Diagnostic and Prognostic Biomarkers in Melanoma

[a]DAVID WEINSTEIN, MD; [a]JENNIFER LEININGER, MD; [b]CARL HAMBY, PhD; [a]BIJAN SAFAI, MD
[a]Department of Dermatology, [a]Department of Microbiology and Immunology, New York Medical College, New York and Valhalla, New York

ABSTRACT

Melanoma is a lethal melanocytic neoplasm. Unfortunately, the histological diagnosis can be difficult at times. Distinguishing ambiguous melanocytic neoplasms that are benign nevi from those that represent true melanoma is important both for treatment and prognosis. Diagnostic biomarkers currently used to assist in the diagnosis of melanoma are usually specific only for melanocytic neoplasms and not necessarily for their ability to metastasize. Traditional prognostic biomarkers include depth of invasion and mitotic count. Newer diagnostic and prognostic biomarkers utilize immunohistochemical staining as well as ribonucleic acid, micro-ribonucleic acid, and deoxyribonucleic acid assays and fluorescence *in situ* hybridization. Improved diagnostic and prognostic biomarkers are of increasing importance in the treatment of melanoma with the development of newer and more targeted therapies. Herein, the authors review many of the common as well as newer diagnostic and prognostic biomarkers used in melanoma.
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Melanoma, an aggressive skin cancer, is currently the fifth most commonly diagnosed cancer in men and seventh in women in the United States with its incidence increasing 194 percent from 1975 to 2011.[1,2] In 2014, approximately 76,100 patients will be diagnosed with melanoma in the United States, accounting for an estimated 9,710 deaths.[1] Though recent advances in therapies for metastatic melanoma have shown some hope,[3,4] melanoma with distant metastasis still carries a grim prognosis with a five-year survival rate of 16 percent.[2] Given the poor prognosis for late stage melanoma, biomarkers are needed to aid in both the diagnosis and prognosis of melanoma and to determine which patients merit more aggressive therapy.

DIAGNOSTIC

Immunohistochemical markers. The histological diagnosis of melanoma occasionally may be difficult due to its variety of cytomorphological variants. Melanoma can resemble different tumors, including carcinomas, neuroendocrine tumors, sarcomas, lymphomas, and germ cell tumors.[5] Therefore, immunohistochemical staining for melanocytic markers of differentiation often are employed in the diagnosis of melanonma.[6–12] Among the markers considered for use in the histological diagnosis of melanoma are Human Melanoma Black-45 (HMB-45), Melan-A, tyrosinase, microphthalmia transcription factor, and S100 as well as several newer ones (Tables 1 and 2).

HMB-45 recognizes a 100 kD glycoprotein known as premelanosome protein (Pmel), Pmel17, gp100, or SILV.[13,14] Mutations in the Pmel gene result in a diluted, silver coat of normally black mice.[15] Pmel is found in pre-melanosomal vesicles and thought to be a necessary component of the fibrillar matrix for the polymerization of eumelanin.[14,16] HMB-45, a mouse monoclonal antibody, reacts with melanoma and junctional nevus cells.[17] Staining appears to be proportional to pigment content with lesions containing less pigment having little to no staining.[8] The sensitivity of HMB-45 has been shown to be 66 to 97 percent with decreased sensitivity in metastatic compared to primary lesions.[8,10,17–22] Specificity of distinguishing melanocytic from nonmelanocytic tumors is 91 to 100 percent.[10,19] Unfortunately, HMB-45 has demonstrated decreased specificity for malignant melanoma in sentinel lymph nodes compared to Melan-A.[23] As is common for many melanocytic biomarkers, HMB-45 demonstrates poor sensitivity for detecting desmoplastic malignant melanoma.[6,18,21,24]

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ADDRESS CORRESPONDENCE TO: David Weinstein, MD, Department of Dermatology, Metropolitan Hospital, 1901 1st Ave, New York, NY 10029; E-mail: davidweinstein82@gmail.com

TABLE 1. Diagnostic biomarkers of primary melanoma, not including desmoplastic melanoma when possible

