Cutaneous Malignant Melanoma: Update on Diagnostic and Prognostic Biomarkers

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Abstract: The incidence of cutaneous malignant melanoma has rapidly increased in recent years in all parts of the world, and melanoma is a leading cause of cancer death. As even relatively small melanomas may have metastatic potential, accurate assessment of progression is critical. Although diagnosis of cutaneous malignant melanoma is usually based on histopathologic criteria, these criteria may at times be inadequate in differentiating melanoma from certain types of benign nevi. As for prognosis, tumor (Breslow) thickness, mitotic rate, and ulceration have been considered the most important prognostic indicators among histopathologic criteria. However, there are cases of thin primary melanomas that have ultimately developed metastases despite complete excision. Given this, an accurate assessment of melanoma progression is critical, and development of molecular biomarkers that identify high-risk melanoma in its early phase is urgently needed. Large-scale genomic profiling has identified considerable heterogeneity in melanoma and suggests subgrouping of tumors by patterns of gene expression and mutation will ultimately be essential to accurate staging. This subgrouping in turn may allow for more targeted therapy. In this review, we aim to provide an update on the most promising new biomarkers that may help in the identification and prognostication of melanoma.

Key Words: malignant melanoma, biomarkers

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LEARNING OBJECTIVES

After completing this CME activity, physicians should be better able to:

1. Identify technologies used in the discovery and identification of different melanoma biomarkers.
2. Apply the appropriate marker in the clinical setting.

INTRODUCTION

Cutaneous malignant melanoma is one of the most aggressive and deadly skin cancers.[1,2] Although histopathologic criteria are usually sufficient for the diagnosis of most melanomas, some tumors may have overlapping histopathologic features with certain types of nevi, making their distinction difficult even for the experienced pathologist.[3,4] In such cases and for some melanoma subsets such as amelanotic or desmoplastic types, immunohistochemistry (IHC) may be needed to confirm diagnosis, though IHC is not required for most melanomas.[5,6] However, there are yet no reliable markers that are both highly sensitive and specific for melanoma diagnosis. Routine markers for ambiguous cases include S100 calcium-binding protein-p, HMB45 antigen (melanocyte lineage-specific antigen gp 100), MART-1 (melan-A protein), and microphthalmia-associated transcription factor (MITF). These proteins, which are mostly components of the melanocyte pigmentation machinery, are highly sensitive for melanoma but show low specificity as they are also expressed in melanocytic nevi.[7,8]

Concerning prognosis, the main clinical and histopathologic predictors of outcome are Breslow thickness, mitotic rate, the presence of ulceration, anatomic site (cutaneous, acral, or mucosal), and sentinel lymph node (SLN) status.[9] However, there exists a small subset of aggressive tumors that are not identified by any of these predictors, that is, some thin, nonmitotically active, nonulcerated lesions.

The problem of melanoma misdiagnosis and potential for metastasis at an early stage warrants the development of more molecular markers with prognostic and therapeutic significance. The ideal biomarker, defined as any measurable molecular change (DNA/chromosomal, epigenetic, mRNA, or protein) in a cancer cell, should be sensitive, specific, reliable, rapidly analyzable, cost effective, and should "add value," prognostically or therapeutically, to our current set of assessment tools. Several molecular and chromosomal events that influence the development and progression of melanoma show promise in improving differentiation of melanomas from benign melanocytic proliferations. These events include tumor initiation [mutations, loss of heterozygosity (LOH), gene amplification, gain and loss of chromosomes], growth (loss of cell cycle control, neovascularization, growth factors), resistance to apoptosis (gain of antiapoptotic and survival factors, inactivation of cell death pathways), invasion and metastasis (cell adhesion and motility, proteolytic enzymes), and immune surveillance escape (loss or gain of immune regulators).[8]

In this review, we provide an update on the most promising new biomarkers that correlate with tumor progression and may aid in improved identification and prognostication of melanoma.

DIAGNOSTIC BIOMARKERS

Tissue-Based Diagnostic Protein Biomarkers Detected Using Immunohistochemistry

With only rare exceptions, the diagnosis of a melanocytic lesion as either benign or malignant does not depend on

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the staining profile of any specific immunohistochemical marker.[5–8] Most evidence on the use of immunohistochemical markers in the differentiation of benign from malignant melanocytic lesions is limited by the small sample size of the studies done and the lack of information on clinical outcome. Among currently commonly available markers, Ki-67 is the best studied and perhaps the most useful marker for differentiating nevi from melanomas.[5–8] The Ki-67 antigen is a nonhistone nuclear protein, and the Ki-67 antibody serves as a proliferation marker by staining the growth fraction of a given cell population in tissue specimens.[10–18] Although its precise function is unknown, it has been detected in the nuclei of proliferating cells during all stages of the cell cycle (late G_1, S, G_2, and M phases), except the G_0-resting phase.[10] In this regard, Ki-67 expression is thought to be a more accurate representation of cell proliferation than mitotic rate. As a diagnostic tool, Ki-67 expression seems to be useful in distinguishing nevi from malignant melanoma.[5–8] In most common nevi, Ki-67 staining is positive in <5% of nevomelanocytes, although up to 15% positivity in some Spitz and dysplastic nevi has been reported (Fig. 1).[11–13] Conversely, Ki-67 expression in melanoma tumor cells is usually between 13% and 30% with some cases even showing 100% nuclear positivity (Fig. 2).[11–13] In addition, the location of Ki-67-positive cells can aid in differentiating benign and malignant lesions, including Spitz nevi from spitzoid melanoma. Melanoma tumor cells tend to express Ki-67 in deeper lesion portions, whereas melanocytic nevi typically show Ki-67-positivity in the superficial portions only.[14]

pHH3 is another immunomarker that aids in quantifying tissue proliferation rate, in this case by staining mitoses specifically. Phosphorylation of histone H3 is an event present throughout mitosis. Anti-pHH3 antibodies have been demonstrated to label mitotic figures in all phases of mitosis including early prophase, a phase that is typically difficult to identify on routine microscopy.[19] The stain has been used to assess mitotic rate in meningiomas and other neural tumors[20,21] and has recently been evaluated on thin melanomas in several studies.

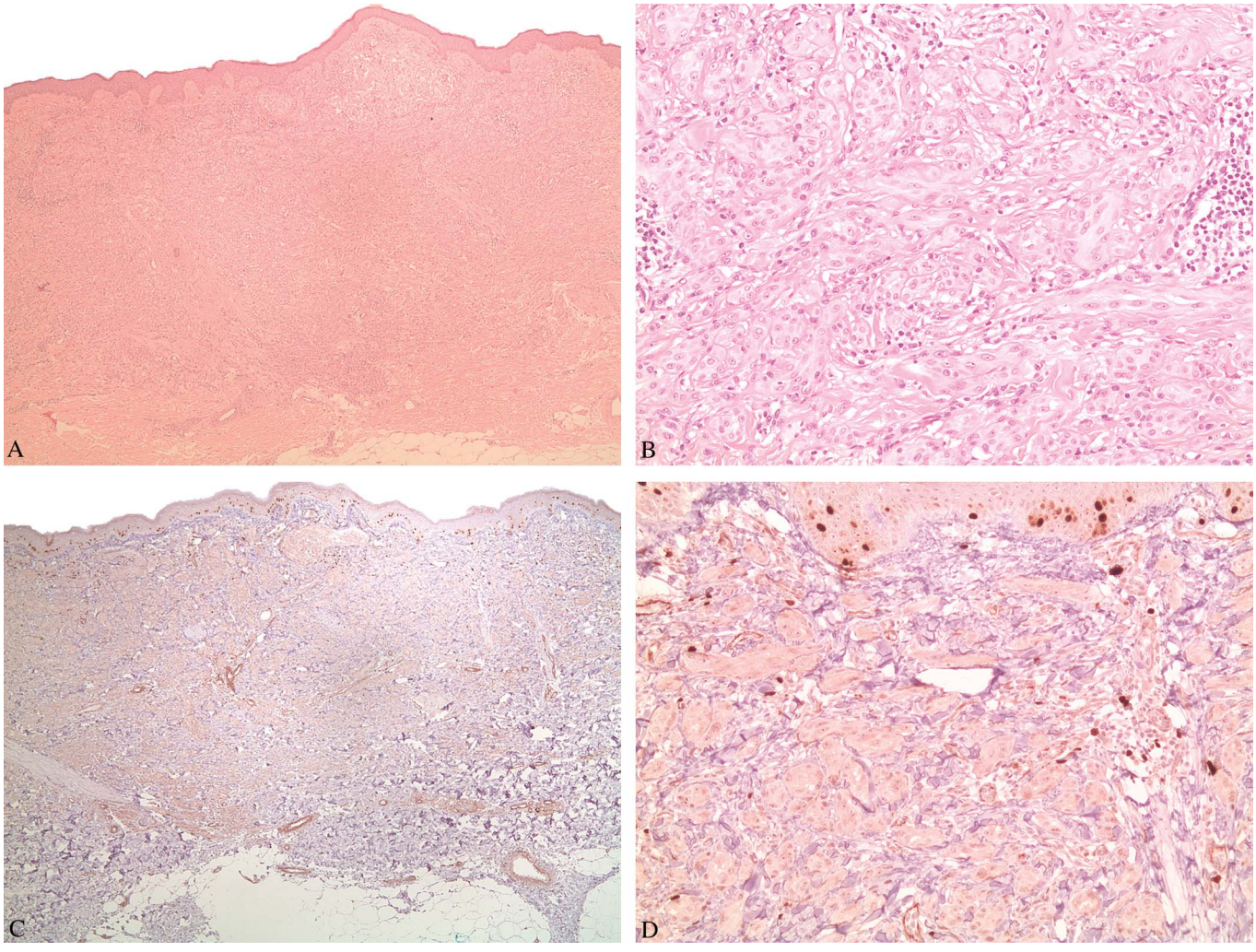

FIGURE 1. Representative case of a dermal spitz nevus showing positive Ki-67 staining in <5% of cells. Hematoxylin and eosin, original magnification: A, ×4; B, ×40; C, Ki-67 stain ×4. D, Ki-67 stain ×40. Note that the cells showing Ki-67-positivity are restricted to the superficial portion of the lesion.

