REVIEW

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Prognostic factors for melanoma progression and metastasis: from Hematoxylin–Eosin to genetics

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Abstract

Researchers have searched for factors that predict the metastatic potential of melanomas for decades. In recent years, the study of their metastatic potential has progressed from routine histological analysis of Hematoxylin–Eosin stained slides to proteomic, genetic, and molecular pathological analyses. As a result, knowledge about the metastatic potential of melanomas has progressed. Hundreds of prognostic factors have been described in literature and it is not possible to mention all of them in a report. Therefore, in the current report, we summarize some of them.

Keywords: melanoma, *AJCC*, metastases, mitoses, Clark, Breslow.

Introduction

In melanoma cases, metastases are the most important predictor of a patient's prognosis. Patients with M1a stage disease have a 1-year survival rate of 59%, whereas those with stage M1b have a 1-year survival rate of 57% [1]. Moreover, patients where metastasis has spread to organs other than the lung have a 1-year survival rate of 41% [1]. The probability of death is related to the initial stage at which the diagnosis is made. Patients in stage IA disease have a 94% 5-year and an 86% 10-year chance of survival, whereas patients with stage IIC have a 53% 5-year and a 41% 10-year likelihood of survival [2]. Patients with IIIA disease have a 67% 5-year survival rate [1] and patients with stage IIIC have a 28% 5-year survival rate [1]. In a very recent study, younger age, lower T status, and lower *American Joint Committee on Cancer* (*AJCC*) stage were associated with better overall survival [3].

For decades, researchers have searched for factors that predict the metastatic potential of melanomas. In recent years, the study of their metastatic potential has progressed from routine histological analysis of Hematoxylin–Eosin stained slides to proteomic, genetic, and molecular pathological analyses.

In the current paper, we review several prognostic factors that have been identified in the recent literature.

Some factors commonly assessed in the Hematoxylin–Eosin slide

Histological type of melanoma

The *World Health Organization* (*WHO*) has classified several morphological types of melanoma (Table 1) [4]. Although such classification is the result of various modifications, it is based on the approach proposed by Clark WH Jr in 1967 [5].

Table 1 – ***Melanoma subtypes (WHO 2006 Classification)***

Superficial spreading melanoma.
Nodular melanoma.
Lentigo maligna melanoma.
Acral lentiginous melanoma.
Desmoplastic melanoma.
Melanoma arising from a blue nevus.
Melanoma arising in a congenital nevus.
Melanoma of childhood.
Nevoid melanoma.
Persistent melanoma.

The principal criticism against such classification is that it is based on clinical, as well as on topographical and morphological criteria [6]. The title of the report by Clark WH Jr (a classification of malignant melanoma in man correlated with histogenesis and biologic behavior) and evidence presented later by Clark WH Jr *et al*. [7–9] pointed to a relationship between the histological subtypes and melanoma prognosis. However, later work demonstrated that the morphological subtype is not an independent prognostic predictor in patients with clinically localized cutaneous melanoma [6]. Nevertheless, research has indicated that the morphological type is related to the possibility of having a positive sentinel lymph node [10].

Debate still surrounds whether the prognosis might differ for specific histological types of melanoma that have been described in recent years. For example, some studies have shown that desmoplastic melanoma has a better prognosis than other variants [11], with some researchers concluding that this type of melanoma is significantly and independently associated with a shorter time of recurrence [12]. However, this finding has been called into question by other groups [13].

According to the clinical management system of the AJCC, the melanoma subtype is not a major consideration in the treatment of primary melanoma [6]. Nevertheless,

some evidence suggests that this idea might be reviewed in the near future. For example, some studies have indicated that the limited width of the excision of desmoplastic melanoma is associated with significantly greater local recurrence and mortality [14], which would mean wider resection margins for this melanoma subtype. However, the difficulty of achieving free margins in this type of melanoma means that radiotherapy may be a complementary therapeutic alternative [15]. Research has also suggested that the behavior of desmoplastic melanomas is closer to that of traditional sarcoma, with hematogenous, rather than lymphatic metastases [12]. As a result, some institutions do not consider the sentinel lymph node as an option in the management protocol of desmoplastic melanoma [16].

