Biomarkers of Melanoma

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Tumour biomarkers can be useful in predicting the risk of metastases and thus prognosis. Some of them can also have a diagnostic use. The use of serum biomarkers, such as lactate dehydrogenase (LDH) or S100b, is recommended in some guidelines, while the use of other serum biomarkers, such as melanoma inhibitory activity (MIA) and vascular endothelial growth factor (VEGF) is limited due to low specificity and limited clinical usability. DNA point mutations in melanoma represent another important biomarker that can guide patient selection and predict treatment response.

Keywords: melanoma ; biomarkers ; molecular pathology ; genetic mutations ; prognosis

1. Prognostic Tissue Biomarkers

In Alexander Breslow's report in 1970, tumour thickness and cross-sectional tumour area were identified as prognostic variables reflecting tumour burden. The thickness of the primary tumour is considered a significant prognostic factor for stage I and II melanoma; overall, 5-year survival rates in stage III melanoma are based on thickness. When the thickness is less than 1 mm, the 5-year survival rate is 53%; while when it is 1–2 mm, 5-year survival rate is 47%; when 2–4 mm, 5-year survival rate is 40%; and when over 4 mm 5-year survival rate falls to 34% [1]. However, thickness of the primary tumour was not found to be prognostic after tumour metastasis (stage IV). Melanoma ulceration is another important prognostic factor. In literature, there have been two possible explanations of the adverse prognostic value of ulceration in primary melanoma. One possibility is that melanoma ulceration could directly enable dissemination of the tumour. Alternatively, it could be that ulceration is a biological attribute of tumours with a predisposition to disseminate.

Proliferative activity of the tumour and overexpression of *c-myc* have been found to favour both dissemination and ulceration of the primary melanoma [2][3][4]. The hypothesis that melanoma ulceration directly enables the dissemination of the tumour through alterations in the local environment has been indicated in studies on the interactions of melanocytes and keratinocytes [5][6]. These studies indicate that ulceration may provide melanoma cells with a very effective way to interrupt the keratinocyte-mediated control that prevents melanocyte transformation. Mitotic activity of the primary tumour has also been investigated as a prognostic factor. In DNA replication genes of two pathways are over-represented: replication origins firing (ROF) genes and the separation of sister-chromatids by securin. For example, overexpression of ROF genes in melanoma is associated with poor prognosis [7]. Expression of *MCM4* and *MCM6* genes is associated with metastasis-free survival and overall survival (OS) [7]. Securin is encoded by the *hPTTG* gene, which acts as an oncogene. Its expression is seen via immunohistochemical staining in the vertical growth phase but not in the radial growth phase of melanoma [8]. Some promising new prognostic tissue biomarkers have also been reported in the literature, including cycloxygenase 1–3 (COX1-3), galectin-3 molecule, matrix metalloproteinases (MMP), and chondroitin sulfate proteoglycan 4 (*CSPG4*). COX1-3 converts arachidonic acid to prostaglandin. In Becker et al., COX-2 staining intensity was found to correlate to Breslow thickness in melanoma [9]. Kuzbicki et al. also showed a higher COX-2 staining intensity in melanoma than in benign nevi [10]. The galectin-3 molecule is secreted by inflammatory cells, and has been associated with tumour progression and metastasis in melanoma [11]. Galectin 3 and tumour size were found to be inversely related and correlated with OS [12]. MMPs are key to remodeling of the tumour tissue microenvironment. MMP-1 and MMP-3 positive melanoma metastases were associated with reduced disease-free survival (DFS) [13]. CSPG4 is believed to be essential in cell adhesion, melanoma migration and metastasis [14]; over 80% of melanomas have been found to be expressing *CSPG4*. However, it can be found in any disease stage and there is no concrete evidence that it correlates to disease progression [15]. Finally, several recent studies have demonstrated that the receptor for advance glycation end products (RAGE) signaling from both melanoma and non-melanoma cells (fibroblasts, immune cells, endothelial cells) in the tumor microenvironment represents an important element in the process of melanoma tumor growth. The RAGE/ligand axis appears to support the association between chronic inflammation and immunosuppression. Therefore, targeting RAGE in melanoma tumors could be therapeutically beneficial [16].

2. Prognostic Serum Biomarkers

The use of serum biomarker assays may identify the presence of residual or recurrent disease prior to imaging studies and relevant radiological evidence. From the therapeutic perspective, this is important, as the prediction or early identification of distant metastasis would enable the timely initiation of systemic therapy in adjuvant or metastatic settings [17].