PROTEIN/ANTIGEN	GENE	FUNCTION	IHC ANTIBODY	SENSITIVITY	SPECIFICITY (MELANOCYTIC VS. NONMELANOCYTIC)
Pmel/Pmel17/SILV/gp100[14]	PMEL	Component of the fibrillar matrix for the polymerization of eumelanin[14]	HMB-45	72–100%[8,10–12,17,22,52]	91–100%[7,10,11,17,19,22]
Melan-A	MLANA	Required for expression, trafficking, processing, and stability of Pmel[29]	A103	83–100%[10,12,52,58]	81–98%[10,19]
Tyrosinase	TYR	Synthesis of melanin.[34,35]	T311	90–100%[9,12,52,58]	97–100%[19,21]
MITF	MITF	Regulates melanocyte development and differentiation.[36]	C5 and D5	100%[11,39]	87–100%[7,11,21,39,40]
S100 protein family	–	Regulate cell growth, cell cycle, cell motility, calcium homeostasis, transcription and differentiation.[45,46]	Polyclonal antibody against S-100 proteins	89–100%[8,10–12,17,22,52]	70–79%[7,10,17,22]
SM5-1	FN1	Contributes to cell adhesion and migration[57]	SM5-1 IgG1 mouse monoclonal antibody[22]	95–99%[22,58]	100%[22]
CSPG4/HMW-MAA	CSPG4	Promotes cell adhesion, motility, and growth[59]	Mouse monoclonal antibodies 763.74, VF1-TP41.2, and VT80.12[62]	89%[60]	NA

TABLE 2. Diagnostic biomarkers in metastatic melanoma lesions

PROTEIN/ANTIGEN	GENE	IHC ANTIBODY	SENSITIVITY
Pmel/Pmel17/SILV/gp100[14]	PMEL	HMB-45	58–95%[7,11,12,17–22,56,62]
Melan-A	MLANA	A103	71–88%[12,18–21,56,58,62]
Tyrosinase	TYR	T311	63–93[9,19–21,56,58]
MITF	MITF	C5 and D5	77–100%[7,11,12,21,40,56,62]
S100 protein family	–	Polyclonal antibody against S-100 proteins	86–100%[7,11,12,17,19–22,62]
SM5-1	FN1	SM5-1 IgG1 mouse monoclonal antibody[22]	91–96%[22,58]
CSPG4/HMW-MAA	CSPG4	Mouse monoclonal antibodies 763.74, VF1-TP41.2, and VT80.12[62]	86–100%[60,62]

Melan-A, also known as melanoma antigen recognized by T-cells-1(MART-1), is a melanocyte differentiation antigen expressed in the cytoplasm of both melanocytes, melanoma, and retinal pigmented epithelium.[25–27] It is a membrane protein located in melanosomes, endoplasmic reticulum, and the trans-Golgi network.[28] Melan-A associates with Pmel and is integral in its expression, trafficking, processing, and stability.[29] A recent study showed Melan-A to be superior to S–100 with a sensitivity of 93 percent and a specificity of 98 percent when differentiating melanoma and nonmelanocytic neoplasms.[10] However, other studies have shown lower sensitivities of 75 to 86 percent, with Melan-A less sensitive for detection of metastatic melanomas compared to primary melanomas.[18–21] Despite this, Melan-A has been found to be one of the most sensitive markers when used in frozen sections obtained during Mohs micrographic surgery.[30,31] While the specificity of Melan-A has been reported to be as high as 95 percent, a few concerns have been raised regarding its specificity.[19] Similar to HMB-45, Melan-A has lower sensitivity for desmoplastic melanoma.[18] Of note, it may be difficult to distinguish melanoma *in situ* from pigmented actinic keratoses and lichenoid reactions in sun-damaged skin based on Melan-A staining.[32,33] Melan-A also has been shown to stain adrenal cortical, Leydig, and granulosa and theca ovary cells as well as tumors derived from these cells.[18,19]