Breslow thickness and Clark level

Among the independent prognostic morphological factors in melanoma cases, thickness remains the single most useful variable [6], a fact noted by Breslow A in 1970 [17]. This allowed to distinguish thin melanomas as low risk [18]. He reported that melanomas less than 0.76 mm in thickness were associated with a very good prognosis, with no metastases observed in his limited initial study [17]. The AJCC selected a cutoff of 1 mm with a similar result. The prognosis of patients with these thin melanomas varies from disease-free survival close to 100% to about 70% [18].

In addition, some studies have demonstrated that in melanomas thinner than 0.75 mm, the sentinel lymph node was always negative [19]. As a result, sentinel lymph node biopsies are not performed in melanomas thinner than 0.75 mm unless they categorized as high risk, i.e., those showing ulceration, a high mitotic rate, and a vertical growth phase [19]. Another study demonstrated that drainage to multiple sentinel lymph nodes is more common when the Breslow depth is greater [20].

Some studies have shown that certain ethnic groups are prone to present with thicker melanomas. For example, although melanoma is rare in Maori and Pacific peoples, after adjustment, melanoma thickness was significantly greater in those populations compared with Europeans [21]. Moreover, the results indicated that the prevalence of melanomas of greater thickness in darker skinned populations might explain why the lesions in such populations were more aggressive [21].

Over the years, the prognostic value of Clark's level has proven to be much less reliable than Breslow thickness, and some studies have shown that Clark's level is not an independent predictor of outcome, even in thin melanomas [22]. In the staging system adopted by the AJCC in 2002, Clark's level was only used for lesions <1 mm. In the 7th edition of the *AJCC's Cancer Staging Manual*, Clark's level was removed from stage grouping because it was considered not to predict patients' outcomes when other melanoma features were evaluated [23]. In a way, Clark's level has been substituted by the mitotic rate. Clark's level is only used when the mitotic index is unavailable for lesions <1 mm.

Ulceration

Ulceration, when evident in a primary melanoma, is one of the strongest negative predictive factors for long-term survival. When ulceration is present, the 10-year survival rate is 50% for stage I and II melanomas, whereas it is 78% if the melanoma is non-ulcerated [24, 25]. For any T in the TNM classification system, the prognosis when ulceration is present is similar to the one of melanomas with an immediate superior T [24], for example, a T1a melanoma with ulceration behaves as a T1b melanoma without ulceration. Ulceration is also one of the most important factors in melanoma without metastases [1].

In a recent study of 522 melanomas, ulceration, as well as clinical staging, was indicative of survival prognosis [26]. Moreover, for melanomas thicker than 1 mm, ulceration seemed to be more predictive of prognosis than thickness [25].

Some studies have suggested, however, that ulceration would not be considered a significant independent factor if the mitotic rate was taken into account [27]. Research has also demonstrated that ulceration, in addition to tumor thickness, is a prognostic factor associated with sentinel lymph node positivity [28]. Other studies have concluded that ulceration is a prognostic factor for the response to adjuvant interferon therapy [29].

Understanding of the importance of ulceration in melanoma remains incomplete. One hypothesis is that ulceration might reflect rapid tumor growth. In this context, some work has identified a correlation between ulceration and the mitotic index [30]. Some studies of the interaction between melanocytes and keratinocytes favor the hypothesis that ulceration influences the local environment and the progression of melanoma [31, 32].

The failure to consider the depth of the ulceration when staging melanoma remains controversial, with some evidence suggesting that differences in the depth of the ulcer could be relevant in the prognosis of melanoma [33, 34]. Nevertheless, in some instances, ulceration has only been considered when the width of the ulcer is more than 0.1 mm; ulcers less than 0.1 mm are categorized as erosions due to lesion trauma [35].

Mitotic index

The mitotic rate, which is the strongest prognostic factor following tumor thickness [27, 36–38] it has been linked with the capability of a melanoma to metastasize in the 10 years following the initial diagnosis [39].

