

PROGNOSTIC BIOMARKERS IN MELANOMA

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Summary

Biomarkers are tumour- or host-related factors that correlate with tumour biological behaviour and patient prognosis. Modern analytical techniques have identified numerous possible biomarkers, but their relevance to melanoma progression, clinical outcome and the selection of optimal treatment strategies still needs to be established. In this review, we discuss common predictive biomarkers of melanoma.

Keywords: biomarkers; melanoma; melanoma prognosis.

Malignant melanoma is one of the most aggressive malignancies in human and is responsible for almost 60% of lethal skin tumors. Its incidence has been increasing in white population in the past two decades. Melanoma metastasizes quickly and only 14% of patients with metastatic disease can expect to live for 5 years [1,2].

Melanoma is a very enigmatic and heterogeneous cancer. There is a complex interaction of environmental and endogenous, including genetic, risk factors in developing malignant melanoma [3]. Deregulation in oncogenes and tumour suppressors, as well as multiple molecular signals, are required for melanoma initiation and progression, leading to a range of interacting pathways. Attempts are ongoing to unravel this complex network, thus allowing the identification of novel genetic and molecular biomarkers, as well as potential therapeutic targets [4].

Current prognostic markers based on the conventional American Joint Committee on Cancer (AJCC) staging system (TNM) are Breslow tumour

thickness, presence of ulceration, mitotic count and extent of nodal involvement for primary cutaneous melanoma, as well as serum lactate dehydrogenase (LDH) and site of metastases for distant metastatic disease [1,5].

Biomarkers are tumour or host related factors that correlate with tumour biological behaviour and patient prognosis. In a very general sense, a biomarker describes any measurable diagnostic indicator that is used to assess the risk or presence of disease. Although longstanding, the quest to identify relevant and useful biomarkers for cutaneous melanoma, assessed by either serum or immunohistochemistry, has yielded few results. Biomarkers in melanoma may serve a variety of purposes, they may serve as a surrogate for identifying present disease burden, as with lactate dehydrogenase (LDH), identifying patients with more aggressive disease, and/or determining disease responsiveness to various therapies [4,5,6].

An explosion of molecular information over the years has unveiled an array of candidate biomarkers for enhanced prognosis and outcome prediction. More than 100 studies have published experiments using DNA microarrays to investigate

the gene expression profiles found in melanoma. Most expression studies designed to investigate the molecular mechanisms associated with melanoma progression used melanoma cell lines or metastatic tumour samples. Although many candidates have been reported, few have proven reliability or predictability at present to allow for routine use. Serum biomarkers are assessed by the peripheral blood, whereas immunohistochemical biomarkers may be evaluated on formalin-fixed paraffin-embedded tissue [7].

Modern personalised medicine intends to use individual molecular markers and patterns of markers to subdivide traditional tumour stages into subsets that behave differently from each other.

As early as in 1954, increased levels of **LDH** (*Lactate dehydrogenase*) were detected in serum of melanoma patients ever since, the value of LDH as a tumour marker for malignant melanoma has been discussed. LDH is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease [8].

LDH was reported to be an indicator for liver metastases, with a respective sensitivity and specificity of 95% and 83% in stage II patients, and 87% and 57% in stage III patients. Patients with abnormal LDH levels had a significantly decreased survival. Taken together, increasing evidence exists to

demonstrate that LDH is elevated in advanced disease, predominantly in cases with liver metastases. LDH might serve as a prognostic factor in late-stage malignant melanoma. This has been discussed in a study where LDH was evaluated in combination with other tumour markers such as S100B and MIA and identified, by multiple logistic regression analysis, as the only statistically significant marker for disease progression. LDH has been included in the AJCC staging system, and patients with distant metastases and elevated LDH are considered stage IV M1c [9,10].

The best-studied melanoma biomarker is currently **S100B**. First described in 1980 in cultured melanoma cells, S100B has quickly become a well-established and widely used immunohistochemical marker of pigmented skin lesions. S-100B protein is a 21-kd thermo-labile acidic dimeric protein consisting of two beta subunits, which was originally isolated from the CNS [11]. In 1995, a first study was published evaluating the clinical significance of serum S100B in melanoma. The study showed that observed death ratio was markedly increased with increasing concentrations of S100B ($P < 0.001$). In other studies, baseline serum S100B protein concentrations correlated with prognosis and stage, rising concentrations of serum S100B indicated progression of the disease and complete decline in serum S100B concentrations reflected remission [12,13].

Although determination of serum biomarkers such as LDH and S100B may have a prognostic value, it does not translate into an adequate therapeutic intervention and survival benefit due to limited efficacy of current treatment options in advanced melanomas.