Tyrosinase, located in melanosomes, is an enzyme involved in the production of melanin.[34,35] Its sensitivity ranges from 90 to 100 percent for primary melanoma with decreasing sensitivity in later stage disease.[9,12,19–21] Specificity typically is very good at 97 to 100 percent when distinguishing melanoma from nonmelanocytic tumors.[19,21] As with other biomarkers, tyrosinase has reduced sensitivity in desmoplastic melanoma.[21]

Microphthalmia-associated transcription factor (MITF), a transcription factor of the MiT family, is a regulator of melanocyte development and differentiation and necessary for melanoblast differentiation from the neural crest.[36] Interestingly, as a transcription factor, MITF has been shown to regulate the transcription of Pmel, Melan-A, and tyrosinase.[37,38] Some early studies have demonstrated excellent sensitivity (100%), at times exceeding that of S-100 and HMB-45, and specificity for distinguishing melanoma from nonmelanocytic carcinomas.[7,11,39] However, later research has highlighted problems with the specificity of MITF due to its ability to stain histiocytes, lymphocytes, fibroblasts, Schwann cells and smooth muscle cells.[21,40] Some of these studies also showed a lower sensitivity of approximately 88 percent, though this was observed in metastatic lesions.[21] Furthermore, like other immunostains, MITF lacks sensitivity and specificity for desmoplastic or spindle cell melanomas.[21,24,39]

S-100, named for its 100 percent solubility in saturated ammonium sulfate, is a family of more than 24 proteins found in several different cell types, including glial cells, Schwann cells, melanocytes, Langerhans cells, and chondrocytes.[41–44] They exist both intracellulary as dimers and are secreted extracellularly. S-100 proteins are involved in many cellular functions including cell growth, cell cycle regulation, cell motility, calcium homeostasis, transcription, differentiation, regulation of cytoskeletal components and inflammatory responses among many others.[45–51] While its sensitivity is >89 percent in formalin fixed tissue,[8,10,19,20,22,52] S-100 staining may be less sensitive when used in frozen sections in Mohs micrographic surgery.[31] Despite high sensitivity, S-100 suffers from low specificity for melanoma, which is estimated to be 70 to 77 percent.[7,10] This lack of specificity stems from the ability of S-100 to stain Schwann cells, chondrocytes, Langerhans cells, and myoepithelial cells among others as well as tumors derived from these cells.[22,53,54] Due to its lack of specificity, S-100 frequently is used simultaneously with more specific stains to distinguish melanoma from other S-100 positive malignancies. S-100 has much greater sensitivity compared to the above-mentioned biomarkers in desmoplastic malignant melanoma and thus, is of great utility in this variant of melanoma.[6,8,21,24,40,55,56]

SM5-1 is a new mouse IgG1 monoclonal antibody directed against two fibronectin isoforms that contribute to cell adhesion and migration and may play a role in melanoma metastasis.[57] In the few studies published so far, SM5-1 appears to be 95 to 99 percent sensitive for primary melanoma and 100 percent specific when distinguishing from other tumors tested; however, it is noted that it does stain perivascular dendritic cells, plasma cells, and myofibroblasts.[22,58] Like other markers, its sensitivity decreased in metastatic lesions, but only to 92 to 96 percent, which is much better than other currently available biomarkers.[22,58]

Chondroitin sulfate proteoglycan 4 (CSPG4), also known as high molecular weight melanoma-associated antigen (HMW-MAA) and melanoma chondroitin sulfate proteoglycan, is a membrane-bound proteoglycan found on melanocytes, endothelial cells, and pericytes among other cell types.[59] CSPG4 promotes cell adhesion, motility, and growth and may play a role in invasion and metastasis.[59]

Immunostaining with CSPG4 has demonstrated a sensitivity of >85 percent for melanoma with less sensitivity for benign melanocytic lesions, such as blue nevi.[60] While CSPG4 may have a lower sensitivity for acral lentignous melanoma, positive immunostaining has been associated with a worse prognosis for these lesions.[61] CSPG4 has a significant sensitivity of >90 percent for metastatic lesions, better than Melan-A, S-100, and HMB-45.[62] Desmoplastic melanoma frequently has diminished staining for many of the biomarkers; however, CSPG4 has recently been shown to have significantly greater sensitivity for detection of both primary and metastatic desmoplastic melanoma compared to HMB-45 and Melan-A.[63] CSPG4 also is interesting for its promising potential in immunotherapy for melanoma.[64,65]

