



Catherine Hickson. *Apples in Golden Barok Red*. Oil on Belgian linen, 76 cm × 121 cm.

New molecules and biomarkers have the potential to clarify and refine the diagnosis, classification, and management of melanoma.

Tumor Biomarkers in Melanoma

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Background: Morphologic and histopathologic markers have been the backbone for the classification and prognostic assessment of melanoma. Availability of an increasing number of molecular markers, however, provides the potential for refining diagnostic and prognostic categories in this disease.

Methods: We reviewed the recent data that are accumulating concerning gene expression and genetic profiling and related these to clinical aspects of the disease.

Results: Multiple biomarkers have now been described, and their biologic significance is being established. In addition, several candidate molecules involved in melanoma pathogenesis have been identified.

Conclusions: The process of biomarker identification and validation is providing a rapidly changing molecular view of melanoma, a strategy that is necessary for developing truly stratified or even personalized prevention or management.

Introduction

Melanoma is the form of skin cancer with the highest death toll in the United States and Europe. Besides morphological and histopathologic biomarkers (anatomic site and type of the primary tumor, tumor size and invasion depth, ulceration, vascular invasion, and mitotic index), an increasing variety of molecular markers have been identified that provide the possibility of a more

detailed diagnostic and prognostic categorization. Recently published gene expression and proteomic profiling data indicate new candidate molecules involved in melanoma pathogenesis, which are currently validated. This ongoing process of biomarker identification and validation results in a rapidly changing molecular view and classification of cutaneous malignancies, which holds the promise of improving not only our prognostic classification systems, but also our diagnostic and therapeutic potential. This article provides an overview of the currently known serologic and immunohistochemical biomarker in melanoma.

Tumor Tissue-Based Biomarkers

Cutaneous malignant melanoma develops in three sequential stages: radial and vertical growth phase and metastases. The prognosis in any stage is only partially explained by morphological and histopathologic parameters such as primary tumor localization, patient gen-

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der and age, mitotic rate, tumor thickness, and ulceration. Additional variables that aid in assigning patients to specific risk groups include immunohistochemistry, gene expression profiling, comparative genomic hybridization, and mutational analysis.

For diagnostic purposes, a small panel of melanocytic lineage markers (S100, MART-1, and gp100/HMB45) is sufficient to distinguish melanoma from other types of nonmelanocytic cancers. However, no marker has proven useful in distinguishing spindle cell and desmoplastic melanomas from other tumors. Ki67 remains the most useful adjunct in distinguishing benign from malignant melanocytic tumors.¹

For prognostic classification, the situation is more complex. The transformation from benign melanocytes to metastatic melanoma is the result of a compilation of genetic alterations contributing to the hallmarks of cancer: uncontrolled proliferation, unlimited replicative potential, apoptosis resistance, and invasion. Several marker molecules involved in these genetic alterations have been identified, and their expression in primary melanoma has been studied and correlated with prognosis. Table 1 summarizes currently established biomarkers whose abnormal expression is associated with the patient's prognosis.²⁻⁴² It is possible that the most detailed prognostic classification will be based on a panel of multiple biomarkers rather than just one biomarker on this list.

In a recent retrospective study, primary melanomas (for which a long-term clinical follow-up was available) were analyzed using a cDNA expression microarray.²¹ The authors described a signature of 174 genes to identify patients at risk of developing distant metastasis. From these genes, 141 were underexpressed and 33 overexpressed in tumors whose host remained free of metastasis for 4 years. Of these 174 genes, 30 had been already studied in melanoma; these genes are involved in cell cycle regulation (CKS2, CDC2, CCNB1, CENPE, and DHFR), mitosis (HCAP-G and STK6), mitotic spindle checkpoint (BUB1), inhibition (BIRC5) or stimulation (GPR105) of apoptosis, DNA replication (TOP2A, RRM2, TYMS, PCNA, MCM4, and MCM6), stress response (GLRX2, DNAJA1, HSPA4, HSPA5, HSPD1, and TXNIP), ubiquitin cycle (SIP), actin and calmodulin binding (CNN3), intracellular signaling (STMN2), negative regulation of the Wnt signaling pathway (CTNNBIP1), inhibition of MITF expression (EMX2), regulation of proteolysis (TNA), testis cancer (CML66), and metastasis suppression (NME1). The authors speculated that by means of antibodies covering the encoded proteins, it would be possible to improve the estimation of prognosis of melanoma patients and thereby allow treatment stratification. Particularly, determination of karyopherin alpha 2, minichromosome maintenance proteins (MCMs), geminin, and PCNA could be used to screen for melanoma patients with a poor clinical outcome.