2.1. Lactate Dehydrogenase (LDH)

LDH catalyzes the conversion of pyruvate to lactate in hypoxic or anoxic conditions. An elevated level of LDH is believed to be due to spillage into the bloodstream when melanoma cells outgrow their blood supply [18]. High levels of LDH are associated with worse prognoses, independently of site or number of metastases [19]. In the American Joint Committee on Cancer (AJCC) melanoma staging system, patients with distant metastasis and elevated LDH levels are considered stage IV M1c [18]. Patients with stage IV disease and normal serum LDH at initial staging have 1-year OS of 65% and 2-year OS of 40%. With elevated LDH levels, 1-year and 2-year OS are 32 and 18%, respectively [18]. Apart from its prognostic value, in patients treated with a combination of dabrafenib and trametinib, LDH was shown to be associated with poorer outcomes [20]. Moreover, when LDH decreases by more than 27.3% from the baseline, this has been associated with radiological response to immunotherapy [21].

2.2. S100 β

S100 proteins are implicated in a multitude of cell functions. As early as the 1980s, S100 β was found to be expressed in human melanoma cell lines and was proposed as a marker that could aid in diagnosis of melanoma [22].

However, S100 β can be found in abnormal levels in many pathological conditions, including liver, brain and renal injury, inflammatory and infectious processes [23].

In 2008, Mocellin et al. published a meta-analysis of 22 series, with a total of 3393 patients with malignant melanoma at all stages. This revealed that positive serum 100B was associated with reduced survival (hazard ratio [HR]: 2.23; connfidence interval [CI]: 1.92–2.58; $p < 0.0001$) [24]. Abraha et al. displayed a correlation between serum S100B levels and Breslow thickness. Serum S100 β > 0.2 µg/L and primary melanoma tumour thickness > 4 mm combined had sensitivity of 91% and specificity of 95% as predictors for disseminated disease, and consequently may inform prognosis at the point of diagnosis [25]. However, this was not confirmed in other studies of multivariate analyses, where levels of serum S100 β did not show clinical prognostic value [26]. Overall, the evidence for routine use of serum S100 β as a prognostic marker in melanoma is limited. This is due to small sample sizes, lack of proven significance in multivariate studies and mismatch of disease stages across studies [24]. However, measurement of serum S100 β in patients with Breslow > 1 mm lesions is recommended every 3–6 months in German and Swiss guidelines [27][28].

2.3. Melanoma Inhibitory Activity (MIA)

Melanoma inhibitory activity (MIA) is secreted by melanoma cells and is a regulatory growth factor [29]. MIA was proposed as a melanoma biomarker, because it is not expressed in benign human melanocytes or benign melanocytic nevi, but is strongly expressed in malignant melanoma cells [30]. Higher levels of MIA were linked with more advanced stages of melanoma and worse prognosis [31]. This was first shown in a German study that included over 830 blood samples of 326 patients with malignant melanoma of all stages. The cutoff was set at 9.8 ng/mL. In stage I and II patients, elevated MIA concentrations were found in 5.6%. This increased to 60% in patients with stage III and 89.5% in stage IV melanoma. Patients at stage III or IV that underwent resection or treatment with irradiation or chemotherapy prior to the study had MIA levels below the cutoff. Notably, all patients with reduced MIA levels in all stages did not develop further metastases during the follow-up period. In patients displaying a significant increase in MIA levels, metastases were detected either at the point of analysis, or in the following 2–6 months [32]. A comparative study in 373 melanoma patients among MIA, S100B, LDH and albumin showed that MIA was not superior to the use of S100 β or LDH; specifically, S100 β had the higher sensitivity (0.86) in newly diagnosed metastatic melanoma while MIA had the second highest (0.80); LDH sensitivity was lower at 0.48 and albumin lowest at 0.15. However, MIA had the lowest specificity (0.62), whilst albumin had the highest (0.99) [33].