A recent study showed that immunostaining of soluble adenylyl cyclase could assist in discriminating benign melanocytic nevi from melanoma. Specifically it was shown that the absence of the dot-like Golgi pattern and the presence of the pannuclear immunostaining was more indicative of melanoma.[66]

Immunohistochemical staining for p16 has recently been

shown to greatly aid in distinguishing spitz nevi from melanoma. Decreased immunohistochemical staining of p16 has been shown to significantly correlate with the diagnosis of melanoma.[67-69]

In addition to the previously mentioned biomarkers used to aid in the diagnosis of melanoma, several other biomarkers currently are under investigation, including MUM-1, Mel-5, melanocortin-1, and PNL2 among others.[70,71]

While these biomarkers do facilitate the histopathological diagnosis of melanoma, Melan-A, HMB-45, and tyrosinase all show diminishing sensitivity with advancing stage disease.[12] Unfortunately, none of these biomarkers are able to distinguish malignant from nonmalignant melanocytic lesions.

Biomarker panels and gene arrays. It is possible that several biomarkers together are needed to distinguish melanoma from melanocytic nevi. Furthermore, not every melanoma harbors the same mutations.[72] However, there may be several different mutations that a melanoma acquires in its progression toward cancer. Thus, particular combinations of mutations that result in upregulation or downregulation of certain biomarkers may be more consistent with or diagnostic of melanoma (Table 3). A study by Lewis et al[73] illustrates this concept. Utilizing real-time quantitative reverse transcriptase-polymerase chain reaction,[73] they characterized the expression profile of 20 genes in melanoma, primary and metastatic, reactive lymph nodes, and benign nevi. Of the 20 genes utilized, three of them, Melan-A, budding uninhibited by benzimidazoles 1 homolog (BUB1), and CD 63, allowed for differentiation among melanoma, benign nevi, and lymphocytes. However, use of this set of genes was performed only on the training set from the study population and was not verified on a larger set of patients.

Another such example, an assay that analyzes multiple genes, has recently been shown to be both sensitive and specific in distinguishing melanoma from benign melanocytic nevi.[74] This innovative system utilizes tape stripping of melanocytic lesions to obtain corneocytes for ribonucleic acid (RNA) analysis in a noninvasive manner. The preliminary data from this study yielded a sensitivity of 100 percent and specificity of 88 percent for detection of melanoma or melanoma *in situ* though further clinical validation is needed.

Recent work by Gerami et al utilizing fluorescence *in situ* hybridization (FISH) assays showed promise in distinguishing ambiguous melanocytic tumors.[75 76] FISH has the benefit of being performed on paraffin-embedded tissue. Alhough the assay has demonstrated some use in discriminating benign from malignant melanocytic lesions, other studies have shown difficulty especially with spitzoid tumors.[77–80] Recent improvement of the assay utilizing the markers CDKN2A (9p21), RREB1 (6p25), MYC (8q24), and CCND1 (11q13) has shown increased sensitivity and specificity in addition to better discrimination of Spitz nevi from Spitzoid melanomas.[75] FISH analysis also can be of use adjunctively to distinguish lymph node nevi from melanoma metastasis.[81]

PROGNOSTIC

Immunohistochemical biomarkers. While some patients will be cured with surgery alone, a significant