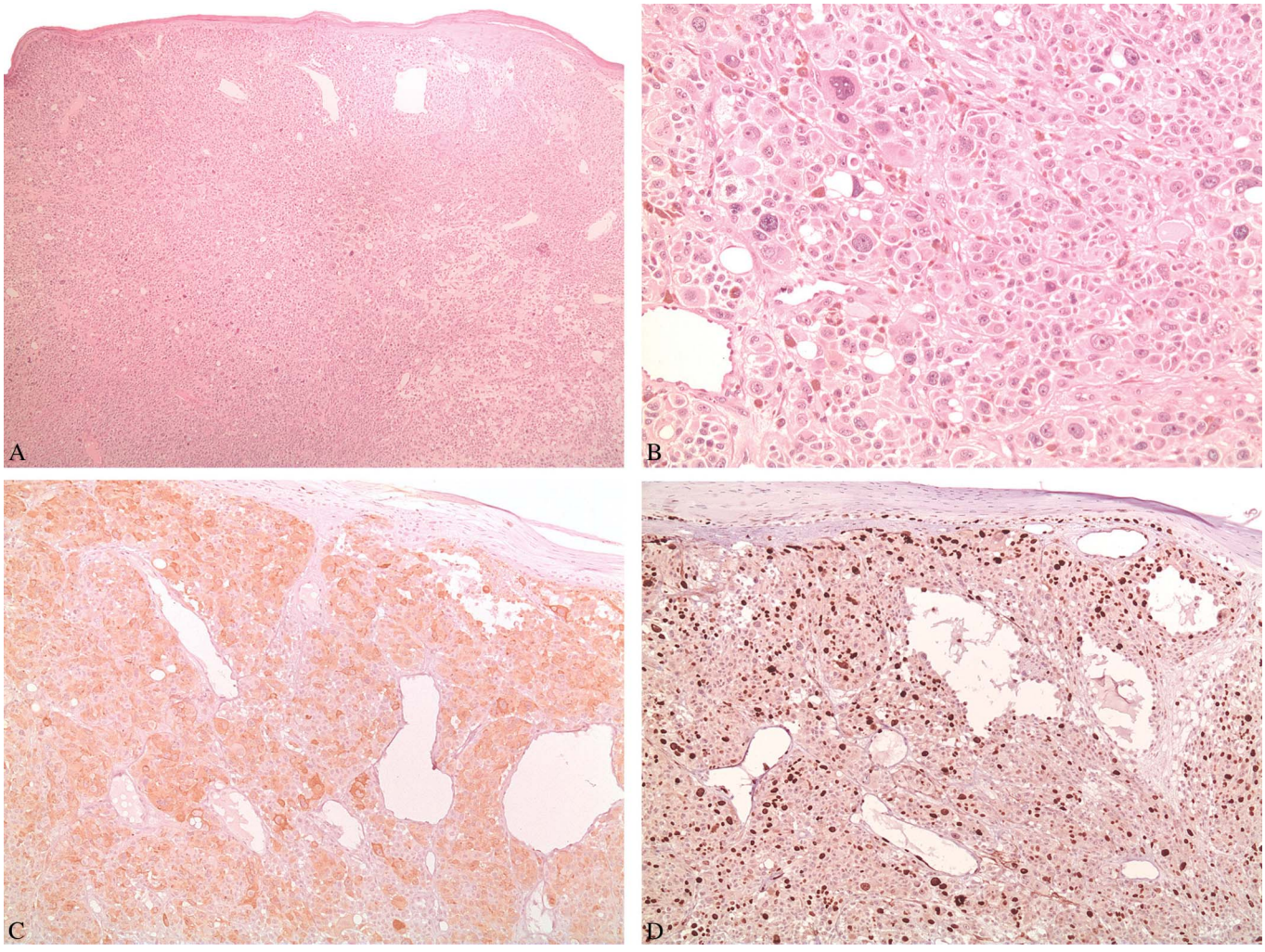

FIGURE 2. Representative case of a nodular melanoma showing positive Ki-67 staining in >30% of cells. Hematoxylin and eosin, original magnification: A, ×4; B, ×40; C, MART-1 stain ×10. D, Ki-67 stain ×10.

Casper et al studied 30 thin melanomas (Breslow depths between 0.45 and 1.2 mm) and demonstrated an average MR of 1.63 per mm^2 with pHH3, compared with 0.67 on routine sections, an increase of 243%.[19] However, in a subsequent study also of thin melanomas, Ikenberg et al[22] found no significant difference in MR between H&E-stained sections and a dual pHH3/Melan-A immunostain, and concluded that the pHH3 stain alone tends to overestimate the MR by capturing both melanocyte and nonmelanocytic mitoses. These authors suggested that the utility of the stain may mostly lie in its time-saving potential when quantifying mitoses for staging purposes. Interestingly, a recent study comparing the predictive value of this pHH3/Melan-A stain with a Ki-67/Melan-A double stain and mitotic count on routine sections identified the pHH3/Melan-A stain as the strongest predictor of progression-free survival and melanoma-specific death among the three.[23] Further study of this stain is clearly warranted.

The p16 protein is another IHC marker that seems to have diagnostic utility in specific situations. The p16 protein is the product of the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene; its loss is believed to contribute to cell cycle dysregulation in melanoma progression.[24] Several large-scale studies have documented decreased nuclear staining within melanomas compared with nevi.[8,25,26] Although nuclei of nevi are usually uniformly p16-positive, 50%–98% of melanomas show loss of nuclear staining. p16 has also been suggested as a useful marker in differentiating Spitz nevi and melanoma. A study of melanocytic lesions in patients under 18 years of age demonstrated loss of p16 in all cases of childhood melanoma (n = 6), but retained strong positive nuclear and cytoplasmic expression in Spitz nevi (n = 18), either in a diffuse or checkerboard pattern, and compound melanocytic nevi (n = 12).[24] The p16 antibody has also been demonstrated as useful in differentiating the desmoplastic variants of Spitz nevi and melanoma. A study of 15 desmoplastic Spitz nevi and 11 desmoplastic melanomas found moderate-to-strong staining in all Spitz nevi, but only weak staining in only 2 of 11 melanomas and absent reactivity in 9 of 11.[26] However, the utility of p16 with respect to Spitzoid neoplasms in general has recently been called into question, as a study by Mason et al[27] recently noted no significant difference in staining patterns between 18 Spitz nevi and 19 Spitzoid melanomas.

TABLE 1. Potential Immunohistochemical Markers That May Become Useful in Distinguishing Nevi From Melanomas

Marker	Description	Staining
PTEN[28,29]	Signaling molecule	Recent study showed positive cytoplasmic expression in 87.7% of 162 primary melanomas versus no cytoplasmic expression in 41 nevi
	Tumor suppressor involved in the phosphatidylinositol-3 kinase pathway and is the main antagonist of phosphoinositide 3-kinase	
Trk-A[30]	Signaling molecule	Membrane and cytoplasmic staining in 21.7% of 152 melanomas versus no staining in 8 nevi
	Nerve growth factor receptor tyrosine kinase involved in activation of major oncogenic signaling pathways in melanoma, including the Ras/MAPK and phosphatidylinositol-3 kinase pathways	
Bcl-2[31]	Cell cycle–related/antiapoptosis markers	Strong, diffuse cytoplasmic staining in compound and dysplastic nevi and thin primary melanomas (<1.0 mm) versus weak diffuse/focal staining in thick primary melanomas (>1.0 mm) and metastatic melanoma
Cdk2[32]	Cell cycle–related/antiapoptosis markers	Significantly increased staining in 46 primary cutaneous invasive melanomas versus 17 benign nevi
Cyclin A[28,33]	Cell cycle–related/antiapoptosis markers	Positive in 42%–99% of melanomas while rarely expressed in nevi
Cyclin B[28,33]	Cell cycle–related/antiapoptosis markers	Expressed in approximately 50% of melanomas while rarely expressed in nevi
Cyclin D3[28]	Cell cycle–related/antiapoptosis markers	Commonly expressed in melanomas while rarely expressed in benign nevi
GADD[34]	Cell cycle–related/antiapoptosis markers	Average staining of 19%–31% of lesional cells in melanomas versus 82%–92% of cells in nevi
	Control transcription factors associated with cell cycle arrest, apoptosis, and cellular differentiation	
HDM2[35]	Cell cycle–related/antiapoptosis markers	>20% of lesional cells stained positive in 1/16 dysplastic nevi, 3/11 melanomas in situ, and 67/102 primary melanomas
	90-kDa zinc finger protein that binds to p53 transcription activation domain inhibiting its function and targeting it for degradation by proteasomes	
P16[8,25,26]	Cell cycle–related/antiapoptosis markers	Loss of nuclear staining in 50%–98% of melanomas Positive in nevi
P21[14,28]	Cell cycle–related/antiapoptosis markers	Increased staining in melanomas Rare staining in nevi
P53[14,33]	Cell cycle–related/antiapoptosis markers	Lack of staining in nevi (rare superficial staining) Positive staining in 25%–58% of melanomas (staining within deeper portions of melanomas)
Retinoblastoma protein (RB)[14,36]	Cell cycle–related/antiapoptosis markers	Statistically significant increased nuclear staining in melanomas compared with nevi (however, difference was too narrow)
	Interacts with p16 and cyclin-dependent kinases to regulate cell cycle progression from G 1 to S phase	
Skp2[37]	Cell cycle–related/antiapoptosis markers	Slightly increased nuclear staining in melanomas compared with nevi
	Fbox protein which aids formation of a larger protein complex that degrades p27	
Cancer/testis antigens[38]	Immune modulatory marker	19 Nevi and 38 primary melanomas distinguished based on the use of a panel of 6 markers
	Proteins that are aberrantly expressed in many types of malignancies	
CD26[39]	Immune modulatory marker—an adenosine deaminase receptor	Increased staining in the radial growth phase of 22 of 66 melanomas compared with 2 of 44 nevi
CD40[40]	Immune modulatory marker-B-cell marker; also a tumor suppressor	Increased expression in melanomas compared with nevi
FLIP[41]	Immune modulatory marker	Positive staining in 1/32 benign nevi versus 24/29 melanomas.
Ki-67[8,10]	Proliferation marker	<5% Staining of cells in nevi 13%–30% In melanomas (some can show higher positivity) Also increased in Spitz
PCNA[6,42]	Proliferation marker	Increased staining in melanomas versus nevi

TABLE 1. (*Continued*) Potential Immunohistochemical Markers That May Become Useful in Distinguishing Nevi From Melanomas

Marker	Description	Staining
	A 36-kDa protein that is a cofactor of DNA polymerase d (expressed in all phases of cell cycle proliferation	Also increased in Spitz tumors
		Nevi show superficial staining, whereas melanomas show staining within deeper dermal component
S100A6[43]	Member of the S100 protein family	All 42 Spitz nevi demonstrated strong diffuse expression in both junctional and dermal components
		Staining was consistently weak and patchy in dermal component and minimal or negative in the junctional component.

GADD, growth arrest DNA damage; PCNA, proliferating cell nuclear antigen.