MIA (*Melanoma-inhibiting activity*) was identified in the early 1990s as a soluble 11 kDa protein with growth-inhibiting activities secreted from malignant melanoma cells. The fact that it was strongly expressed in malignant melanocytic tumours, but not in benign human skin melanocytes or benign melanocytic nevi, indicated that MIA may represent a novel tumour marker for malignant melanoma [14,15].

TA90-IC (*Tumour-associated antigen 90 immune complex*) is a 90kD glycoprotein found in the serum and urine of 63% to 68% patients with melanoma. Since TA90 binds to endogenous anti TA90 monoclonal antibody immune complex (TA90-IC) may be detected in the serum of patients with melanoma by ELISA assay. Multivariate regression analysis revealed that TA90IC was an independent predictor of survival when elevation occurred between 2 weeks and 3 months, whereas MIA was an independent predictor

appearing at 4–6 months. In general, elevation of TA90IC preceded increase of MIA in patients who developed recurrence. Additional studies in populations not receiving vaccines will further clarify the clinical utility of these assays [16,17].

YKL-40 is a heparin- and chitin-binding lectin secreted by activated neutrophils and macrophages during the late stages of differentiation, but also by arthritic chondrocytes, differentiated vascular smooth muscle cells and fibroblast-like synovial cells. Elevated serum levels of YKL-40 are seen in a number of non- malignant diseases characterised by inflammation and remodelling of the extracellular matrix, and were shown to be an independent prognostic factor for poor survival in patients with cancer of the breast, colon, ovary, kidney and lung. Study analysis showed that serum YKL- 40 ($P = 0.004$) and serum LDH ($P = 0.004$) were independent prognostic factors for survival. A combination variable of elevated serum YKL-40 and LDH quadrupled the risk of early death ($P < 0.001$) compared with that of patients with normal levels of the markers. The use of serum YKL-40 has not received Food and Drug Administration approval for use as a biomarker for cancer [18,19].

Melanoma is a complex genetic disease, and multiple genetic alterations have been reported to play a role during disease progression. The mitogen-activated protein (MAP) kinase pathway is an important driver in melanoma and is made up of several potential targets providing therapeutic options. In this pathway, the activation of RAS proteins stimulates the RAF kinases ARAF, BRAF, and RAF1. This process causes phosphorylation of the MEK kinases, which phosphorylate the ERK kinases. Activated ERK regulates cyclin D1, which, in turn, regulates multiple cellular processes involved in cell division. Dysregulation of BRAF signaling has been shown to be one of these key drivers of the disease [20,21].

In 2002, Davies et al. first reported that **BRAF** is mutated in approximately 8% of human tumors, most frequently in melanoma where the BRAF^{V600} mutation is observed in approximately 50% of tumors. Mutations in BRAF^{V600E} may cause the protein to become oncogenic. In preclinical studies, oncogenic BRAF signaling that is a result of this mutation may lead to increased and uncontrolled cell proliferation and resistance to apoptosis (programmed cell death) [22].

Drugs that treat cancers driven by *BRAF* have been developed. Two of these drugs, vemurafenib and dabrafenib are approved by FDA for treatment of late-stage melanoma. Vemurafenib (PLX4032) was the first drug to come

out of fragment-based drug discovery. BRAF V600E mutations are associated with increased sensitivity to BRAF inhibitors [23].

The novel BRAF V600E mutant-specific antibody, VE1 is currently used to detect the presence of the BRAF V600E mutation in patients with metastatic melanoma on paraffin-embedded, formalin-fixed melanoma biopsies. The antibody had a sensitivity of 97% and a specificity of 98% for detecting the presence of BRAF in immunohistochemical (IHC) analysis (*Figures 1, 2*). Clinical use of the V600E BRAF antibody should be a valuable supplement to conventional mutation testing and allow V600E mutant metastatic melanoma patients to be triaged rapidly into appropriate treatment pathways [24].

Since the discovery of BRAF^{V600E} mutations in melanoma in 2002, scientists and clinicians have learned much about the role of mutated BRAF^{V600E}, but many questions remain unanswered and research is ongoing. The rapidly increasing incidence of melanoma, coupled with its highly aggressive metastatic nature and limited current treatment options, make this an active and exciting area of research.