The mitotic rate has also been shown to be an independent predictor of post-recurrence survival [40]. In one study, fewer mitoses and the absence of ulceration were associated with improved overall survival in melanoma of the head and neck [3]. Some groups have concluded that a high mitotic rate in melanoma is associated with a lower survival probability [38], whereas others have asserted that the mitotic rate is weakly predictive of sentinel lymph node status and that it is not an independent predictor of survival for melanomas 1 mm or thicker [41].

Moreover, although thin melanomas have in general

a better prognosis, it is well recognized that around 5% of patients with such melanomas will die. Some have suggested that the mitotic rate could play a role in these thin melanomas with poor outcomes [42].

Since 2003, the *College of American Pathologists* has recommended that the mitotic rate should be included as a relevant factor in reports of cutaneous melanoma [43].

Furthermore, in the 2010 staging classification for melanoma of the *AJCC* (7^{th} edition), the presence of =1 mitosis/mm^2 has been added as a modifier of risk for patients with T1 melanomas [44]. The addition reflects the fact that in such a group, the presence of mitoses has the greatest influence on tumor recurrence.

Not all researchers agree on the inclusion of the mitotic rate as a prognostic parameter, mainly because of the fact that such procedures are much time consuming [45].

Invasion pathways

Melanomas are known to be able to metastasize via several routes. For example, they can produce so-called satellites, which are nests 0.05 mm or bigger in diameter that occur in the reticular dermis or hypodermis beneath the tumor but at a distance from it of at least 0.3 mm [46]. Satellites are associated with an increased frequency of regional lymph node metastasis (from 12% to 53%) in tumors greater than 1.5 mm [46].

In-transit metastases comprise another route. These refer to the metastatic nodules in the lymphatic pathway between the primary tumor and its draining lymph nodes [47, 48].

Melanomas may also metastasize *via* the lymph nodes [49], including the sentinel lymph node, but this latter subject is beyond the remit of this report. Some studies have shown that the status of the sentinel lymph node seems to be the most important prognostic factor in patients with thick melanomas [50].

In addition to the lymphatic route, particular histological variants of melanoma are more prone to metastasize through a hematogenous route [12, 16]. Recent work has suggested that aberrant expression of vimentin by melanoma could be clinically used as a predictor of the hematogenous metastasizing capability of a melanoma [51]. Other work has proposed that apoptosis 24 to 48 hours after the commencement of metastasis may play a crucial role in the spontaneous disappearance (metastatic inefficiency) of metastasis [52].

In the literature, several works have related vascular invasion to a significantly increased risk of relapse, lymph node involvement, distant metastases, and death, with an impact on melanoma outcomes even similar to that of ulceration [53–55].

An alternative mechanism of infiltration known as perivascular may also be important in melanoma dissemination. In 1995, Shea CR *et al.* presented a case of angiotropic metastatic malignant melanoma in which melanoma cells surrounded but did not invade the dermal vessels [56]. The pericytic location of the malignant cells without evidence of intravasation suggested that the melanoma cells had migrated along the external surface of the vessels [57, 58].

Distant metastases represent an additional route by which melanomas may metastasize. The occurrence of distant metastases is associated with a median survival time of about 7.5 months [59]. The disease-free interval prior to the occurrence of distant metastases, as well as the stage at which the disease was at, seems to have predictive value in survival [60].

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) are believed to represent the body's immune response to melanoma cells. For many years, TILs were categorized as brisk, non-brisk, or absent [61]. A brisk infiltrate signifies a better prognosis in terms of 5- and 10-year survival rates for melanoma with a vertical growth phase [62]. Moreover, a favorable clinical outcome seems to be associated with the presence of GrB+ and CD4+ TILs, with the expression of MHC class I antigen on tumor cells and MHC class II antigen on intratumor antigen-presenting cells [63]. However, some recent reports have suggested that the presence of TILs is a significant predictor of sentinel lymph node metastasis but that it is not a major predictor of disease-free survival or of overall survival [64]. One promising area of therapeutic interest is how the transfer of TILs generated from the primary tumor might be used to treat melanoma metastases [64].