TABLE 3. Diagnostic multiple biomarker assays

MULTIPLE BIOMARKER ASSAY	BIOMARKERS	SENSITIVITY	SPECIFICITY (MELANOMA VS. BENIGN MELANOCYTIC NEOPLASMS)
3 gene qRT-PCR profile*[73]	Melan-A, BUB1, and CD 63	100%[73]	100%[73]
Epidermal tape stripping gene analysis[74]	17 gene classifier	100%[74]	88%[74]
4 loci FISH assay[75,76,78,79]	RREB1 (6p25), MYB (6q23), Cep6 (Centromere 6), CCND1 (11q13)	43–86.7%[75,76,78,79]	50–96%[75,76,78,79]
4 loci FISH assay[75]	CDKN2A (9p21), RREB1 (6p25), MYC (8q24), CCND1 (11q13)	94%[75]	98%[75]

TABLE 4. Prognostic biomarkers				
BIOMARKER	**GENE**	**ANTIBODY**	**FUNCTION**	**PROGNOSIS**
Mitotic rate	N/A	N/A	N/A	Higher rate associated with worse prognosis[83,84]
Ki-67	MKI-67	Ki-67 (MIB-1 clone)	Nuclear antigen expressed during proliferation[85]	Higher expression associated with worse prognosis[86,87]
MCAM	MCAM	Anti-MCAM	Cell adhesion molecule.[96]	Higher expression associated with worse prognosis[100,101]
Metallothionein I and II	Family of genes on chromosone 16q13[102]	Dako E9 mouse monoclonal antibody[103-105]	Homeostasis of heavy metal ions and protection against oxidative stress.[102]	Higher expression associated with worse prognosis[103–105]

number will not. Even patients with thin melanomas occasionally develop metastatic disease.[82] To help elucidate which patients are more likely to have disease progression and need adjuvant therapy, investigators have searched for histological prognostic biomarkers (Table 4).

While not considered a biomarker, the Breslow depth, or tumor thickness, on histopathology is the most accurate prognostic marker for patient survival in early stage cutaneous melanoma and hence, its inclusion in the American Joint Commission on Cancer (AJCC) melanoma staging system.[83,84]

Mitotic rate is currently included in the AJCC as one of the staging criteria because of its correlation with patient survival.[84] It is the second most significant predictor of patient survival, ranked behind only tumor thickness in localized primary cutaneous melanoma.[83]

Ki-67, a nuclear antigen, is a marker of proliferation that is expressed during the active phases of the cell cycle (G1, S, G2, and M).[85] For thin melanomas (<1mm), Ki-67 expression has been shown to correlate directly with prognosis and may correlate more highly with prognosis than mitotic count.[86,87] In addition, it has been shown that Ki-67 may be superior to mitotic count as a prognostic factor for survival in thicker melanomas (≥1mm).[88] Furthermore, there is a high degree of interobserver variability among histopathologists in recognition of mitoses.[89]

Tumors with higher mitotic rates, Breslow thickness, and the absence of tumor infiltrating lymphocytes all are associated with an increased risk of sentinel lymph node involvement.[90] These markers take on even more importance since the number of nodal metastases is the single most significant predictor of patient survival in patients with stage III disease.[91]

BRAF mutations are found in more than 50 percent of melanomas, and of these, more than 90 percent consist of the V600E mutation.[92,93] These mutations can lead to constitutive activation of the MAPK pathway.[94] BRAF mutations, more specifically the V600E mutation, have not been associated with any significant difference in patient survival compared to those melanomas that lack this mutation.[95] However, the introduction of the BRAF inhibitor, vemurafenib, has been shown to improve survival in patients with late-stage melanoma that have the V600E mutation. Therefore, patients with tumors that are positive for this BRAF mutation may have improved survival with treatment due to this new treatment.[4]

Melanoma cell adhesion molecule (MCAM), also know as MUC18 and CD146, is a 113-kDa cell adhesion molecule normally expressed on endothelial and smooth muscle cells in adult tissue.[96] While rarely expressed in carcinomas, it is strongly expressed in advanced primary and metastatic melanoma and less so in nevi.[97–99] MCAM expression has been shown to be an independent predictor of prognosis in primary melanoma.[100, 101]

Metallothioneins are a family of heavy metal-binding low molecular weight proteins.[102] They contribute to the homeostasis of heavy metal ions and protect against oxidative stress as well as have several other roles.[102] Several studies have demonstrated that overexpression of metallothioneins in primary melanoma is associated with progression of disease and hematogenous metastasis.[103–105]