Similarly, genetic abnormalities have recently been recognized to influence the prognosis of cancer patients. A new classification system has been proposed by Curtin et al^{43,44} and Viros et al⁴⁵ that combines genetic aberrations with histomorphologic characteristics, resulting in new insights into the pathogenesis of this malignancy.

Moreover, the chemosensitivity profile determined by an in vitro ATP-based chemosensitivity assay has been shown to differentiate between chemosensitive and chemoresistant melanoma patients and can be used as a biomarker of chemotherapy response and survival outcome. A phase II study testing this assay in 53 metastatic melanoma patients followed by a sensitivity-directed individualized chemotherapy demonstrated that the chemosensitivity profile of an individual patient, reflected by the best individual chemosensitivity index (BICSI), correlated with therapy outcome.⁴⁶ Interestingly, a surprisingly high proportion (42%) of the investigated patient cohort were classified as chemosensitive, and the remaining 58% were classified as chemoresistant. The objective response rate was 36.4% in chemosensitive patients compared to 16.1% in chemoresistant patients ($P = .114$). Progression arrest (complete response, partial response, and stable disease) was 59.1% vs 22.6% ($P = .01$). Chemosensitive patients showed an increased overall survival of 14.6 months compared with 7.4 months in chemoresistant patients ($P = .041$).

Serologic Markers

Despite a large research effort, the prognosis of metastasized melanoma is still poor; best results have been achieved in cases when the tumor is still amendable to surgical intervention. Thus, the search for reliable methods that will not only detect metastases in such early states, but also identify patients with high risk of disease progression who should undergo more vigorous follow-up is important. Serological markers for tumor progression combine several advantages such as the ease of obtaining serum samples and the availability of numerous methods to detect respective small molecules or proteins correlated with the tumor burden; hence, several serological biomarkers have been established. In several European countries, the melanocyte lineage/differentiation antigens S100-beta and melanoma inhibitory activity (MIA) are frequently or almost routinely used for early detection of tumor relapse or metastasis during follow-up of melanoma patients (Table 2).⁴⁷⁻⁷⁷ Both proteins are with high but not exclusive specificity expressed by melanoma cells, and both correlate with the patient's tumor load.

The S100 protein is a 21-kd thermo-labile acidic dimeric protein that was originally isolated from central nervous system. It consists of two subunits, alpha and beta, in any pairing, ie, alpha/alpha, alpha/beta, and

Table 1. — Immunohistochemical Markers of Malignant Melanoma Associated With Impaired Prognosis