For most other potential markers (Table 1), large studies are needed, before reliable clinical application becomes possible as evidence of significant ability to separate benign and malignant melanocytic lesions is scant.[6,8,14,28–43]

Genetic Biomarkers

Although melanocytic nevi rarely show chromosomal aberrations, melanoma is characterized by frequent and numerous chromosomal aberrations with most melanomas demonstrating aneuploidy or losses and gains of portions of or whole chromosomes (Table 2).[44,45]

Accurate quantification of DNA copy number variations down to detection of single copy deletions and duplications has recently become possible using comparative genomic hybridization (CGH), which can be performed on paraffin-embedded tissues.[46–48,49] For histologically challenging melanocytic cases, this method improves distinction of melanoma from melanocytic nevi in specific situations, and can identify genetic differences among melanoma subtypes. In a study comparing melanocytic nevi with melanoma by CGH, Bastian et al[44] demonstrated a significant difference in the frequencies and types of aberrations in melanomas (96.2% melanomas had some form of aberration, with a mean of 7.5 anomalies), versus aberrations in only 13% of nevi, all of which were Spitz nevi and contained only a single aberration. In addition, acral melanomas have significantly more focused gene amplifications and aberrations involving chromosomes 5p, 11q, 12q, and 15, whereas lentigo maligna melanomas had more frequent chromosomal losses of 17p and 13q.[45]

Another method for detecting the presence of deletions or gains of specific alleles is the analysis of allelic imbalance (AI) or LOH, which uses polymerase chain reaction (PCR) amplification of microsatellite polymorphic markers followed by gel electrophoresis. This assay can be performed on DNA obtained from formalin-fixed paraffin-embedded (FFPE) tissues. In a study in which 32 benign melanocytic nevi and 41 primary cutaneous melanomas were allelotyped using 45 microsatellite markers that spanned all autosomal arms, Healy et al demonstrated frequent AI on several arms including 9p, 10q, 6q, and 18q in primary melanomas, whereas only 2 dysplastic nevi showed AI, one of which was loss of 9p.[46] Similarly, only 2 of 27 Spitz nevi showed deletions, also of 9p, suggesting that AI of 9p may not be confined to melanoma, whereas other genetic lesions such as loss of 10q, 6q, and 18q could be malignant phenotype markers.

TABLE 2. Genetic Biomarkers and Their Detection Methods

Method	Method Description	Application
CGH[45]	Accurate quantification of DNA copy number variations over a wide dynamic range with detection of single copy deletions and duplications	In histologically difficult cases, this method may allow distinction between melanoma and melanocytic nevi
	FFPE	96.2% Melanomas had some form of aberration compared with only 13% nevi, all of which were Spitz nevi
Analyses of AI[49]	Detects the presence of deletions or gains of specific alleles	Primary melanomas demonstrate frequent AI on several arms including 9p, 10q, 6q, and 18q
	Uses PCR amplification of microsatellite polymorphic markers followed by gel electrophoresis	Only 2 dysplastic nevi of 32 nevi showed AI, one of which was loss of 9p.
	Performed on DNA from FFPE tissues	
MLPA[50]	Measures the copy number of up to 45 nucleic acid sequences in one single reaction	86% concordance with CGH
	Performed on DNA extracted from routinely processed FFPE sections	−22 Of 24 primary melanomas showed multiple (>3) copy number gains and losses, whereas all Spitz and banal nevi showed copy number changes at <2 loci
FISH[53]	Utilizes a fluorescent probe or group of probes to search for preselected genomic abnormalities in tumors.	In the case of cutaneous melanoma, a group of 4 probes (6p25, centromere of chromosome 6, 6q23 and 11q13) have been studied and validated as 87% sensitive and 95% specific for the diagnosis of melanoma.

Cdk2, cyclin-dependent kinase 2; FFPE, formalin-fixed paraffin-embedded tissues.

Multiplex ligation-dependent probe amplification (MLPA) is another novel method that measures the copy number of up to 45 nucleic acid sequences in a single reaction.[50] This technique uses sequence-specific probe hybridization to genomic DNA followed by multiplex-PCR amplification of the hybridized probe, and then semiquantitative analysis of the resulting PCR products. The assay is fast, can be performed on DNA extracted from routinely processed FFPE sections, and multiple samples can be tested in one reaction; thus, it has several practical advantages over other adjunctive tests such as CGH. In a study evaluating MLPA on DNA isolated from archival melanocytic tumors, Van Dijk et al compared their results with those simultaneously determined by CGH, and found 86% concordance between the 2 methods.[51] In another MLPA study examining copy number alterations of 17 banal nevi, 14 Spitz nevi, and 24 primary melanomas, Takata et al[52] showed multiple (>3) copy number gains and losses in 22 of 24 primary melanomas, whereas all Spitz and banal nevi showed copy number changes at <2 loci.

Fluorescence in situ hybridization (FISH) is another emerging diagnostic aid for histologically challenging melanocytic lesions. FISH assays use a fluorescent probe or group of probes to search for preselected genomic abnormalities in tumors (Fig. 3). With respect to cutaneous melanoma, a group of 4 probes (6p25, centromere of chromosome 6, 6q23, and 11q13) has been studied and validated as 87% sensitive and 95% specific for the diagnosis of melanoma.[53] After initial validation studies, FISH has also been demonstrated as useful in multiple specific histopathologic quandries, including nevi with atypical epithelioid components versus melanoma arising within a nevus,[54] desmoplastic melanoma versus sclerosing nevus,[55] epithelioid blue nevus versus blue nevus-like melanoma metastasis,[56] intranodal nevus versus melanoma in a lymph node,[57] and cellular blue nevus versus melanoma.[58] Additionally, a recent study described improved detection of spitzoid melanomas via FISH with the addition of a second assay with a probe to 9q21; in 27 cases in which both assays were performed, sensitivity was improved from 70% (4-probe FISH assay only) to 85% (combined assays).[59] A 2010 study of 22 diagnostically ambiguous melanocytic lesions noted relatively low sensitivity (60%) and specificity (50%) for the prediction of future metastases, when FISH with this same 4-probe assay was used.[60] Subsequent to this publication, Gerami et al revised their FISH probe set for melanoma to include loci targeting 9p21, 6p25, 11q13, and 8q24, and reported improved sensitivity (94%) and specificity (98%) in discriminating a set of 51 melanomas and 51 nevi.[61]

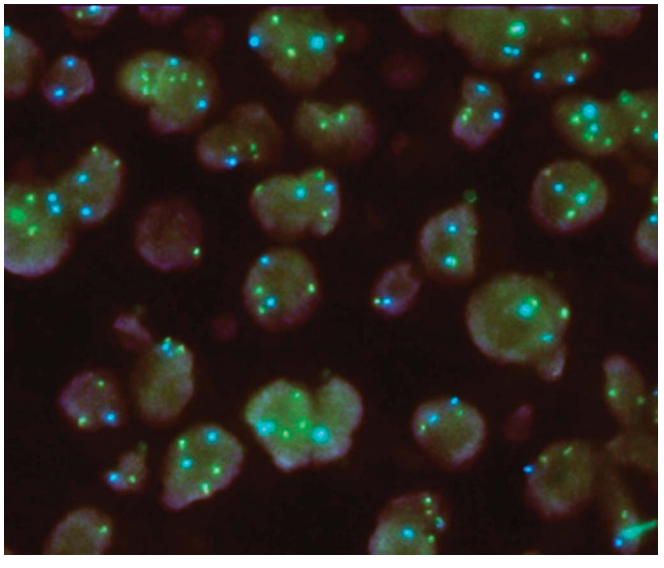

FIGURE 3. FISH assay on melanoma cells; 2 color probes to CCND1 (green; chromosome 11q) and CEP6 (blue; chromosome 6, centrosome). Multiple copy number changes are present, including gains of CCND1 and several cells with losses of CEP6. Photograph courtesy of Drs Boris Bastian and Philip LeBoit (UC-San Francisco Departments of Dermatology and Pathology).

Finally, in histologically ambiguous spitzoid melanocytic lesions, testing for specific mutations in BRAF, NRAS, and HRAS oncogenes via direct DNA sequencing may also contribute to a more accurate diagnosis. BRAF and NRAS mutations have generally been described in melanoma and melanocytic nevi, but are present only in a small minority of Spitz nevi.[62–72] HRAS mutations are found in a minority of Spitz nevi (~20% of cases), but not in melanoma (Table 3).[64,65,68,71,72]

Epigenetic Biomarkers

In addition to structural genetic changes, malignant transformation of melanocytes requires epigenetic alterations, which describe heritable changes in gene expression that are not caused by alterations in the primary DNA sequence. Epigenetic alterations are associated with the development of various human cancers, including melanoma, and they consist of DNA methylation aberrations, microRNAs (miRNA) expression patterns, posttranslational histone modifications, and chromatin remodeling.[73]

Ample evidence currently exists on the important role of abnormal DNA methylation in the development and progression of malignant melanoma. Enzymes involved in DNA methylation including DNA methyltransferases DNMT3A and DNMT3B have been shown to be significantly upregulated during melanoma progression.[74] More than 70 genes have also been shown to be hypermethylated in melanoma with aberrant promoter DNA hypermethylation preferably occurring at CpG islands and leading to a decreased expression of tumor suppressive genes.[75–79] As a result,

TABLE 3. BRAF, NRAS, and HRAS Mutations in Melanocytic Lesions

BRAF and NRAS Mutations[63,65–72]	Rare (9%) in Spitz and Atypical Spitz Nevi
	Present in 37% of primary spitzoid melanomas, 59% of common primary melanoma, and 67% of spitzoid melanoma metastasis
	Detected in 29% of spitzoid tumors of uncertain malignant potential (probably a significant number of these tumors were actual melanomas)
HRAS mutation[64,65,68,71,72]	Present in 8% of Spitz nevi, 8% of atypical Spitz nevi, and 6% of spitzoid tumors of uncertain malignant potential
	Not detected in melanomas

numerous essential pathways in melanoma are affected by this mechanism, including Mitogen-activated protein kinase (MAPK), WNT, pRb, cell cycle, DNA repair, apoptosis, growth, invasion, and metastasis. Detection of CpG islands hypermethylation in archival FFPE tissue using methylation-specific PCR is currently becoming one of the most prevalent molecular melanoma markers. By using methylation-specific MLPA to detect CpG methylation of 25 tumor suppressor genes commonly present in human cancers,[80] Takata et al[71] examined a series of melanomas and spitzoid tumors and found CpG methylation in 10 of 24 primary melanomas and in no Spitz nevi or atypical Spitz tumors. Thus, testing for CpG methylation may be a promising adjunctive diagnostic tool for melanocytic tumors.