2.4. Vascular Endothelial Growth Factor (VEGF)

VEGF is elevated in patients with advanced-stage melanoma. This was associated with negative immune effects, such as impaired dendritic cell function [34][35]. It was also linked with both elevated and decreased T helper 2 (Th2) cytokines. These were found to result in suppression of effective antitumour immunity. VEGF inhibitors can lead to improved dendritic cell function and reverse Th2 dominance, leading to Th1 polarity. These changes should in theory enhance

tumour rejection [36]. Ugurel et al., in a study including 125 patients with stages I-IV melanoma, concluded that VEGF was found to be an independent prognostic marker for OS [37]. However, VEGF has not been found to be effective as a marker of disease progression. This was replicated in a study in 2005, although healthy individuals were found to have higher VEGF levels [38]. When used to monitor patients, VEGF potentially has high negative predictive value (90%) with low sensitivity, specificity and positive predictive value of 57.1%, 78% and 34.5% respectively.

2.5. Other Serum Biomarkers

Apart from those already discussed, there is a multitude of other promising serum biomarkers. Tumor associated antigen 90 immune complex (TA90IC) and its utility in melanoma has been indicated in several studies. In a comparative study between TA90IC, MIA and S100 β in stage III melanoma patients undergoing adjuvant immunotherapy, TA90IC was the earliest elevated marker and an independent predictor for survival and recurrence of melanoma [5]. Further studies support this, indicating that antiTA90 IgM can be an independent prognostic factor for melanoma [39]. The expression of TA90IC can be elevated in inflammatory processes, such as hepatitis with liver cirrhosis [5]. Tyrosinase is a marker specific for melanocytes and Schwann cells, which are normally not found in peripheral blood [40]. Several studies have been performed, with conflicting results. Some have shown that the presence of microRNAs (miRNA) in tyrosinase correlates with melanoma relapse progression [41][42]. However, variable levels of miRNA in stage III and IV melanoma indicate that blood tyrosinase level is not a dependable marker in metastatic disease [43][44]. Osteopontin's role in cell death, tumour cell growth and recruitment of tumor promoting stromal cell has also been described [45][46][47]. In Maier et al., it was shown that a combination of S100 β and osteopontin may correlate with disease relapse and help identify patients at high risk of metastasis [48]. However, osteopontin can also be elevated in several autoimmune conditions. Interleukin-8 is a chemokine associated with inflammatory processes; it has been shown to promote angiogenesis and correlate with disease stage, survival, tumour burden and response to treatment [49]. Melanoma Antigen Gene A3 protein (MAGE-A3) is part of a family of proteins whose genes are normally silent, except in male germline cells. This is not the case in melanoma and other tumours though [50]; indeed, elevated levels are found in early melanoma stages. Nevertheless, its use as a prognostic factor has yet to be proved [51]. YKL-40 is a glycoprotein secreted by activated neutrophils and macrophages. Elevated levels can be seen in non-malignant diseases, but were also found to be an independent prognostic biomarker for poor survival in breast, lung, colon, ovary and kidney cancers [52]. Their use alone or in conjunction with LDH as an independent prognostic marker was shown by Schmidt et al. [53]. In the same study, serum level of YKL-40 at diagnosis was found to be an independent factor for survival [54]. However, YKL-40 has yet to receive approval by the Food and Drug Administration (FDA) to be used as a biomarker in the United States [55]. In addition, medications such as IL-2 and IFN-a2b increase YKL-40 expression and can cause false negative results [56]. Cytoplasmic melanoma-associated antigen (CYT-MAA) is produced in normal and tumour cells alike. However, its levels are elevated in melanoma. Although it is not sensitive or specific, it has been linked with disease recurrence and progression, as well as potentially with response to immunotherapy [57]. Melanotransferrin (MTF) is expressed in normal adult, fetal and tumour cells [58]. It is commonly found in exocrine tissues, such as salivary glands and the pancreas, as well as the epididymis [59]. Its exact role in melanoma is not yet known. However, it is thought to contribute to angiogenesis, tumour proliferation and tumour genesis [59][60]. Microphthalmia-associated transcription factor (MITF) contributes to the regulation of melanocytes' development, differentiation and function [61]. MITF is sensitive and specific in identifying melanoma cells [62]. Its levels are inversely proportional to melanoma cell invasiveness [63]. The identification of MITF after treatment indicates metastatic disease and worse outcomes in melanoma patients [64]. Glycoprotein 100 (gp100) is normally expressed in adult melanocytes. However, levels are increased in neonatal cells and melanoma, albeit those levels vary [65]. Despite this, gp100 is not specific and was not proven to correlate with response to treatment [66][67]. Lastly, elevated C reactive protein (CRP) and interleukin 6 (IL-6) levels were found to be linked to reduced survival and treatment resistance [67][68]. Despite this, CRP was found to be an independent predictor for survival [69].