	Association With Impaired Prognosis	References
Melanocyte Lineage/Differentiation Antigens		
gp100 / HMB45	increased expression	Niezabitowski et al ²
Tumor Suppressors/Oncogenes/Signal Transducers		
AP-2 (activator protein-2 alpha) transcription factor	loss of nuclear AP-2 expression	Berger et al ³
bcl-6	expression	Alonso et al ⁴
c-Kit	expression	Janku et al ⁵
c-met	expression	Cruz et al ⁶
c-myc	increased expression	Kraehn et al ⁷
CYLD	decreased expression	Massoumi et al ⁸
EGFR (epidermal growth factor receptor)	increased expression	Udart et al ⁹
ERK (extracellular signal-regulated kinase)	absence of cytoplasmic ERK activation	Jovanovic et al ¹⁰
HER3	increased expression	Reschke et al ¹¹
HDM2 (human homologue of murine mdm2)	increased expression	Polsky et al ¹²
ING3	decreased nuclear expression	Wang et al ¹³
MITF (microphthalmia-associated transcription factor)	gene amplification	Ugurel et al ¹⁴
p16 ^{INK4A}	decreased expression	Mihic-Probst et al ¹⁵
p-Akt (activated serine-threonine protein kinase B)	increased expression	Alonso et al ⁴
pRb (retinoblastoma protein)	inactivation due to protein phosphorylation	Dai et al ¹⁶
PTEN	decreased expression	Roesch et al ¹⁷ Mikhail et al ¹⁸
Cell Cycle Associated Proteins		
cyclin A, B, D, E	increased expression	Flørenes et al ¹⁹ Flørenes et al ²⁰
geminin	increased expression	Winnepenninckx et al ²¹
Ki67 (detected by Mib1)	increased expression	Gimotty et al ²²
p21 ^{CIP1}	decreased expression	Alonso et al ⁴
PCNA (proliferating cell nuclear antigen)	increased expression	Ostmeier et al ²³ Alonso et al ⁴ Winnepenninckx et al ²¹
Regulators of Apoptosis		
APAF-1 (apoptotic protease activating factor-1)	decreased expression	Fujimoto et al ²⁴
bak	decreased expression	Fecker et al ²⁵
bax	decreased expression	Fecker et al ²⁵
bcl-2	increased expression	Tas et al ²⁶
survivin	increased expression	Tas et al ²⁶
Molecules Involved in Angiogenesis		
LYVE-1 (lymphatic vascular endothelial hyaluronan receptor-1)	increased expression	Dadras et al ²⁷
PTN (pleiotrophin)	increased expression	Wu et al ²⁸
Molecules Involved in Cell Adhesion and Motility		
beta-catenin	loss of nuclear staining	Bachmann et al ²⁹
CEACAM1 (carcinoembryonic antigen-related cell-adhesion molecule 1)	increased expression	Thies et al ³⁰
dysadherin	increased expression	Nishizawa et al ³¹
E-cadherin	decreased expression	Andersen et al ³²
integrins beta-1 and beta-3	increased expression	Saalbach et al ³³
MMPs (matrix metalloproteinases)	increased expression	Redondo et al ³⁴
osteonectin (also termed BM40 or SPARC [secreted protein, acidic and rich in cysteine])	increased expression	Massi et al ³⁵
P-cadherin	strong cytoplasmic expression	Bachmann et al ²⁹
Immunoregulators		
HLA allele frequency	specific expression	Luongo et al ³⁶ Ostmeier et al ²³
Others		
ALCAM/CD166 (Activated leukocyte cell adhesion molecule)	increased expression	Swart et al ³⁷
CXCR4 receptor	increased expression	Scala et al ³⁸
melastatin	decreased expression	Duncan et al ³⁹
metallothionein	increased expression	Weinlich et al ⁴⁰
osteopontin	increased expression	Rangel et al ⁴¹
TA (telomerase activity)	increased expression	Carvalho et al ⁴²

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beta/beta. It affects the assembly and disassembly of microtubules and also interacts in a calcium-dependent manner with the p53 tumor suppressor gene. The beta subunit is expressed in cells of the central nervous system and of the melanocytic lineage. Initially, the presence of S100-beta in the cerebrospinal fluid was used as a marker of central nervous system damage.⁷⁸ In the following years, it was observed that S100-beta was also elevated in the serum of melanoma patients.⁴⁷ MIA was originally detected in melanoma cell culture supernatant⁵² and was shown to exert an important role in cell-matrix interaction and metastasis.⁵³

Studies comparing both serum markers demonstrated that S100-beta is superior to MIA as an early indicator of tumor progression, relapse, or metastasis^{79,50}; hence S100-beta is used more often.⁸⁰ Both markers have been shown to be useful prognostic markers in melanoma patients with distant metastases^{48,49} but they fail to pro-

vide prognostic significance in early stages of melanoma, especially in patients who are tumor-free after surgical procedures.⁵⁴ Moreover, S100-beta fails to identify patients with lymph node micrometastases detected by sentinel node procedure.⁸¹ The correlation of S100 serum concentrations with the patient's tumor load, however, makes it a useful marker for monitoring therapy response in the patient with advanced metastatic melanoma.⁴⁹

The strongest prognostic serum biomarker in advanced metastatic melanoma is lactate dehydrogenase (LDH), an unspecific marker indicating high tumor load in a variety of tumor entities, including melanoma. Studies comparing LDH, S100-beta, and MIA using multivariate data analysis showed LDH as the strongest independent prognostic factor in stage IV melanoma patients.⁷⁹ Due to its high prognostic significance paired with its easy, cost-efficient, and widely distributed detection methodology, serum LDH is the only