Tumor regression

Tumor regression refers to the replacement of tumor tissue with fibrosis, degenerated melanoma cells, lymphocytic proliferation, and telangiectasia formation [65]. The prognostic value of regression in melanoma remains controversial. Although some studies have shown that regression is associated with an adverse prognostic outcome in predicting survival in thin melanoma [66], others have suggested that tumor regression is not a predictor of sentinel lymph node metastasis in patients with thin melanomas [67]. Another study reported an absence of metastasis in 73 patients who had thin melanomas without histological evidence of regression [68]. In addition, some researchers have concluded that regression is not an independent predictor of the risk of sentinel lymph node metastasis in melanoma [69].

Molecular pathology and melanoma

In the late 1980s, Holzmann B *et al.* proposed a model of melanoma in which benign melanocytes gradually evolved into melanoma cells with metastatic capability, in which every step was defined by the acquisition or loss of certain cellular markers that were easily detected by immunohistochemical analysis [70]. Unfortunately, the model was not as clinically useful as its simplicity suggested it would be. However, in the last decade, knowledge of genetic and molecular events associated with melanoma has increased dramatically.

N-RAS and BRAF were two of the first melanoma-related genes to be identified. In 2005, Curtin JA *et al.* demonstrated that 81% of melanomas lacking chronic sun-induced damage (intermittent sun exposure) had BRAF or N-RAS mutations, whereas the majority of melanomas in other groups (long-term sun exposure) had mutations in neither gene [71].

N-RAS mutations are found in 15–20% of cutaneous melanomas [72–74]; BRAF mutations are present in 50% of cutaneous melanomas, and c-KIT aberrations occur in 2% of melanomas [6]. Moreover, N-RAS and BRAF seem to be mutually exclusive [71, 75], with only some rare cases of coexistence of both mutations [76, 77].

It is also known that mutation of p16INKK4a – an inhibitor of cyclin-dependent kinase 4a – is the most common known cause of inherited susceptibility in familial melanoma [78, 79].

It appears clear that not all melanomas develop following the same molecular pathways. To shed light on this issue, some groups have tried to classify melanoma into several molecular types. Research conducted, thus far, has pointed to potential alterations in the mechanisms controlling cell proliferation, cell senescence, and apoptosis [80].

Table 2 shows some of the proposed molecular subtypes of melanoma.

Table 2 – ***A melanoma molecular disease model (from Vidwans SJ et al. [81])***

Subtype 1: Aberrations in the MAPK pathway.
Subtype 2: Mutations in the c-KIT pathway.
Subtype 3: Mutations in the G proteins, GNAQ and GNA11.
Subtype 4: RAS gene abnormalities.
Subtype 5: Abnormalities in the melanocyte development and survival pathway.
Subtype 6: Abnormalities in the AKT/PI3K signaling pathway.
Subtype 7: Aberrations in the G1/S Cyclin/CDK pathways.
Subtype 8: Aberrations in the p53-regulated intrinsic cell death pathway.

MAPK: Mitogen-activated protein kinase.

Figure 1 provides a simplified diagram depicting several of the pathways that can be altered to induce melanoma. The MAPK pathway is the most commonly altered and accounts for 70% of melanomas [71, 82]. In the MAPK pathway, Ras triggers the formation of a RAF/MEK/ERK kinase complex, which drives the transcription of key regulators through protein phosphorylation [81].

Research has also revealed that BRAF mutations are present in up to 82% of benign nevi; such mutations are, therefore, not sufficient for malignant transformation [83].

The study of these molecular pathways has several implications, the most important of which is probably the potential prognostic value of specific markers and the identification of therapeutic targets.

These therapeutic tools are not universal for all melanoma types. For example, while BRAF inhibitors are useful to treat melanomas with BRAF alterations, MEK-inhibitors are more useful in treating melanomas with mutation of the GNA gene [82]. The results of trials have suggested that RG7204 (previously known as RO5185426/PLX-4032) appears promising in treating patients with BRAF mutant metastatic melanoma [84, 85].