Knowledge is also rapidly increasing on the role of miRNA in the development of melanoma.[81–83] These noncoding RNAs can interfere with gene regulation on the RNA level and regulate melanoma target genes that mainly affect cell cycle, invasion, and metastasis. For instance, the let-7 family, which is the first family of miRNAs identified in humans and is highly conserved across species, has been recognized as a key regulator in cancer and its members have been shown to be downregulated in primary cutaneous melanoma when compared with benign nevi.[84,85]

Finally, posttranslational histone modifications can disrupt contacts within and between nucleosomes and recruit proteins leading to the formation of a higher-order chromatin structure. Despite the lack of strong data on their role in melanoma,[86] it is thought that hypoacetylation of histones is involved in regulating melanoma biology by affecting the same pathways involved by mutations and CpG island hypermethylation. For example, histone modifications of genes involved in cell cycle regulation and apoptosis have been described such as histone hypoacetylation-mediated downregulation of CDKN1A,[87] and upregulation of the proapoptotic proteins BAX, BAK, caspase-3, and caspase-8.[88]

Diagnostic mRNA Markers of Melanoma

At the diagnostic level, differential gene expression in benign versus malignant melanocytic lesions has only been investigated in few studies. Haqq et al[89] carried out the first important in vivo investigation where comparison of gene expression profiles of a series of normal skin samples, melanocytic nevi, primary melanomas, and metastatic melanomas was done. Several transcripts useful in discriminating between these lesions were identified, including ARPC2, FN1, RGS1, WNT2, and osteopontin, which were each found to be overexpressed in melanomas. Kashani-Sabet et al,[90] in a follow-up study, described an IHC-based diagnostic assay for melanocytic tumors using the products of the above-mentioned 5 transcripts as markers. For each of those 5 markers, both the intensity and expression pattern were significantly different between melanomas and melanocytic nevi.[90] Furthermore, this multimarker assay is reported, based on comparison with the actual microscopic diagnoses, to show 97% sensitivity and 95% specificity for diagnosing melanomas arising in melanocytic nevi, 75% accuracy in appropriately diagnosing formerly misinterpreted melanocytic lesions, and 95% accuracy in identifying both dysplastic nevi and Spitz nevi.[89,90] In another study evaluating gene expression profiles of normal skin samples, melanocytic nevi and primary melanomas, Talantov et al[91] identified novel genes specifically overexpressed in melanoma and reported, similar to that in Haqq et al, a set of transcripts that can distinguish melanoma from benign nevi including prostate differentiation factor (PLAB), kinesin-like 5 (KNSL5), cadherin 3 (CDH3), osteopontin (SPP1), Cbp/p300-interacting transactivator 1 (CITED1), cathepsin B (CSTB), and presenilin 2 (PSEN2). Interestingly, among these transcripts, CITED1 and CDH3 were determined to be differentially expressed in early melanoma progression stages.[89,91]

PROGNOSTIC BIOMARKERS

Immunohistochemically Detectable Tissue-Based Prognostic Protein Biomarkers

In current clinical practice, microscopic diagnosis of melanoma is followed by the assessment for regional and systemic disease using clinicopathologic criteria defined by the TNM classification for tumor staging [2009 American Joint Committee on Cancer guidelines].[92] In the absence of regional or systemic disease, Breslow thickness, mitotic rate, and presence or absence of ulceration are the most important prognostic factors for primary melanoma.[85] The single most important parameter for outcome is SLN status, assessment of which includes micrometastases detected by IHC.[92–94] This evidence-based clinicopathologic staging system assigns patients to risk categories, but it does not predict individual patient outcomes. This detail is highlighted by the existence of some thin (<1 mm) melanomas that eventually metastasize; a significant proportion of melanoma deaths ($\sim$15%) result from metastases of thin primary melanomas according to National Cancer Institute Surveillance Epidemiology and End Results.[92,95] Thus, assays which could identify early-stage tumors with high metastatic risk are needed.

Several potentially applicable protein biomarkers (Table 4) have been identified, which demonstrate statistically significant associations with all-cause mortality, melanoma-specific mortality, or disease-free survival (DFS) on multivariate analysis.[7,96–144] However, no immunohistochemical markers for metastatic risk assessment have yet been validated in sufficiently large and repeatable clinical studies. This is partly due to the fact that the American Joint Committee on Cancer currently recommends cohort studies of $>$30,000 patients with extensive follow-up data to accept a new biomarker in routine clinical practice.[68] In addition, any new melanoma biomarker must show significantly improved predictive power beyond our current prognostic tools.[93,94]

Among these, many studies assessed the possible prognostic correlation between melanoma outcome and Ki-67 expression with variable results.[6,15–18] Although Ostmeier et al[15] described Ki-67 staining as an independent prognostic factor in a multivariate analysis of 399 primary melanomas with tumors showing lower Ki-67 rates being associated with increased metastasis-free survival, many other studies have shown that the direct correlation of increased recurrences and mortality with increasing Ki-67 positivity was not independent of Breslow thickness.[16–18]

TABLE 4. Protein Biomarkers With Independent Prognostic Significance

Marker	Function	Staining
AP-2 (alpha)[96,97]	Transcription factor	High level of AP-2 expression in the cytoplasm relative to the nucleus correlates with poor prognosis and the loss of nuclear
	52-kd DNA-binding protein	AP-2 expression is associated with malignant transformation and progression of melanoma[96]
	Self-sufficiency in growth signals	Decreased AP-2 expression independently associated with elevated risk of subsequent metastatic of stage I cutaneous malignant melanoma[97]
ATF-2[98]	Transcription factor	In primary cutaneous melanomas, strong nuclear staining and weak cytoplasmic staining was an independent poor outcome predictor
	Self-sufficiency in growth signals	
NCOA3[99]	Steroid receptor coactivator family member	Expression was associated with increased SLN metastases, reduced relapse-free and disease-specific survival
	Stimulates transcriptional activity in a hormone-dependent fashion by direct binding to nuclear receptors	NCOA3 was shown to be a stronger disease-specific survival predictor than all other variables, including tumor thickness.
	Self-sufficiency in growth signals	
PRKCA[100]	Belongs to the epithelial–mesenchymal transition group	Increased cytoplasmic expression in melanoma cells
	Regulates cell growth and progression	Predicts melanoma metastasis independent of Breslow index
	Self-sufficiency in growth signals	
Bcl-2[101]	Evasion of apoptosis	High expression was associated with a better outcome in the entire cohort and among metastatic specimens only
		Expression was higher in primary than in metastatic melanomas
Survivin[102]	Inhibitor of apoptosis protein family	Nuclear expression is associated with disease recurrence and poor survival in patients with stage I and II melanoma
CEACAM-1[103]	Required for the intercellular adhesion and subsequent signal transduction events	28 of 40 patients with CEACAM1-positive primary melanomas developed metastatic disease, compared with only 6 of 60 patients with CEACAM1-negative melanomas.
	Tissue invasion and metastasis	Highly significant association between CEACAM1 expression and metastasis
CXCR4[104]	Seven-domain transmembrane chemokine receptor recently implicated in cancer metastasis	Expression in melanoma cells correlated with unfavorable prognosis and correlated with a decreased median disease-free and overall survival.
	Tissue invasion and metastasis	
CD44[105]	Cell surface glycoprotein	Reduced CD44 expression associated with short recurrence-free survival and unfavorable prognosis in stage I cutaneous melanoma
	Tissue invasion and metastasis	
MCAM[106,107]	Adhesion molecule	Expression was an independent prognostic indicator inversely correlated with patient survival
	Mediates interactions between melanoma cells and between melanoma cells and endothelial cells	5-yr Survival was 92% for MCAM-negative patients compared with 40% for MCAM-positive patients.
	Tissue invasion and metastasis	MCAM expression was a stronger prognostic indicator than Breslow thickness.
L1-CAM[108]	Adhesion molecule	Overexpression associated with metastasis in malignant melanoma
	Binds to integrin alpha5-beta3	
	Tissue invasion and metastasis	
MMP-2[109,110]	Tissue invasion and metastasis	MMP-2 overexpression (>20% of malignant cells positive) was an independent prognostic marker for melanoma related death
		10-yr Disease-specific survival rate was only 51% in patients with MMP-2 overexpression compared with 79% in patients with a primary melanoma with low expression for MMP-2
OPN osteopontin[111]	Glycoprotein expressed by various tissues and cells—tissue invasion and metastasis	Expression was associated with reduced disease-specific and recurrence-free survival and was significantly predictive of SLN metastasis and burden
Tenascin-C[112]	Tissue invasion and metastasis	In primary cutaneous melanoma, absence of tenascin-C expression in the stroma of invasion fronts and within melanoma cells seems to be related to a more benign disease behavior with a lower risk of developing metastases

TABLE 4. (*Continued*) Protein Biomarkers With Independent Prognostic Significance

Marker	Function	Staining
tPA[113]	Tissue invasion and metastasis	Stage II melanomas with 51%–100% tPA-positive tumor cells were found to have the best prognosis, whereas lesions with 6%–50% tPA-positive cells had the worst.
		The extent of tPA tumor cell positivity was shown in multivariate analyses to be an independent prognostic factor for distant metastasis-free interval and for the duration of survival
HMB45[114]	Melanoma-associated antigens	HMB45 expression correlated with disease-free and overall survival and was an independent prognostic factor for DFS
iNOS[115]	Produces nitric oxide, which has growth promoting activity	In untreated stage III melanoma patients, significant association exists between tumor iNOS expression and shortened survival
	Sustained angiogenesis	iNOS expression was shown to be an independent predictor of poor survival
p16/INK4a[28,116]	CDKN2A (p16INK4alpha) cell cycle-inhibitory gene has been associated with development of familial melanoma.	In vertical growth phase melanomas, loss of nuclear p16 expression is associated with increased tumor cell proliferation and independently predicts decreased patient survival
	p16 Alterations occur frequently in sporadic melanomas	
	Insensitivity to antigrowth signals	
p27[28,117]	Cyclin-dependent kinase inhibitor	Cytoplasmic p27 expression was significantly increased in primary melanomas and further in melanoma metastases when compared with dysplastic nevi
	Insensitivity to antigrowth signals	
Cyclin A[118]	Mitotic cyclin necessary for DNA replication during cell cycle S-phase	Cyclin A expression in 0%–5% of tumor cells was independently associated with poor relapse-free survival
	Limitless replicative potential	
MAP-2[119]	Limitless replicative potential	Primary MAP2-positive melanomas had significantly improved survival
	Neuron-specific protein	
	Involved in the assembly of the mitotic spindle during cell division	
Metallothionein[120,121]	Limitless replicative potential	Expression independently associated with both progression to metastasis and poor survival rate.
	Heavy-metal binding proteins.	Thin (<1-mm thickness) metallothionein-positive melanomas were associated with higher risk of progression to advanced disease when compared with metallothionein-negative melanomas (5.30% vs. 0.28%)
	Their main function is heavy metal detoxification, free radical modulation, ultraviolet protection, and apoptosis inhibition	
p53[116]	Limitless replicative potential	In multivariate analysis, p53 expression was an independent prognostic factor
		Cases without p53 expression had improved survival
Ki-67[122]	Proliferation marker	Elevated Ki-67 index predicts poor clinical outcome for primary thick nodular melanomas (>1 mm).[10]
		Ki67 expression has been shown to have prognostic value in segregating high-risk from low-risk thin melanomas as thin melanomas with an intratumoral Ki67 expression rate of >20% were associated with a 10-yr metastasis rate of 39%.
bFGF[123,124]	Neoplastic progression and angiogenesis	bFGF expression in tumor-associated endothelial cells (79%) of 202 vertical growth phase cutaneous melanomas was an independent prognostic factor.
β-Catenin[125–127]	Cellular adhesion,	One study showed that there is higher nuclear β-catenin expression in melanomas compared with benign nevi and loss of nuclear expression was an independent poor prognostic factor[108]
	Wnt signaling cytoplasmic β-Catenin	Another study however showed that loss of nuclear β-catenin was not associated with poor prognosis in acral melanomas[109]
	Transcription factor nuclear β-catenin	
Bcl-6[28]	Transcriptional repressor	One study showed that although only a small number of invasive melanomas (8%) expressed Bcl-6, all positive cases were strongly and independently associated with poor prognosis[21]