Table 2. — Serologic Markers of Malignant Melanoma

	Serologic Marker	References
Melanocyte lineage/differentiation antigens	S100-beta	Guo et al ⁴⁷ Schultz et al ⁴⁸ Hauschild et al ⁴⁹ Krähn et al ⁵⁰ Garbe et al ⁵¹
MIA (melanoma inhibitory activity)		Bogdahn et al ⁵² Blesch et al ⁵³ Stahlecker et al ⁵⁴ Garbe et al ⁵¹
Tyrosinase		Agrup et al ⁵⁵
5-S-cysteinyl-dopa		Wimmer et al ⁵⁶
L-Dopa/L-tyrosin		Stoitchkov et al ⁵⁷
Proangiogenic factors	VEGF (vascular endothelial growth factor) bFGF (basic fibroblast growth factor) IL-8 (interleukin-8)	Ugurel et al ⁵⁸ Ugurel et al ⁵⁸ Ugurel et al ⁵⁸
Molecules involved in cell adhesion and motility	sICAM-1 (soluble intracellular adhesion molecule 1) sVCAM (soluble vascular cell adhesion molecule 1) matrix metalloproteinases (MMP)-1 and -9 Tissue inhibitor of metalloproteinases (TIMP-1 and -2)	Hirai et al ⁵⁹ Vuoristo et al ⁶⁰ Franzke et al ⁶¹ Vuoristo et al ⁶⁰ Nikkola et al ⁶² Yoshino et al ⁶³
Cytokines and cytokine receptors	IL-6 (interleukin-6) IL-10 (interleukin-10)	Mouawad et al ⁶⁴ Dummer et al ⁶⁵ Nemunaitis et al ⁶⁶
HLA molecules	sIL-2R (soluble interleukin-2-receptor) sHLA-DR (soluble HLA-DR) sHLA-class I (soluble HLA-class I)	Boyano et al ⁶⁷ Rebmann et al ⁶⁸ Westhoff et al ⁶⁹
Others	LDH (lactate dehydrogenase) CRP (C-reactive protein) albumin TuM2-PK (tumor pyruvate kinase type M2) CD95 (sFas/CD95) YKL-40 CYT-MAA (cytoplasmic melanoma-associated antigen) HMW-MAA (high-molecular-weight melanoma-associated antigen) human endogenous retrovirus K	Sirott et al ⁷⁰ Deichmann et al ⁷¹ Sirott et al ⁷⁰ Ugurel et al ⁷² Ugurel et al ⁷³ Schmidt et al ⁷⁴ Schmidt et al ⁷⁵ Vergilis et al ⁷⁶ Vergilis et al ⁷⁶ Hahn et al ⁷⁷

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molecular marker that has been included into the current melanoma staging and classification system of the American Joint Committee on Cancer (AJCC).⁸² In fact, it serves as a stratification parameter in most randomized clinical trials testing therapeutic interventions in advanced melanoma, and it also can be used to monitor therapy response in these patients.

Several reports suggest that a variety of additional potential biomarkers present in the serum may be correlated with tumor load and disease progression in melanoma. These are ascribed to different characteristic of melanoma such as melanocytic differentiation (eg, tyrosinase), tumor angiogenesis (eg, VEGF, bFGF, IL-8), cell adhesion and motility (eg, ICAM-1, MMPs), cytokines and their receptors (eg, IL-6, IL-10), antigen presentation (eg, HLA molecules), tumor cell metabolism (eg, TuM2-PK), and apoptosis (eg, Fas/CD95) (Table 2). However, none of these markers has been demonstrated to be superior to S100-beta or LDH in reflecting the prognosis of patients in advanced disease stages. Moreover, these markers failed to be of prognostic relevance in early-stage tumor-free patients.

An innovative approach to identify new and better serological biomarkers in melanoma is the serum proteomic profiling. This methodology offers the possibility to screen the whole serum proteome for markers that match different criteria such as prognostic significance and prediction of therapy response. Using this technology, marker proteins from thematic fields different from those mentioned above might be found and thereafter validated for their clinical use. The first promising results showed that patients with stage I and stage IV disease can be differentiated by their serum proteomic profiles.⁸³ A recent study using proteomic profiling in larger sets of sera succeeded in the identification of serum amyloid A as a new prognostic serum biomarker in melanoma.⁸⁴

Conclusions

Melanoma is a highly aggressive form of skin cancer that is difficult to treat once the tumor has metastasized beyond the locoregional area. Recently established biomarkers such as morphological and histopathologic characteristics, as well as molecular markers, allow a detailed diagnostic and prognostic categorization that is mandatory for stratified or even personalized therapy.

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