Recently, the biomarker CD10 has shown a significant correlation to progression and prognosis in patients with melanoma.[106–108] It has been proposed that CD10, a zinc-dependent endopeptidase, may affect prognosis by degradation of substances, such as enkephalin and substance P, which are known to suppress tumor progression in melanoma.[109,110] In one study, positive staining of CD10 correlated with a shorter five-year survival, although the majority of melanomas were acral lentiginous melanomas.[107]

Hundreds of studies investigating the myriad of molecular markers have been performed to better stratify patient risk

and obtain improved prognostic information. Rothberg et al[111] performed a systematic review of these studies and identified more than 100 proteins that represent potential candidates for prognostic markers in melanoma. However, many of these proteins had been evaluated only in a single study and not further substantiated.[111] Further research studies need to be conducted to evaluate their clinical utility as independent predictors of outcome in patients with melanoma.[111,112]

More recent research has utilized tissue microarrays to screen a wide panel of immunohistochemical markers and to conduct gene expression profiling to search for prognostic biomarkers and gene expression signatures.[72,113–118] As with the diagnostic biomarkers, it is unlikely that a single biomarker alone will be sufficient to determine prognosis. Perhaps a signature from an array or panel of biomarkers will most accurately predict prognosis. As such, several multimarker assays have recently been developed to more accurately predict prognosis (Table 5).

The combined score of the three biomarkers, NCOA3, SPP1, and RGS1, is significantly correlated with disease-specific survival and sentinel lymph node metastasis, making it an independent risk factor in primary cutaneous melanoma.[119] After evaluating 38 different markers, Rothberg et al[120] recently designed a five-marker assay utilizing automated quantification of immunofluorescence that correlated significantly with reduced survival.[120]

Researchers likewise are investigating RNA, MicroRNA (miRNA), and deoxyribonucleic acid (DNA) assays, but those studies remain in the investigational phases of research. They currently are used to assist in selection of promising immunohistochemical biomarkers that may help determine prognosis and further elucidate the mechanisms of disease progression.[114–117]

RNA studies screening large numbers of genes have already revealed biomarkers that are significantly associated with prognosis. However, these studies are limited by the fact that they must be performed on cryopreserved tissue.[114–116] Nonetheless, recent studies have been able to ascertain profiles of RNA expression in paraffin-embedded, formalin-fixed melanoma tissue utilizing cDNA and new RNA extraction and isolation techniques.[117,121]

miRNA and DNA assays have the advantage of being conducted using paraffin-embedded, formalin-fixed tissue. Several studies utilizing miRNA already have shown that different profiles of miRNA are significantly associated with disease progression and survival.[107–110] Alhough fewer in number, DNA studies likewise have shown a correlation of different gene profiles with prognosis.[122]

FISH assays also may yield prognostic information. Positivity of one of the FISH assays already mentioned has been shown to be an independent risk factor for metastasis and melanoma-related death.[123] Another FISH assay evaluating copy number changes at CCND1 (11q13) and MYC(8q34) showed a gain of copy number in those melanomas that metastasized compared to those that did not.[124] Ideally, a complete genomic profile of the melanoma combined with the relevant prognostic information for each aberrantly expressed gene would give the most accurate prognosis, but the availability of such a prognostic indicator is still far off.

Serologic biomarkers. Serologic biomarkers have gained momentum in melanoma research in the search for the best markers of disease onset, progression, and therapeutic response. Using serologic markers is ideal as their testing is less invasive while providing a test with the potential to provide valuable information to clinicians treating melanoma patients. Melanoma-associated antigens, melanin-related metabolites, adhesion molecules, angiogenesis factors, and cytokines are among the serological biomarkers that are under current investigation.[125]