(*continued on next page*)

TABLE 4. (*Continued*) Protein Biomarkers With Independent Prognostic Significance

Marker	Function	Staining
Dysadherin[128]	Membrane glycoprotein	In a study on 115 melanomas (55% of which were acral melanomas), dysadherin expression correlated with reduced patient survival and was an independent prognostic factor
	Downregulates expression and function of E-cadherin in a posttranscriptional manner	
The human natural killer antigen (HNK-1)[129,130]	May be crucial for cell migration	HNK-1 expression was a significantly independent worse prognostic factor
		A significantly higher metastatic risk was also present in stage I melanomas that showed positive HNK-1 expression
HIF2α[131]	Induced by hypoxia, which has a role in tumor growth by activating cell migration, angiogenesis, and glycolysis.	High HIF2α expression was associated with poorer prognosis in melanoma
GADD153[132]	Assist in DNA repair	In one study on 106 primary melanomas, *GADD153* was the only marker to show independent prognostic significance
Melastatin[133–135]	Cell-cycle control and cell survival	Expressed by nevi and in situ melanomas
		Downregulated in invasive and metastatic melanomas
		Loss of melastatin was associated with a 6-fold increase in metastasis risk and a worse 8-yr DFS
MITF[136]	Transcription factor required for the formation of normal melanocytes	Loss of MITF expression was shown to inversely correlate with overall and DFS.
p-Akt[137,138]	Known as PKB	AKT activity increases dramatically with melanoma progression and invasion
	Serine/threonine kinase	Strong AKT activity correlated inversely with both overall and disease-specific 5-yr survival of primary melanoma patients
	Stimulates cell cycle progression, cell proliferation, and apoptosis inhibition.	p-AKT was shown to be an independent prognostic factor in low-risk melanomas.
RGS1[139]	Codes for a member of the regulator of G protein family	Overexpressed in melanoma
		High expression significantly correlated with increased tumor thickness, mitotic rate, presence of vascular involvement, and SLN metastasis,
		High expression significantly associated with reduced relapse-free survival and disease-specific survival
RUNX3[140]	Tumor suppressor gene	Loss of expression correlated with a worse 5-yr survival of melanoma patients
	Important role in cell proliferation, apoptosis, and metastasis	
RBM3[141]	RNA- and DNA-binding protein	Strong nuclear expression in primary melanoma was significantly associated with prolonged overall and recurrence free survival and with favorable clinicopathological parameters
		High nuclear expression in primary melanoma was shown to be an independent marker of a prolonged overall survival
PUMA[142,143]	Mitochondrial protein	Loss of expression was independently associated with both disease-specific and overall 5-yr survival in melanoma
	Induces apoptosis when upregulated by E2 family of transcription factors 1 (E2F1)	
Mitotic marker PHH3[22,144]	Mitotic marker	In a study on nodular melanoma, PHH3 value was associated with tumor thickness and ulceration and was shown to be an independent prognostic indicator[144]
	Facilitate counting of mitosis	However, another study demonstrated that pHH3/MART double staining essentially shows no difference compared with mitotic count on H&E staining[22]

AP-2 (alpha), activator protein-2 alpha; ATF-2, activating transcription factor-2; Bcl-2, B-cell lymphoma 2; Bcl-6, B-cell lymphoma 6 protein; bFGF, basic fibroblastic growth factor; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule-1; CXCR4, chemokine (C-X-C motif) receptor 4; GADD153, growth arrest and DNA-damage-inducible protein 153; HIF2α, hypoxia-inducible factor 2 α; iNOS, nitric oxide synthase 2, inducible; L1-CAM, L1 cell adhesion molecule; MAP-2, microtubule-associated protein-2; MCAM, melanoma cell adhesion molecule; MMP-2, matrix metalloproteinase-2; NCOA3, nuclear receptor coactivator; p-Akt, phosphorylated AKT; PHH3, phosphohistone H3; PKB, protein kinase B; PRKCA, protein kinase C, alpha; PUMA, p53 Upregulated modulator of apoptosis; RGS1, regulator of G protein signaling 1; tPA, tissue plasminogen activator.

Genetic Biomarkers

Few studies exist currently regarding the ability of CGH to predict melanoma outcomes. The majority of these studies have been performed on uveal melanoma (Onken, White, Trolet).[47,48,49] In CGH analysis of 82 uveal melanomas, White et al identified 6 chromosomal regions that had prognostic significance for survival, the most predictive of which was a gain in 18q, which portended a 50% decreased

survival compared with a normal copy number at this region. Multivariate analyses using combinations of the 6 most predictive regions yielded more detailed data regarding survival.[48] In a more recent study using array-CGH on uveal melanomas, Trolet et al[49] identified a group of alterations including gains of 8q and losses of chromosome 3, 8p, and 16q, which taken together were 85.9% predictive of liver metastasis. These results for uveal melanomas seem promising and warrant further studies on other melanoma subtypes.

Recent evidence also suggests that, in addition to contributing to a more accurate diagnosis, testing for specific mutations in BRAF and NRAS oncogenes may also have prognostic implications in melanoma.[145–147] A recent meta-analysis on 674 patients with melanoma, in which the average BRAF mutation prevalence was 47.8%, showed that BRAF mutation increases the risk of mortality in melanoma patients by 1.7 times (95% confidence interval, 1.37 to 2.12), suggesting that BRAF mutation is an absolute risk factor for patient survival in melanoma.[145] Similarly, in a recent retrospective study evaluating the prognostic value of BRAF (V600) mutations in 105 consecutive patients with stage III cutaneous melanomas, BRAF mutations were detected in 40% of patients. The overall survival of patients with BRAF mutations (median of 1.4 years) was significantly lower than patients without BRAF mutations (median of 2.8 years). On multivariate analysis, BRAF status was shown to be an independent risk factor.[146] Furthermore, in a recent study testing the prognostic significance of BRAF and NRAS mutations in 677 patients with metastatic melanoma, the investigators showed that NRAS mutation status was an independent predictor of shorter survival after stage IV melanoma diagnosis and that patients with BRAF or NRAS mutations were more likely to have central nervous system involvement at the time of diagnoses of distant metastasis.[147]

Epigenetic Biomarkers

Recent evidence is also accumulating on epigenetic biomarkers that have prognostic significance.[148,149] For instance, a recent study assessed the association of promoter methylation status in long interspersed nucleotide element-1 and absent in melanoma-1 (AIM1) in paraffin-embedded archival tissue with melanoma progression and disease outcome. Results showed that high long interspersed nucleotide element-1 U-Index and/or AIM1 methylation in melanomas were significantly associated with DFS and overall survival in stage I/II patients. In multivariate analysis, AIM1 methylation status in melanoma was a significant prognostic factor of overall survival.[148] Similarly, the methylation status of Methylguanine-DNA Methyltransferase (MGMT) gene promoter, which is considered of prognostic significance by enhancing chemosensitivity to alkylating drugs in melanomas, was evaluated in 29 primary melanomas and 74 metastases using a standard methylation-specific PCR-based method to identify any correlation with the patients' outcome. Patients with methylated metastases had both significantly longer disease free and overall survival, irrespective of therapy.[149]

Prognostic mRNA Markers of Melanoma

Winnepennickx et al used an oligonucleotide-based microarray on 83 primary melanomas and identified 254 genes associated with distant metastasis-free survival.[139] Protein expression of 23 of these genes was studied with IHC, and overall survival was significantly associated with the expression of 5 markers (KPNA2, MCM3, MCM4, MCM6, and geminin).[150]

In addition, several other gene expression profiling studies on primary melanomas have demonstrated notable upregulation of osteopontin and specific DNA repair genes, and these were significantly associated with poor prognostic histopathologic features, metastatic progression, and reduced relapse-free survival.[151–153]

CONCLUSIONS

In summary, the diagnostic and prognostic utility of several melanoma biomarkers have been evaluated with promising results, although none has yet proven to be clinically useful in large-scale studies. Thus, there currently exists a major need for the melanoma biomarkers with prognostic significance that may eventually guide patient management and lead to new therapeutic targets.

REFERENCES

1. Elwood JM, Koh HK. Etiology, epidemiology, risk factors, and public health issues of melanoma. *Curr Opin Oncol.* 1994;6:179–187.
2. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer—the role of sunlight. *Adv Exp Med Biol.* 2008;624:89–103.
3. Magro CM, Crowson AN, Mihm MC. Unusual variants of malignant melanoma. *Mod Pathol.* 2006;19:S41–S70.
4. Barnhill RL, Mihm MC Jr. The histopathology of cutaneous malignant melanoma. *Semin Diagn Pathol.* 1993;10:47–75.
5. Mangini J, Li N, Bhawan J. Immunohistochemical markers of melanocytic lesions: a review of their diagnostic usefulness. *Am J Dermatopathol.* 2002;24:270–281.
6. Ohsie SJ, Sarantopoulos GP, Cochran AJ, et al. Immunohistochemical characteristics of melanoma. *J Cutan Pathol.* 2008;35:433–444.
7. Gould Rothberg BE, Bracken MB, Rimm DL. Tissue biomarkers for prognosis in cutaneous melanoma: a systematic review and meta-analysis. *J Natl Cancer Inst.* 2009;101:452–474.
8. Carlson JA, Ross JS, Slominski A, et al. Molecular diagnostics in melanoma. *J Am Acad Dermatol.* 2005;52:743–775.
9. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol.* 2001;19:3622–3634.
10. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol.* 2000;182:311–322.
11. Li LXL, Crotty KA, McCarthy SW, et al. A zonal comparison of MIB1-Ki67 immunoreactivity in benign and malignant melanocytic lesions. *Am J Dermatopathol.* 2000;22:489–495.
12. Kanter-Lewensohn L, Hedblad MA, Wejde J, et al. Immunohistochemical markers for distinguishing Spitz nevi from malignant melanomas. *Mod Pathol.* 1997;10:917–920.
13. Chorny JA, Barr RJ, Kyshtoobayeva A, et al. Ki-67 and p53 expression in minimal deviation melanomas as compared with other nevomelanocytic lesions. *Mod Pathol.* 2003;16:525–529.
14. Stefanski C, Stefanski K, Antoniou C, et al. G1 cell cycle regulators in congenital melanocytic nevi. Comparison with acquired nevi and melanomas. *J Cutan Pathol.* 2008;35:799–808.
15. Ostmeier H, Fuchs B, Otto F, et al. Prognostic immunohistochemical markers of primary human melanomas. *Br J Dermatol.* 2001;145:203–209.
16. Sparrow LE, English DR, Taran JM, et al. Prognostic significance of MIB-1 proliferative activity in thin melanomas and immunohistochemical analysis of MIB-1 proliferative activity in melanocytic tumors. *Am J Dermatopathol.* 1998;20:12–16.