Another promising BRAF inhibitor is GSK2118436 [86]. In addition, the inhibition of some other pathways such as MEK or CDK4 results in massive apoptosis of tumoral melanocytes [87]. Moreover, investigators have reported some success with c-Kit inhibitors in particular types of metastatic melanomas [88].

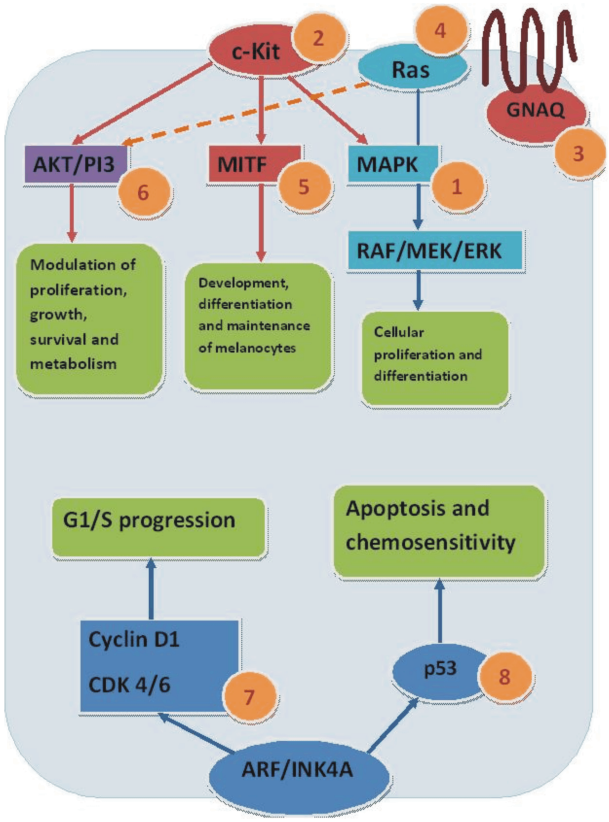

Figure 1 – ***A very simplified depiction of some of the main molecular pathways associated with melanoma. The orange circles signify the eight main molecular melanoma subtypes, which are enumerated in Table 2 [81]. MAPK: Mitogen-activated protein kinase. PI3K: Phosphatidylinositol 3-kinase. MITF: Microphthalmia-associated transcription factor. CDK: Cyclin-dependent kinase. INK4A: Inhibitor of cyclin-dependent kinase 4A.***

The various molecular subtypes of melanoma have implications for treatment with conventional chemotherapy. Mutant p53 cell lines appear to be refractory to drugs such as cisplatin, vincristine, and camptothecin [89, 90].

Many of these molecular alterations can also be correlated with morphological features routinely identified in melanoma. For example, BRAF mutations have been correlated with increased upward migration and nest formation of intra-epidermal melanocytes; thickening of the involved epidermis; sharper demarcation of the surrounding skin; and larger, rounder, and more pigmented tumor cells [91]. Some morphological subtypes of melanoma can also be correlated with certain specific molecular alterations. For example, melanomas with BRAF mutations usually exhibit superficial spreading, whereas those with c-KIT pathway alterations are generally mucosal and acral lentiginous [6]. In contrast, molecular alterations specific to nodular melanomas have not been identified so far [92].

The variation in molecular melanoma subtypes also has prognostic significance, with BRAF mutations, for example, apparently not affecting the melanoma prognosis at the time of diagnosis of the primary tumor; however, such mutations are associated with a poorer prognosis in metastatic melanoma [6].

Melanoma and genetics

One of the most active lines of investigation in the last few years has been the potential prognostic utility of genetic maps and genetic profiling in melanoma.