Lactate dehydrogenase (LDH), one of the earliest studied biomarkers in melanoma research, is a cytoplasmic enzyme responsible for the conversion of pyruvate to lactate. Cancer cells that replicate via anaerobic or glycolytic mechanisms have reduced dependence on oxygen for energy production creating a survival advantage.[126,127] This is relevant as tumors often have rapid growth resulting in necrosis and hypoxia as they quickly outgrow their vascular supply.[126] LDH elevations occur due to upregulation of LDH by tumor cells and by tumor cell necrosis causing spillover of the enzyme into the bloodstream.[126,127] In early melanoma research, elevated serum LDH levels were thought to be solely associated with liver metastasis; however, this has since been disproven.[128,129] Instead, elevated LDH levels have been consistently associated with adverse prognosis and directly correlate with survival in patients with stage IV disease.[84,129–131] Deichmann et al[129] showed LDH to be the most specific biomarker for disease progression in stage IV melanoma patients with a 92

TABLE 5. Prognostic multiple biomarker assays

MULTIPLE BIOMARKER ASSAYS	PROTEINS	PROGNOSIS
Hashani-Sabet 3 marker score[119]	NCOA3, SPP1, RGS1	Positive net multimarker index score associated with worse prognosis
Gould Rothberg 5 marker genetic algorithm[120]	ATF2, $p21^{WAF1}$, $p16^{INK4A}$, β-catenin, fibronectin	Score above a certain threshold associated with worse prognosis

percent specificity though only 79 percent sensitivity.[129] False positives LDH in high-risk patients have been as high as 1.6 percent and can be due to hemolysis or other disease states, such as myocardial infarction.[132] LDH is the only current biomarker to be included in the AJCC 2009 staging system due to its significant prognostic value. This is evident in patients with stage IV disease and elevated LDH levels who have approximately 50 percent shorter one- and two-year survival rates compared to those patients with normal LDH levels.[84]

S100, used commonly as an immunohistochemical biomarker, as mentioned previously, also can be used as a serologic biomarker. While of limited value in early melanoma detection, elevated S100B levels have been found to be an indicator of advanced clinical disease stage.[133] Elevated S100B levels in advanced melanoma patients have been associated with metastasis, treatment response, relapse, and overall survival.[43,134–141] While LDH is a recognized important independent prognostic factor in advanced melanoma, it is primarily of value only in stage IV disease; S100 may be of equal if not superior value in monitoring and prognosis in stage III and IV disease.[132,141–144] S100B has been shown to have a false-positive rate of 1.9 percent and can also be elevated in cases of ischemic stroke, cerebrovascular disorders, and complications of cardio-bypass surgery.[132,133,145]

C-reactive protein (CRP) is a member of the pentraxin protein family that binds phosphocholine on bacteria and autologous ligands from necrotic and apoptotic cells and can activate complement.[146] As an acute phase reactant, it is a nonspecific marker of inflammation, infection, and tissue injury that is synthesized principally by hepatocytes in response to circulating cytokines including IL-6.[147–149] Elevated CRP has been associated with several malignancies in addition to a worse prognosis for those malignancies.[150] Since IL-6 levels correlate with tumor burden in melanoma, it is not surprising that IL-6 and, therefore CRP, correlate with disease progression.[151,152] Furthermore, increased CRP is associated with progression from stage I, II, or III to stage IV melanoma.[153] In patients receiving IL-2 immunotherapy, elevated CRP prior to initiating therapy was associated with a lack of response.[154]

Melanoma-inhibiting activity (MIA) is an 11 kd soluble protein, which has been characterized as an autocrine growth factor.[155] Despite the name MIA, hamster melanoma cells transfected with recombinant human MIA cDNA demonstrate increased invasiveness and metastasis of melanoma cells.[156] Higher levels of MIA are observed in melanoma compared to benign melanocytic nevi and normal skin.[157] Serum levels of MIA have been shown to correlate not only with disease stage, but also progression and response to therpay.[158–162] MIA is not specific for melanoma and can be elevated in other neoplasms, such as squamous cell carcinoma, late in pregnancy, and in children.[163,164]