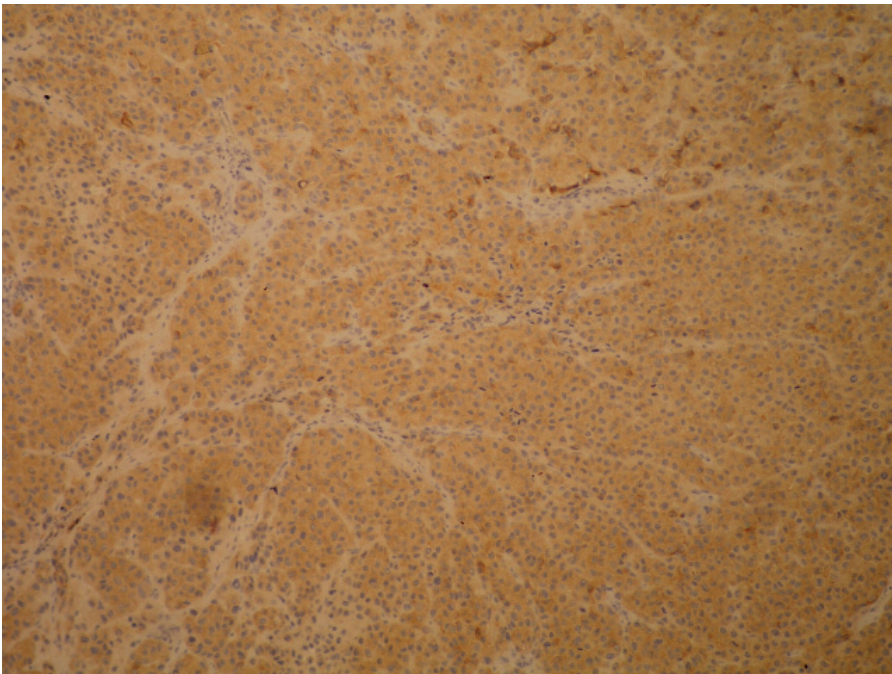


Figure 1. Metastatic melanoma positive with BRAF V600E (dot-like cytoplasmic positivity X200).

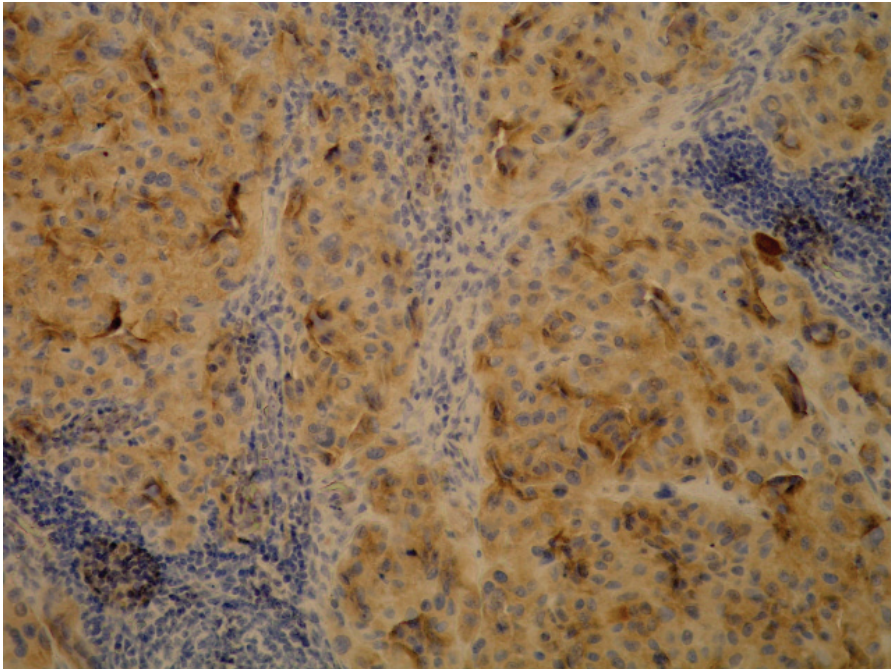


Figure 2. Metastatic melanoma in lymph node positive with BRAF V600E (dot-like cytoplasmic positivity X400).

Current molecular information indicates that melanoma should be viewed as a heterogeneous group of disorders with molecularly distinct defects in important cellular processes that include cell cycle regulation, cell signalling, cell adhesion, cell differentiation and cell death. The heterogeneity of these molecular signatures has two important implications: first, it accentuates the need for individualisation of melanoma diagnosis, prognosis and treatment; and second, it provides an array of potential biomarkers and novel putative drug targets to attain this individualisation. Careful dissection of melanoma into more homogeneous subgroups may be essential for identification of treatment benefits in specific subcategories of patient. At present, only LDH has been included in the AJCC staging system, no identified potential biomarker has undergone a large, rigorous, prospective trial with multivariate analysis that would allow it to be fully validated and developed for clinical practice. As such, there still remains an acute need for such markers in melanoma [25].

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Sažetak

Prognostički biomarkeri melanoma kože

Biomarkeri su faktori vezani za tumor ili domaćina koji koreliraju s biološkim ponašanjem tumora ili prognozom bolesnika. Moderne analitičke tehnike su dosada otkrile brojne moguće biomarkere, ali njihova važnost u razvoju i progresiji melanoma kože kao i kliničkom ishodu bolesti i odabiru najbolje terapije tek se treba utvrditi. U ovom preglednom članku navedeni su najčešće korišteni biomarkeri melanoma kože.

Ključne riječi: biomarkeri; melanom kože; prognoza.

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