17. Frahm SO, Schubert C, Parwaresch R, et al. High proliferative activity may predict early metastasis of thin melanomas. *Hum Pathol.* 2001;32: 1376–1381.
18. Hazan C, Melzer K, Panageas KS, et al. Evaluation of the proliferation marker MIB-1 in the prognosis of cutaneous malignant melanoma. *Cancer*. 2002;95:634–640.
19. Casper DJ, Ross KI, Messina JL, et al. Use of anti-phosphohistone H3 immunohistochemistry to determine mitotic rate in thin melanoma. *Am J Dermatopathol.* 2010;32:650–654.
20. Ribalta T, McCutcheon IE, Aldape KD, et al. The mitosis-specific antibody anti-phosphohistone-H3 (PHH3) facilitates rapid reliable grading of meningiomas according to WHO 2000 criteria. *Am J Surg Pathol.* 2004;28:1532–1536.
21. Colman H, Giannini C, Huang L, et al. Assessment and prognostic significance of mitotic index using the mitosis marker phosphohistone H3 in low and intermediate-grade infiltrating astrocytomas. *Am J Surg Pathol.* 2006;30:657–664.
22. Ikenberg K, Pfaltz M, Rakozy C, et al. Immunohistochemical dual staining as an adjunct in assessment of mitotic activity in melanoma. *J Cutan Pathol.* 2012;39:324–330.
23. Nielsen PS, Riber-Hansen R, Jensen TO, Schmidt H, Steiniche T. Proliferation indices of phosphohistone H3 and Ki67: strong prognostic markers in a consecutive cohort with stage I/II melanoma. *Mod Pathol*. 2013;26(3):404–414.
24. Talve L, Sauroja I, Collan Y, et al. Loss of expression of the p16 INK4/CDKN2 gene in cutaneous malignant melanoma correlates with tumor cell proliferation and invasive stage. *Int J Cancer.* 1997;74:255–259.
25. Hilliard NJ, Krahl D, Sellhayer K. p16 Expression differentiates between desmoplastic Spitz nevus and desmoplastic melanoma. *J Cutan Pathol.* 2009;36:753–759.
26. Al Dhaybi R, Agoumi M, Gagne I, et al. p16 Expression: a marker of differentiation between childhood malignant melanomas and Spitz nevi. *J Am Acad Dermatol.* 2011;65:357–363.
27. Mason A, Wititsuwannakul J, Klump VR, Lott J, Lazova R. Expression of p16 alone does not differentiate between Spitz nevi and Spitzoid melanoma. *J Cutan Pathol*. 2012;39(12):1064–1072.
28. Alonso SR, Ortiz P, Pollan M, et al. Progression in cutaneous malignant melanoma is associated with distinct expression profiles. *Am J Pathol.* 2004;164:193–203.
29. Slipicevic A, Holm R, Nguyen MT, et al. Expression of activated Akt and PTEN in malignant melanomas: relationship with clinical outcome. *Am J Clin Pathol.* 2005;124:528–536.
30. Florenes VA, Mælandsmo GM, Holm R. Expression of activated TrkA protein in melanocytic tumors: relationship to cell proliferation and clinical outcome. *Am J Clin Pathol.* 2004;122:412–420.
31. Zhuang L, Lee CS, Scolyer RA, et al. Mcl-1, Bcl-XL and Stat3 expression are associated with progression of melanoma whereas Bcl-2, AP-2 and MITF levels decrease during progression of melanoma. *Mod Pathol.* 2007;20:416–426.
32. Kuźbicki L, Aladowicz E, Chwirot B. Cyclin dependent kinase 2 expression in human melanomas and benign melanocytic skin lesions. *Melanoma Res.* 2006;16:435–444.
33. Tran TA, Ross JS, Carlson JA, et al. Mitotic cyclins and cyclin dependent kinases in melanocytic lesions. *Hum Pathol.* 1998;29:1085–1090.
34. Korabiowska M, Betke H, Kellner S, et al. Differential expression of growth arrest, DNA damage genes and tumour suppressor gene p53 in naevi and malignant melanomas. *Anticancer Res.* 1997;17: 3697–3700.
35. Polsky D, Melzer K, Hazan C, et al. HDM2 protein overexpression and prognosis in primary malignant melanoma. *J Natl Cancer Inst.* 2002;94: 1803–1806.
36. Karim RZ, Li W, Sanki A, et al. Reduced p16 and increased cyclin D1 and pRb expression are correlated with progression in cutaneous melanocytic tumors. *Int J Surg Pathol.* 2009;17:361–367.
37. Li Q, Murphy M, Ross J, et al. Skp2 and p27kip1 expression in melanocytic nevi and melanoma: an inverse relationship. *J Cutan Pathol.* 2004;31:633–642.
38. Luftl M, Schuler G, Jungbluth AA. Melanoma or not? Cancer testis antigens may help. *Br J Dermatol.* 2004;151:1213–1218.
39. van den Oord JJ. Expression of CD26/dipeptidyl peptidase IV in benign and malignant pigment-cell lesions of the skin. *Br J Dermatol.* 1998; 138:615–621.
40. van den Oord JJ, Maes A, Stas M, et al. CD40 is a prognostic marker in primary cutaneous malignant melanoma. *Am J Pathol.* 1996;149: 1953–1961.
41. Bullani RR, Huard B, Viard-Leveugle I, et al. Selective expression of FLIP in malignant melanocytic skin lesions. *J Invest Dermatol.* 2001; 117:360–364.
42. Niemann TH, Argenyi ZB. Immunohistochemical study of Spitz nevi and malignant melanoma with use of antibody to proliferating cell nuclear antigen. *Am J Dermatopathol.* 1993;15:441–445.
43. Ribe A, McNutt NS. S100A6 protein expression is different in Spitz nevi and melanomas. *Mod Pathol.* 2003;16:505–511.
44. Bastian BC, Olshen AB, LeBoit PE, et al. Classifying melanocytic tumors based on DNA copy number changes. *Am J Pathol.* 2003; 163:1765–1770.
45. Bauer J, Bastian BC. Distinguishing melanocytic nevi from melanoma by DNA copy number changes: comparative genomic hybridization as a research and diagnostic tool. *Dermatol Ther.* 2006;19:40–49.
46. Healy E, Belgaid CE, Takata M, et al. Allelotypes of primary cutaneous melanoma and benign melanocytic nevi. *Cancer Res.* 1996;56:589–593.
47. Onken MD, Worley LA, Person E, et al. Loss of heterozygosity of chromosome 3 detected with single nucleotide polymorphisms is superior to monosomy 3 for predicting metastasis in uveal melanoma. *Clin Cancer Res.* 2007;13:2923–2927.
48. White JS, McLean IW, Becker RL, et al. Correlation of comparative genomic hybridization results of 100 archival uveal melanomas with patient survival. *Cancer Genet Cytogenet.* 2006;170:29–39.
49. Trolet J, Hupe P, Huon I, et al. Genomic profiling and identification of high-risk uveal melanoma by array CGH analysis of primary tumors and liver metastases. *Invest Ophthalmol Vis Sci.* 2009;50; 2572–2580.
50. Schouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 2002;30:e57.
51. van Dijk MC, Rombout PD, Boots-Sprenger SH, et al. Multiplex ligation-dependent probe amplification for the detection of chromosomal gains and losses in formalin-fixed tissue. *Diagn Mol Pathol.* 2005;14:9–16.
52. Takata M, Suzuki T, Ansai S, et al. Genome profiling of melanocytic tumors using multiplex ligation dependent probe amplification (MLPA): its usefulness as an adjunctive diagnostic tool for melanocytic tumors. *J Dermatol Sci.* 2005;40:51–57.
53. Gerami P, Jewell SS, Morrison LE, et al. Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. *Am J Surg Pathol.* 2009;33:1146–1156.
54. Pouryazdanparast P, Haghighat Z, Beilfuss BA, et al. Melanocytic nevi with an atypical epithelioid cell component: clinical, histopathologic, and fluorescence in situ hybridization findings. *Am J Surg Pathol.* 2011; 35:1405–1411.
55. Gerami P, Beilfuss B, Haghighat Z, et al. Fluorescence in situ hybridization as an ancillary method for the distinction of desmoplastic melanomas from sclerosing melanocytic nevi. *J Cutan Pathol.* 2011;38: 329–334.
56. Pouryazdanparast P, Newman M, Mafee M, et al. Distinguishing epithelioid blue nevus from blue nevus-like cutaneous melanoma metastasis using fluorescence in situ hybridization. *Am J Surg Pathol.* 2009;33: 1396–1400.
57. Dalton SR, Gerami P, Kolaitis NA, et al. Use of fluorescence in situ hybridization (FISH) to distinguish intranodal nevus from metastatic melanoma. *Am J Surg Pathol.* 2010;34:231–237.
58. Gammon B, Beilfuss B, Guitart J, et al. Fluorescence in situ hybridization for distinguishing cellular blue nevi from blue nevus-like melanoma. *J Cutan Pathol.* 2011;38:335–341.
59. Gammon B, Beilfuss B, Guitart J, et al. Enhanced detection of spitzoid melanomas using fluorescence in situ hybridization with 9p21 as an adjunctive probe. *Am J Surg Pathol.* 2012;36:81–88.
60. Gaiser T, Kutzner H, Palmedo G, et al. Classifying ambiguous melanocytic lesions with FISH and correlation with clinical long-term follow up. *Mod Pathol.* 2010;23:413–419.
61. Gerami P, Li G, Pouryazdanparast P, et al. A highly specific and discriminatory FISH assay for distinguishing between benign and malignant melanocytic neoplasms. *Am J Surg Pathol*. 2012;36:808–817.
62. Takata M, Saida T. Genetic alterations in melanocytic tumors. *J Dermatol Sci.* 2006;43:1–10.