In a pioneering gene expression profiling study, Winnepenninckx V *et al.* identified 254 genes that were associated with distant metastasis-free survival of patients with primary melanoma [93]. Most of these genes were implicated in activating DNA replication, such as mini-chromosome maintenance genes and geminin [93]. Many of the genes were also correlated with melanoma thickness [94]. Bogunovic D *et al.* identified a group of 266 genes associated with post-recurrence survival [95]. Some of those they identified have already been correlated with morphological peculiarities. For example, Lugassy C *et al.* recently identified 128 genes that are differentially expressed in angiotropic *vs.* nonangiotropic melanomas [96]. They identified 15 genes that were directly involved in extravascular migratory metastases [96]. Some of these gene profiles have resulted in the identification of proteins easily identifiable by immunohistochemical analysis. For example, using cDNA microarrays, Alonso SR *et al.* confirmed that the expression of a set of proteins included in the epithelial-mesenchymal transition group (N-cadherin, osteoponin, and SPARC/osteonectin) was associated with metastatic development [97].

Immunohistochemical markers

Obviously, increased knowledge of genetics alone is not the solution to therapeutic and prognostic problems in melanoma. For example, melanoma is not always a genetic related phenomenon but many times an epigenetic one. In one study, 64% of the melanomas studied showed methylation of RASSF1a, and 75% showed methylation of CDKN2a [98].

Other work has revealed that promoter methylation is different in melanomas and nevi [99].

Some proteins coded by genes related to melanoma such as Bcl-2 and PTEN and PI3K can be studied by immunohistochemistry in daily practice using paraffin-embedded tissue. C-KIT is also identifiable by immunohistochemistry, and a higher expression of this marker has been found in lentiginous acral melanoma [100].

In univariate regression analysis, Ostmeier *et al.* found that the following immunohistochemical markers were related to disease-free survival: VLA-2; HLA-A, B, C; HLA-DR; gp100; Mel 14; ICAM-1; K-1-2; G-7-E2; and H-2-4-7 [30].

Multivariate analysis, however, failed to yield similar findings. More recent studies have demonstrated the independent prognostic value of certain immunohistochemical markers such as particular molecules involved in cell proliferation, matrix degradation, adhesion, transcription, and cell differentiation [101–109]. Alonso SR *et al.* reported that a combination of four antibodies – Ki67, p16(INK4a), p21(CIP1), and Bcl-6 – was associated with shorter overall survival in patients with vertical growth phase melanoma [110].

Gould Rothberg BE *et al.* conducted an exhaustive review of the literature and summarized the role of several such markers in melanoma prognosis related to tumor progression [111]. The main markers can be grouped as follows:

(1) Markers related with all-cause mortality [111]:

(a) Limitless replicative potential: cyclin E [110], Ki67 [103, 110], Ku70 [112], and Ku80 [112];

(b) Insensitivity to antigrowth signals: p16/INK4A [110], p27/KIP1 [110], and PCNA [103];

(c) Tissue invasion and metastasis: chemokine receptor CXCR4 [113], matrix metalloproteinase (MMP)-2, MCAM/MUC18 [114], and tissue plasminogen activator [106].

(2) Markers associated with overall and disease-free survival [111]:

(a) Limitless replicative potential: PNCA [103] and metallothionein [115, 116];

(b) Self-sufficiency in growth signals: NCOA3/AIB-1 [117] and AP-2a [118];

(c) Tissue invasion and metastasis: CXCR4 [113] and MCAM/MUC18 [119].

(3) Markers associated with melanoma-specific mortality:

(a) Evading apoptosis: Bcl-2 [120];

(b) Insensitivity to antigrowth signals: p16/INK4a [105];

(c) Limitless replicative potential: Ki67 [105], metallothionein [115], and p53 [105];

(d) Melanocyte differentiation: gp100 [121];

(e) Self-sufficiency in growth signals: AP-2a [122], ATF-2 [123], and NCOA3/AIB-1 [124];

(f) Sustained angiogenesis: iNOS [125];

(g) Tissue invasion and metastasis: matrix metalloproteinase-2 [126] and osteopontin [124].

Serum markers

In oncological pathology, several serum markers are related to melanoma and, therefore, are of interest in the follow up of this malignancy.

S100

The S100 protein is intensively expressed by most melanomas [127–129]. This protein was first discovered in cultured melanoma cells in 1980 [127]. Its clinical significance in relation to melanomas was first suggested in the 1990s [130].