Interestingly enough, protein levels can vary significantly between serum and plasma, due to the storage conditions, the method of blood fractionation and the properties of the specific analysed proteins. As such, there are significant discrepancies in the literature regarding protein levels in plasma and serum; nevertheless, it has been shown that plasma has better reproducibility in protein measurement. **Table 1** summarises the serum biomarkers that were described here. Among them, at least S100 β, MIA, VEGF, osteopontin, and interleukin 8 (IL-8) are relevant in plasma [70].

Table 1. Serum biomarkers.

Biomarker	Correlation	Limitation	Laboratory Methodology	References	
Enzymes	LDH	Increased levels with worse prognosis Increased LDH levels with distant metastases are classed as stage IV M1C in AJCC Radiological response to immunotherapy on LDH decrease	LDH can be elevated in other conditions	Photometric assay	[18] [19] [21]
	Tyrosinase	May correlate with melanoma relapse	Conflicting results in studies done	RT-PCR	[5][40] [41] [42] [43] [44]

Biomarker	Correlation	Limitation	Laboratory Methodology	References	
Secreted proteins/antigens	MIA	Increased levels with advanced disease and worse prognosis	Low specificity in newly diagnosed metastatic melanoma	ELISA	[31] [33] [32]
	TA90 Antigen	May be an independent predictor for survival, prognosis and recurrence	Can be elevated in inflammatory processes		[5][39]
	VEGF	Elevated in advanced stage melanoma Associated with negative immune effects Could be an independent prognostic marker for survival	Levels can also be elevated in healthy individuals Low sensitivity, specificity and positive predictive value in monitoring	ELISA, RT-PCR	[34] [35] [37] [38]
	Osteopontin	May be used in conjunction with S100b to predict relapse of high risk for metastases	Can be found in autoimmune conditions	IHC, TMA	[45] [46] [47] [48]
	IL-8	Increased levels with disease stage, survival tumour burden and response to treatment	Can be elevated in other inflammatory processes	ELISA, IHC, RT-PCR, TMA, HPLC	[49]
	MAGE-A3	Elevated levels can be found in early melanoma stages	May be elevated in other tumours Its use as a prognostic factor is not yet proven	RT-PCR	[50] [51]
	Glycoprotein YKL-40	Found to be an independent prognostic marker correlating with disease-free and overall survival	Has not received FDA authorisation Can yield false-negative results Can be associated with other tumours or inflammatory processes	ELISA	[52] [53] [54] [55] [56]
	CYT-MAA	Linked with disease recurrence and progression May be related to response to immunotherapy	Not sensitive or specific		[57]
	MTF	Thought to contribute to angiogenesis, tumour proliferation	It is also excreted in exocrine tissues	ELISA, IHC, RT-PCR	[57] [58] [59] [60]
	MITF	Has a diagnostic role in melanoma Increased levels with reduced invasiveness	Exact physiological role not yet discovered	ELISA, IHC, RT-PCR, HPLC	[61] [62] [64]
	GP100	Increased levels are found in neonatal cells and melanoma cells	Not specific Not proven to correlate with response to treatment	ELISA, IHC, RT-PCR	[65] [66] [67]
	CRP	Elevated CRP and IL6 are linked to reduced survival and treatment resistance CRP may be an independent predictor for survival	Can also be elevated by a multitude of other factors	IP	[67] [68] [69]
S100 Proteins	S100 β	Increased levels with reduced survival May be related with disseminated disease Recommended in some German and Swiss guidelines as surveillance	S100b can also be elevated in liver, brain, renal injury, inflammatory and infectious conditions	ELISA, LIA	[23] [24] [26] [27] [28]

AJCC: American Joint Committee on Cancer; RT-PCR: reverse transcription polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; IHC: immunohistochemistry; TMA: tissue microarray; IL-8: interleukin-8; HPLC: high performance liquid chromatography; MAGE-A3: MAGE Family Member A3; CYT-MAA: cytoplasmic melanoma-associated

antigen; MTF: melanotransferrin; MITF: microphthalmia-associated transcription factor; GP100: glycoprotein 100; CRP: C reactive protein; IP: immunoprecipitation; LIA: luminescence immunoassay.

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