63. Da Forno PD, Fletcher A, Pringle JH, et al. Understanding spitzoid tumours: new insights from molecular pathology. *Br J Dermatol.* 2008;158:4–14.
64. Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features. *Am J Pathol.* 2000;157:967–972.
65. Da Forno PD, Pringle JH, Fletcher A, et al. BRAF, NRAS and HRAS mutations in spitzoid tumours and their possible pathogenetic significance. *Br J Dermatol.* 2009;161:364–372.
66. Fullen DR, Poynter JN, Lowe L, et al. BRAF and NRAS mutations in spitzoid melanocytic lesions. *Mod Pathol.* 2006;19:1324–1332.
67. Emley A, Yang S, Wajapeyee N, et al. Oncogenic BRAF and the tumor suppressor IGFBP7 in the genesis of atypical spitzoid nevomelanocytic proliferations. *J Cutan Pathol.* 2010;37:344–349.
68. Gill M, Cohen J, Renwick N, et al. Genetic similarities between Spitz nevus and Spitzoid melanoma in children. *Cancer*. 2004;101:2636–2640.
69. Palmedo G, Hantschke M, Rutten A, et al. The T1796A mutation of the BRAF gene is absent in Spitz nevi. *J Cutan Pathol.* 2004;31:266–270.
70. Lee DA, Cohen JA, Twaddell WS, et al. Are all melanomas the same? Spitzoid melanoma is a distinct subtype of melanoma. *Cancer*. 2006; 106:907–913.
71. Takata M, Lin J, Takayanagi S, et al. Genetic and epigenetic alterations in the differential diagnosis of malignant melanoma and spitzoid lesion. *Br J Dermatol.* 2007;156:1287–1294.
72. van Dijk MC, Bernsen MR, Ruiter DJ. Analysis of mutations in B-RAF, N-RAS, and H-RAS genes in the differential diagnosis of Spitz nevus and spitzoid melanoma. *Am J Surg Pathol.* 2005;29:1145–1151.
73. Richards HW, Medrano EE. Epigenetic marks in melanoma. *Pigment Cell Melanoma Res.* 2009;22:14–29.
74. Nguyen T, Kuo C, Nicholl MB, et al. Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma, *Epigenetics* 2011;6:388–394.
75. Rothhammer T, Bosserhoff AK. Epigenetic events in malignant melanoma. *Pigment Cell Res.* 2007;20:92–111.
76. Howell PM Jr, Liu S, Ren S, et al. Epigenetics in human melanoma. *Cancer Control.* 2009;16:200–218.
77. Schinke C, Mo Y, Yu Y, et al. Aberrant DNA methylation in malignant melanoma. *Melanoma Res.* 2010;20:253–265.
78. Sigalotti L, Covre A, Fratta E, et al. Epigenetics of human cutaneous melanoma: setting the stage for new therapeutic strategies. *J Transl Med.* 2010;8:56.
79. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation, *N Engl J Med.* 2003;349:2042–2054.
80. Nygren AO, Ameziane N, Duarte HM, et al. Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences. *Nucleic Acids Res.* 2005;33:e128.
81. Bonazzi VF, Stark MS, Hayward NK. MicroRNA regulation of melanoma progression, *Melanoma Res.* 2012;22;101–113.
82. Mueller DW, Rehli M, Bosserhoff AK. miRNA expression profiling in melanocytes and melanoma cell lines reveals miRNAs associated with formation and progression of malignant melanoma. *J Invest Dermatol.* 2009;129:1740–1751.
83. Philippidou D, Schmitt M, Moser D, et al. Signatures of microRNAs and selected microRNA target genes in human melanoma. *Cancer Res.* 2010;70:4163–4173.
84. Roush S, Slack FJ. The let-7 family of microRNAs. *Trends Cell Biol.* 2008;18:505–516.
85. Schultz J, Lorenz P, Gross G, et al. MicroRNA let-7b targets important cell cycle molecules in malignant melanoma cells and interferes with anchorage-independent growth. *Cell Res.* 2008;18:549–557.
86. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128:693–705.
87. Fan T, Jiang S, Chung N, et al. EZH2-dependent suppression of a cellular senescence phenotype in melanoma cells by inhibition of p21/CDKN1A expression. *Mol Cancer Res.* 2011;9:418–429.
88. Zhang XD, Gillespie SK, Borrow JM, et al. The histone deacetylase inhibitor suberic bishydroxamate regulates the expression of multiple apoptotic mediators and induces mitochondria-dependent apoptosis of melanoma cells. *Mol Cancer Ther.* 2004;3:425–435.
89. Haqq C, Nosrati M, Sudilovsky D, et al. The gene expression signatures of melanoma progression. *Proc Natl Acad Sci U S A.* 2005;102:6092–6097.
90. Kashani-Sabet M, Rangel J, Torabian S, et al. A multimarker assay to distinguish malignant melanomas from benign nevi. *Proc Natl Acad Sci U S A.* 2009;106:6268–6272.
91. Talantov D, Mazumder A, Yu JX, et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res.* 2005;11:7234–7242.
92. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol.* 2009;27: 6199–6206.
93. Cochran AJ. A glimpse of future management of melanoma. *Arch Dermatol*. 2009;145:1176–1177.
94. Carlson JA, Ross JS, Slominski AJ. New techniques in dermatopathology that help to diagnose and prognosticate melanoma. *Clin Dermatol.* 2009;27:75–102.
95. Gimotty PA, Elder DE, Fraker DL, et al. Identification of high-risk patients among those diagnosed with thin cutaneous melanomas. *J Clin Oncol.* 2007;25:1129–1134.
96. Berger AJ, Davis DW, Tellez C, et al. Automated quantitative analysis of activator protein-2alpha subcellular expression in melanoma tissue microarrays correlates with survival prediction. *Cancer Res.* 2005;65: 11185–11192.
97. Karjalainen JM, Kellokoski JK, Eskelinen MJ, et al. Downregulation of transcription factor AP-2 predicts poor survival in stage I cutaneous malignant melanoma. *J Clin Oncol.* 1998;16:3584–3591.
98. Berger AJ, Kluger HM, Li N, et al. Subcellular localization of activating transcription factor 2 in melanoma specimens predicts patient survival. *Cancer Res.* 2003;63:8103–8107.
99. Rangel J, Torabian S, Shaikh L, et al. Prognostic significance of nuclear receptor coactivator-3 overexpression in primary cutaneous melanoma. *J Clin Oncol.* 2006;24:4565–4569.
100. Alonso SR, Tracey L, Ortiz P, et al. A high-throughput study in melanoma identifies epithelial-mesenchymal transition as a major determinant of metastasis. *Cancer Res.* 2007;67:3450–3460.
101. Divito KA, Berger AJ, Camp RL, et al. Automated quantitative analysis of tissue microarrays reveals an association between high Bcl-2 expression and improved outcome in melanoma. *Cancer Res.* 2004;64:8773–8777.
102. Piras F, Murtas D, Minerba L, et al. Nuclear survivin is associated with disease recurrence and poor survival in patients with cutaneous malignant melanoma. *Histopathology*. 2007;50:835–842.
103. Thies A, Moll I, Berger J, et al. CEACAM1 expression in cutaneous malignant melanoma predicts the development of metastatic disease. *J Clin Oncol.* 2002;20:2530–2536.
104. Scala S, Ottaiano A, Ascierto PA, et al. Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. *Clin Cancer Res.* 2005;11:1835–1841.
105. Karjalainen JM, Tammi RH, Tammi MI, et al. Reduced level of CD44 and hyaluronan associated with unfavorable prognosis in clinical stage I cutaneous melanoma. *Am J Pathol.* 2000;157:957–965.
106. Pacifico MD, Grover R, Richman PI, et al. Development of a tissue array for primary melanoma with long-term follow-up: discovering melanoma cell adhesion molecule as an important prognostic marker. *Plast Reconstr Surg.* 2005;115:367–375.
107. Pearl RA, Pacifico MD, Richman PI, et al. Stratification of patients by melanoma cell adhesion molecule (MCAM) expression on the basis of risk: implications for sentinel lymph node biopsy. *J Plast Reconstr Aesthet Surg.* 2008;61:265–271.
108. Thies A, Schachner M, Moll I, et al. Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma. *Eur J Cancer.* 2002;38:1708–1716.
109. Vaisanen AH, Kallioinen M, Turpeenniemi-Hujanen T. Comparison of the prognostic value of matrix metalloproteinases 2 and 9 in cutaneous melanoma. *Hum Pathol.* 2008;39:377–385.
110. Vaisanen A, Kallioinen M, Taskinen PJ, et al. Prognostic value of MMP-2 immunoreactive protein (72 kD type IV collagenase) in primary skin melanoma. *J Pathol.* 1998;186:51–58.
111. Rangel J, Nosrati M, Torabian S, et al. Osteopontin as a molecular prognostic marker for melanoma. *Cancer*. 2008;112:144–150.
112. Ilmonen S, Jahkola T, Turunen JP, et al. Tenascin-C in primary malignant melanoma of the skin. *Histopathology*. 2004;45:405–411.
113. Ferrier CM, Suciu S, van Geloof WL, et al. High tPA expression in primary melanoma of the limb correlates with good prognosis. *Br J Cancer.* 2000;83:1351–1359.