Research has revealed that there is a very strong correlation between serum S100 values and the total tumoral burden and showed that a decrease in the serum S100 concentration is associated with tumoral remission [131–133]. S100 serum values are useful as a follow-up marker of a patient's response to treatment in metastatic stage [134–136], although they are not valid for the follow up of patients with stages I, II or III disease [137]. Increasing concentrations of serum S100 precede the detection of melanoma progression by several weeks [138]. Therefore, some clinicians recommend the determination of serum S100 in patients with melanoma widths more than 1 mm every 3–6 months [139–141]. S100 is also useful in the immunohistochemical detection of metastatic melanoma cells in sentinel lymph nodes [142, 143].

Lactate dehydrogenase (LDH)

It has been known since the 1950s that serum lactate dehydrogenase (LDH) increases with the melanoma tumoral burden [144]. Serum LDH is an independent prognostic factor in stage IV disease [145, 146] in which metastases and LDH levels are the most important predictors of survival [147].

LDH was also the only molecular marker for stage IV melanoma included by the *AJCC* in its 6th edition [1]. LDH is indicative of liver metastasis of melanoma; it has a sensitivity of 95% and a specificity of 83% in patients with stage II disease and a sensitivity of 87% and a specificity of 57% in patients with stage III disease [148]. Two or more elevated LDH levels drawn more than 24 hours apart will upgrade a patient to M1c status, regardless of the site of metastasis [147].

Melanoma-inhibiting activity (MIA)

Melanoma-inhibiting activity (MIA) was identified as a protein secreted from melanoma cells with growth-inhibiting properties [149–151]. Although serum MIA levels are increased in a low percentage of patients with stages I or II melanomas (13% to 23%), MIA is increased in 100% of patients with stages III and IV disease [152]. Moreover, MIA is useful as a predictor of the non-progression of melanoma. In a previous study, none of the patients with melanoma and normal MIA levels exhibited metastasis in a follow-up study at 6 and 12 months [152]. However, the sensitivity and specificity of MIA are lower than those of S100 [153].

Additional serum markers

A number of other serum markers have been investigated [154]. Four of the most important are highlighted below:

(a) Melanoma-associated antigens (neuron-specific enolase and lipid-bound sialic acid-P);

(b) Antigens related with melanocytic differentiation (tyrosinase);

(c) Antigens of angiogenesis (vascular endothelial growth factor and interleukin 8);

(d) Adhesion molecules (intercellular adhesion molecule 1, soluble vascular cell adhesion molecule-1, and some metalloproteinases);

(e) Cytokines (IL-6 and IL-10);

(f) Presentation antigens (HLA class I membrane antigens);

(g) Miscellaneous (tumor-associated antigen 90 immune complex and YKL-40).

However, the prognostic value of almost all of these markers in metastatic melanoma stages has been shown to be inferior to S100 or to LDH [154]. Many of these serum markers also have important limitations in the diagnosis of melanoma in stages I, II or III [154].

Some groups have studied the serum protein profiles of patients with early-stage melanomas and found that levels of transthyretrin and angiotensin were increased in the serum of those with melanoma compared with controls, whereas vitamin D binding protein (DBP) was decreased [155].

Although transthyretin alteration might be related to dysregulation of vitamin A homeostasis, the decrease in levels of the DBP could be due to the enzymatic activity of N-acetylgalactosaminidase (NAGA) by tumoral melanocytes and to DBP's glycosylation activity. Work has shown that glycosylated DBP hampers macrophage function, thereby, favoring tumor progression [155]. As a result, NAGA enzymatic activity can be correlated with the Breslow thickness, and it decreases after surgical resection of the tumor [155, 156]. Research has also demonstrated that the L-DOPA/tyrosine ratio significantly increases during the progression from stage I to III to higher disease stages [157].

Conclusions and future perspectives

Since the first publications by Breslow and Clark in the 1960s and 1970s, knowledge on prognostic factors in melanoma has increased dramatically, with the most pertinent data appearing mainly in the last decade. Although the high mortality rate still associated with melanoma might suggest that advances have been fruitless, an effective therapeutic target is much closer than before. Such progress is commendable given the almost complete absence of any effective treatment until recently.

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