Vascular endothelial growth factor (VEGF) is an angiogenic cytokine that regulates endothelial proliferation, differentiation, and survival.[165] Angiogenesis has been associated with solid-tumor growth, migration, and metastasis.[166,167] VEGF is secreted not only by melanoma cells, but also by peripheral blood lymphocytes and platelets, thus complicating its potential prognostic and clinical value.[168–170] While elevated VEGF is associated with disease stage, overall survival, progression of disease, and metastasis,[171,172] other studies have been less encouraging, showing elevation of VEGF when compared to controls, but no association with disease progression or therapeutic response.[173] In view of these limitations and the fact that VEGF has persistently shown lower sensitivity and specificity when compared to well-established biomarkers, its utility as a biomarker in melanoma is questionable.[173–175]

Reverse transcriptase-PCR (RT-PCR) and real-time quantitative PCR (qPCR) are techniques used to detect and quantify DNA and RNA expression, and have many applications within a wide variety of fields.[176] RT-PCR has been used on peripheral blood samples to assess for the presence of circulating melanoma cells through the detection of mRNA of the melanocyte specific gene tyrosinase.[177] Various melanoma markers in addition to tyrosinase, gp100, melan-A/MART1, MIA, p97, β1è4-*N*-acetylgalactosaminyltransferase (GalNAc-T), paired box homeotic gene transcription factor 3 (PAX-3), and melanoma antigen A3 (MAGE-A3) have been used to detect circulating melanoma cells through both single and multimarker RT-PCR or qPCR.[177–181] While Palmieri et al showed that the presence of serum markers did not assist in prognosis, Arenberger et al found a rise in markers prior to disease progression and Koyanagi et al showed that the number of positive markers correlated with stage of disease.[178,179,181] Rarely, melanoma can metastasize across the placenta from mother to infant. qPCR was used successfully in a case to determine maternal tumor cell origin for proper diagnosis, prognosis, and management of the affected infant.[182]

Soluble BRAF V600E DNA mutations have been detected using RT-PCR and qPCR in patients with known cutaneous melanoma.[183,184] BRAF V600E, as mentioned previously, is currently used as a histological prognostic indicator and to guide available therapeutic options that target the BRAF-MEK-ERK pathway. Unfortunately, as a serological biomarker, BRAF V600E has not been useful in monitoring for disease progression. Among patients with melanoma, Pinzani et al[184] found no correlation between serum BRAF V600E DNA levels and Breslow thickness, Clark level, presence of ulceration, nor sentinel lymph node positivity.[184]

Serum miRNAs are non-coding short RNA elements important in the regulation of gene expression and subsequent protein synthesis.[185] They help regulate cell proliferation, differentiation, and apoptosis and also affect expression of oncogenes and tumor suppressor genes.[186] Given the known dysregulation of miRNAs in cancer, they are currently being investigated for diagnostic, prognostic, and therapeutic utility.[187] Expression of miRNAs in tissue specimens already show correlation with diagnosis and prognosis.[188–191] Recently it has been discovered that miRNAs can be detected in the blood.[192] Circulating miRNAs show diagnostic and progonsotic utility in several different types of cancer, but have yet to be investigated in melanoma.[193]

CONCLUSION

Currently most diagnostic biomarkers of melanoma rely on detection of melanocytes rather than melanoma itself. Newer biomarkers depend on cytogenetic markers of carcinogenesis and signatures of mutations utilizing panels of biomarkers. There are no current serologic markers for the early detection of melanoma, and there may never be. Such evidence may be possible only in advanced stage disease that has metastasized from the primary site. As such, current serologic biomarkers detect circulating melanoma cells or secondary evidence of advanced disease, such as LDH.

Future research into serological and histological methods to detect early stages of melanoma hopefully will improve prognosis through earlier intervention. Such research might investigate markers of melanoma stem cells or markers of melanoblast differentiation that indicate a survival advantage and progression toward neoplasia.[194] Microarrays could be utilized to screen for similarities between melanoma and stem cells. Genes in common among melanoma and stem cells could be investigated to design better diagnostic and prognostic assays, perhaps using FISH, as well as to suggest new therapeutic targets.

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