114. Niezabitowski A, Czajecki K, Rys J, et al. Prognostic evaluation of cutaneous malignant melanoma: a clinicopathologic and immunohistochemical study. *J Surg Oncol.* 1999;70:150–160.
115. Ekmekcioglu S, Ellerhorst JA, Prieto VG, et al. Tumor iNOS predicts poor survival for stage III melanoma patients. *Int J Cancer.* 2006;119: 861–866.
116. Straume O, Sviland L, Akslen LA. Loss of nuclear p16 protein expression correlates with increased tumor cell proliferation (Ki-67) and poor prognosis in patients with vertical growth phase melanoma. *Clin Cancer Res.* 2000;6:1845–1853.
117. Chen G, Cheng Y, Zhang Z, et al. Prognostic significance of cytoplasmic p27 expression in human melanoma. *Cancer Epidemiol Biomark Prev.* 2011;20:2212–2221.
118. Florenes VA, Maelandsmo GM, Faye R, et al. Cyclin A expression in superficial spreading malignant melanomas correlates with clinical outcome. *J Pathol.* 2001;195:530–536.
119. Soltani MH, Pichardo R, Song Z, et al. Microtubule associated protein 2, a marker of neuronal differentiation, induces mitotic defects, inhibits growth of melanoma cells, and predicts metastatic potential of cutaneous melanoma. *Am J Pathol.* 2005;166:1841–1850.
120. Weinlich G, Eisendle K, Hassler E, et al. Metallothionein—overexpression as a highly significant prognostic factor in melanoma: a prospective study on 1270 patients. *Br J Cancer.* 2006;94:835–841.
121. Weinlich G, Topar G, Eisendle K, et al. Comparison of metallothionein-overexpression with sentinel lymph node biopsy as prognostic factors in melanoma. *J Eur Acad Dermatol Venereol.* 2007;21:669–677.
122. Gimotty PA, Van Belle P, Elder DE, et al. Biologic and prognostic significance of dermal Ki67 expression, mitoses, and tumorigenicity in thin invasive cutaneous melanoma. *J Clin Oncol.* 2005;23:8048–8056.
123. Straume O, Akslen LA. Importance of vascular phenotype by basic fibroblast growth factor, and influence of the angiogenic factors basic fibroblast growth factor/fibroblast growth factor receptor-1 and ephrin-A1/EphA2 on melanoma progression. *Am J Pathol.* 2002;160:1009–1019.
124. Giehl KA, Nägele U, Volkenandt M, et al. Protein expression of melanocyte growth factors (bFGF, SCF) and their receptors (FGFR-1, c-kit) in nevi and melanoma. *J Cutan Pathol.* 2007;34:7–14.
125. Bachmann IM, Straume O, Puntervoll HE, et al. Importance of P-cadherin, β-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. *Clin Cancer Res.* 2005;11:8606–8614.
126. Kageshita T, Hamby CV, Ishihara T, et al. Loss of β-catenin expression associated with disease progression in malignant melanoma. *Br J Dermatol.* 2001;145:210–216.
127. Arozarena I, Bischof H, Gilby D, et al. In melanoma, beta-catenin is a suppressor of invasion. *Oncogene*. 2011;30:4531–4543.
128. Nishizawa A, Nakanishi Y, Yoshimura K, et al. Clinicopathologic significance of dysadherin expression in cutaneous malignant melanoma: immunohistochemical analysis of 115 patients. *Cancer*. 2005;103: 1693–1700.
129. Thies A, Schachner M, Berger J, et al. The developmentally regulated neural crest-associated glycotope HNK-1 predicts metastasis in cutaneous malignant melanoma. *J Pathol.* 2004;203:933–939.
130. Vincent M, Thiery JP. A cell surface marker for neural crest and placodal cells: further evolution in peripheral and central nervous system. *Dev Biol.* 1984;103:468–481.
131. Giatromanolaki A, Sivridis E, Kouskoukis C, et al. Hypoxia-inducible factors 1alpha and 2alpha are related to vascular endothelial growth factor expression and a poorer prognosis in nodular malignant melanomas of the skin. *Melanoma Res.* 2003;13:493–501.
132. Korabiowska M, Cordon-Cardo C, Betke H, et al. GADD153 is an independent prognostic factor in melanoma: immunohistochemical and molecular genetic analysis. *Histol Histopathol.* 2002;17:805–811.
133. Hammock L, Cohen C, Carlson G, et al. Chromogenic in situ hybridization analysis of melastatin mRNA expression in melanomas from American Joint Committee on Cancer stage I and II patients with recurrent melanoma. *J Cutan Pathol.* 2006;33:599–607.
134. Duncan LM, Deeds J, Cronin FE, et al. Melastatin expression and prognosis in cutaneous malignant melanoma. *J Clin Oncol.* 2001;19: 568–576.
135. Deeds J, Cronin F, Duncan LM. Patterns of melastatin mRNA expression in melanocytic tumors. *Hum Pathol.* 2000;31:1346–1356.
136. Salti GI, Manougian T, Farolan M, et al. Microphthalmia transcription factor: a new prognostic marker in intermediate-thickness cutaneous malignant melanoma. *Cancer Res.* 2000;60:5012–5016.
137. Robertson GP. Functional and therapeutic significance of Akt deregulation in malignant melanoma. *Cancer Metastasis Rev.* 2005;24:273– 285.
138. Dai DL, Martinka M, Li G. Prognostic significance of activated Akt expression in melanoma: a clinicopathologic study of 292 cases. *J Clin Oncol.* 2005;23:1473–1482.
139. Rangel J, Nosrati M, Leong SP, et al. Novel role for RGS1 in melanoma progression. *Am J Surg Pathol.* 2008;32:1207–1212.
140. Zhang Z, Chen G, Cheng Y, et al. Prognostic significance of RUNX3 expression in human melanoma. *Cancer*. 2011;117:2719–2727.
141. Jonsson L, Bergman J, Nodin B, et al. Low RBM3 protein expression correlates with tumour progression and poor prognosis in malignant melanoma: an analysis of 215 cases from the Malmö Diet and Cancer Study. *J Transl Med.* 2011;9:114.
142. Hao H, Dong Y, Bowling MT, et al. E2F-1 induces melanoma cell apoptosis via PUMA up-regulation and Bax translocation. *BMC Cancer*. 2007;7:24–35.
143. Karst A, Dai DL, Martinka M, et al. PUMA expression is significantly reduced in human cutaneous melanomas. *Oncogene*. 2005;24:1111–1116.
144. Ladstein RG, Bachmann IM, Straume O, et al. Prognostic importance of the mitotic marker phosphohistone h3 in cutaneous nodular melanoma. *J Invest Dermatol.* 2012;132:1247–1252.
145. Safaee Ardekani G, Jafarnejad SM, Tan L, et al. The prognostic value of BRAF mutation in Colorectal cancer and melanoma: a systematic review and meta-analysis. *PLoS One*. 2012;7:e47054.
146. Moreau S, Saiag P, Aegerter P, et al. Prognostic value of BRAF (V600) mutations in melanoma patients after resection of metastatic lymphnodes. *Ann Surg Oncol*. 2012;19:4314–4321.
147. Jakob JA, Bassett RL Jr, Ng CS, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer*. 2012; 118:4014–4023.
148. Hoshimoto S, Kuo CT, Chong KK, et al. AIM1 and LINE-1 epigenetic aberrations in tumor and serum relate to melanoma progression and disease outcome. *J Invest Dermatol.* 2012;132:1689–1697.
149. Cesinaro AM, Sartori G, Migaldi M, et al. Prognostic significance of MGMT gene promoter methylation in differently treated metastatic melanomas. *Pathology*. 2012;44:313–317.
150. Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst.* 2006;98:472–482.
151. Conway C, Mitra A, Jewell R, et al. Gene expression profiling of paraffin-embedded primary melanoma using the DASL assay identifies increased osteopontin expression as predictive of reduced relapse-free survival. *Clin Cancer Res.* 2009;15:6939–6946.
152. Kauffmann A, Rosselli F, Lazar V, et al. High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene*. 2008;27:565–573.
153. Jewell R, Conway C, Mitra A, et al. Patterns of expression of DNA repair genes and relapse from melanoma. *Clin Cancer Res.* 2010;16: 5211–5221.

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CME EXAMINATION
MAY 2014

Please mark your answers on the ANSWER SHEET.

After completing this CME activity, physicians should be better able to utilize appropriate systematic criteria to diagnose Spitz nevi and apply histologic criteria in the differential diagnosis of intradermal Spitz nevi.

1. Which of the following clinical and histopathologic features have not been shown to be predictors of outcome in malignant melanoma?

A. Breslow thickness
B. Mitotic rate
C. Ulceration
D. Sentinel lymph node status
E. None of the above

2. Which of the following is not a characteristic of an ideal biomarker?

A. High sensitivity and specificity
B. Reliable,
C. Slowly analyzable
D. Cost-effective
E. Of prognostic or therapeutic value

3. Which of the following statements concerning Ki67 is false?

A. Ki-67 antigen is a non-histone nuclear protein.
B. Ki-67 antigen has been detected in the nuclei of proliferating cells during all stages of the cell cycle, except the G_1.
C. Ki-67 antibody serves as a proliferation marker by staining the growth fraction of a given cell population in tissue specimens.
D. Ki67 expression is thought to be a more accurate representation of cell proliferation than mitotic rate.
E. Diagnostically, Ki-67 expression appears to be useful in distinguishing nevi from malignant melanoma.

4. Which of the following is not true about comparative genomic hybridization?

A. Comparative genomic hybridization allows accurate quantification of DNA copy number variations down to detection of single copy deletions and duplications.
B. Comparative genomic hybridization can be performed on paraffin embedded tissues.
C. This method improves distinction of melanoma from melanocytic nevi in histologically challenging melanocytic cases.
D. Among nevi, only blue nevi may show aberrations, usually single.
E. All of the above are true.

5. Which of the following is not true about Fluorescence in situ hybridization?

A. Emerging diagnostic aid for histologically challenging melanocytic lesions.
B. FISH assays utilize a fluorescent probe or group of probes to search for pre-selected genomic abnormalities in tumors.
C. Group of four probes has been studied and validated as 87% sensitive and 95% specific for melanoma diagnosis.
D. The group of four probes commonly used includes: 6p25, centromere of chromosome 16, 6q23 and 11q13.
E. All of the above are true.

ANSWER SHEET FOR THE AMERICAN JOURNAL OF DERMATOPHATHOLOGY
CME PROGRAM EXAM
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Please answer the questions on page 377 by filling in the appropriate circles on the answer sheet below. Please mark the one best answer and fill in the circle until the letter is no longer visible. To process your exam, you must also provide the following information:

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1. (A) (B) (C) (D) (E)
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Your evaluation of this CME activity will help guide future planning. Please respond to the following questions below.

Please rate these activities (1 — minimally, 5 — completely)	1 2 3 4 5
These activities were effective in meeting the educational objectives	O O O O O
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These activities were relevant to my practice	O O O O O

Please rate your ability to achieve the following objectives, both before and after this activity: 1 (minimally) to 5 (completely)

	Pre	Post
	1 2 3 4 5	1 2 3 4 5
1. Identify technologies used in the discovery and identification of different melanoma biomarkers.	O O O O O	O O O O O
2. Apply the appropriate marker in the clinical setting.	O O O O O	O O O O O

How many of your patients are likely to be impacted by what you learned from this activity?
O <20% O 20-40% O 40-60% O 60-80% O >80%

Do you expect that these activities will help you improve your skill or judgment within the next 6 months? (1 — definitely will not change, 5 — definitely will change)	1 2 3 4 5
	O O O O O

How will you apply what you learned from these activities (mark all that apply):

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In monitoring patients O	As a foundation to learn more O
In educating students and colleagues O	In educating patients and their caregivers O
As part of a quality or performance improvement project O	To confirm current practice O
For maintenance of board certification O	For maintenance of licensure O

How committed are you to applying these activities to your practice in the ways you indicated above? (1 — minimally, 5 — completely)	1 2 3 4 5
	O O O O O

Did you perceive any bias for or against any commercial products or devices?	Yes	No
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What are your biggest clinical challenges related to dermatopathology?

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