanomas.[10] Although *ras* may have a role in proliferation of melanoma cells, its function in the development of melanoma remains to be clarified. Altered expression of the nuclear oncogenes c-*myb*, c-*myc*, c-fos, and c-jun was reported in cultured melanoma cells.[10,12,99] The KIT receptor has an important role in the migration and differentiation of melanoblasts toward melanocytes.[100] The KIT receptor acts as a regulator of melanocyte proliferation and differentiation, but its potential role in melanoma development remains unclear. Recently, a new nuclear protein Mader, which may accompany the malignant transformation of melanocytes, has been described.[101]

Differentiation Markers of Melanoma

Markers widely used in immunocytochemical diagnostic pathology are S-100 and HMB-45 antigens. The S-100 antigen is not specific because it also is expressed in other neuroectodermal cells and in Langerhans cells. However, the HMB-45 antigen is more specific, since it is localized in the premelanosomes and melanosomes of normal and malignant melanocytes. The gene for HMB-45 antigen (gp100) and for the antigens recognized by melanocyte specific antibodies, HMB-50 and NKI-beteb, has been cloned and identified as the Pmel 17 gene, which encodes a melanosomal enzyme converting the dihydroxyindole carboxylic acid form (DHICA) into melanin.[102–104] Other melanogenesis-related proteins include tyrosinase and tyrosinase-related proteins (TRP1 and TRP2) that also may serve as markers for cells of melanocytic lineage.[105–109]

Tyrosinase, a product of the c-locus, catalyzes the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-dopa), oxidation of L-dopa to dopaquinone, and oxidation of DHI to 5,6-dihydroxyquinone.[109] It has been suggested that tyrosinase also acts as a regulatory protein by controlling intracellular levels of L-dopa,[110] a potential intracrine regulator of gene expression.[111,112] Tyrosinase seems to be a better marker for melanoma than the widely used HMB-45. Serum tyrosinase activity and protein levels progressively increase in advanced melanotic melanoma.[113]

Immune Markers of Melanoma

It has been proposed that a decrease in the expression of HLA class I molecules, which present antigens and activate $CD8^+$, or changes in HLA-I specific subclasses may be associated with progression of melanoma. Specifically, a selective loss of HLA-A2 and/or A28 was found in 21% of primary tumors and in 44% of metastatic melanoma.[114] Within HLA class II molecules that activate $CD4^+$ lymphocytes, it has been reported that the expression of the HLA-DR and HLA-DP on melanoma cells increases with the progression from the radial growth phase to the vertical and metastatic growth phase. The number of Langerhans cells increases around in situ and early invasive melanomas and declines around deeply invasive tumors or cutaneous metastases.[115] Immunization protocols for the systemic treatment of melanoma are being developed that use recombinant viruses and bacteria, naked DNA containing tumor antigen genes, and genetically modified cells expressing or presenting tumor antigens.[116–119] Newer techniques include the administration of cytokines, such as IL-2, IL-10, IL-12, and INF-γ, that would augment immunization of tumor recognition

or delivery of recombinant DNA containing cytokine or HLA and B7 molecule genes into the tumor.[116–119] In addition, tumor-reactive T cells stimulated with specific tumor antigens or T cells modified by transfection of complementary DNA for T-cell receptors recognizing tumor antigens can be transferred to melanoma patients.[116–119]

Stimulation of antibody production against cell surface antigens represents another immunotherapeutic approach for the treatment of melanoma.[117,118] Gangliosides are good candidates as substrates for antimelanoma vaccines.[118] Immunotherapy has used antiganglioside antibodies or immunization with ganglioside-containing antimelanoma vaccine,[118] and high molecular weight melanoma-associated antigens have been proposed as targets in antimelanoma therapy.[12,120,121]

Molecular Markers of Metastatic Melanoma

Metastatic disease causes a dramatic decrease in the survival of patients with stage III and IV melanomas.[2,3,122] The first step in the metastatic cascade is dissemination of melanoma cells from the primary tumor; therefore, the expression of factors involved in cell adhesion, matrix degradation, migration, and colonization in relation to tumor development have been studied. For example, plasminogen activation can be an important step in the progression of malignant melanoma.[123,124] The expression of the cell adhesion molecules ICAM-1 and MUC18 on melanoma cells increases with increased tumor thickness and development of metastatic disease.[125,126] Increased serum levels of ICAM-1 suggest a poor prognosis.[126] Similarly, the expression of a particular class of integrins has been correlated positively with the level of invasion in localized melanoma or with the development of metastases.[12,127] Expression of CD44, a lymphocyte homing and cartilage attachment cell adhesion molecule, has been associated with increased cell motility and metastatic potential in malignant melanoma.[128] Thus, the expression of plasminogen activation components, ICAM-1, or MUC18, or of selected integrins deserves further clinical testing for usefulness as markers of melanoma progression.

It has been reported that the serum activity of tyrosinase[113] and enolase[129] and the serum and urine levels of precursors to melanin[130–133] correlate, to some degree, with the progression of human melanoma. The successful detection of mRNA for tyrosinase using the reverse transcription polymerase chain reaction technique in blood from patients with melanoma demonstrates that the expression of the tyrosinase gene can act as a specific and sensitive marker of melanoma progression.[134]

Researchers have documented that patients with advanced stages of melanoma demonstrate increased plasma or urine levels of melanin precursors including dopa, 5-S-cysteinyldopa, DHI and DHICA, and O-methyl derivatives of DHI and DHICA.[130–133] Precursors to melanin also have intrinsic biologic activity as potent immunosuppressors,[135] whereas tyrosinase also may impair immune system function via extracellular oxidation of tyrosine and dopa to generate lymphocytotoxic precursors of melanogenesis.[136] Thus, increased serum levels of intermediate metabolites of melanogenesis or tyrosinase enzyme also may serve as potential indicators of impairment in the host immune response against melanoma.

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