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Biomarkers in melanoma

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Biomarkers are tumour- or host-related factors that correlate with tumour biological behaviour and patient prognosis. High-throughput analytical techniques—DNA and RNA microarrays—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. The review discusses a possible molecular basis for predictive tissue biomarkers such as melanoma thickness, ulceration and mitotic activity, and provides a list of promising new biomarkers identified from tissue microarrays that needs confirmation by independent, prospectively collected clinical data sets. In addition, common predictive serum biomarkers—lactate dehydrogenase, S100B and melanoma-inhibiting activity—as well as selected investigational serum biomarkers such as TA90IC and YKL-40 are also reviewed. A more accurate, therapeutically predictive classification of human melanomas and selection of patient populations that would profit from therapeutic interventions are among the major challenges expected to be addressed in the future.

Key words: biomarkers, melanoma, LDH, MIA, S100

introduction

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. For example, current prognostic biomarkers based on the conventional American Joint Committee on Cancer (AJCC) staging system (TNM) are Breslow tumour thickness, presence of ulceration and extent of nodal involvement for primary cutaneous melanoma, as well as serum lactate dehydrogenase (LDH) and site of metastases for distant metastatic disease.

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. In melanoma, sentinel node status might be the most relevant information for selection of adjuvant therapy. In some instances, biomarkers can predict the effect of an intervention, most commonly a systemic treatment. In other situations, unfortunately rare in oncology, the biomarker serves as a reliable indicator of the treatment response. Tests for hormone receptors (estrogens and progesterone) are some of the most long-standing predictive biomarker assays for treatment selection in breast and prostate cancers; recently, the FDA approved assays for HER2, epidermal growth factor receptor and KIT [1]. In addition, many new targeted agents such as imatinib and cetuximab are effective only if their respective molecular markers are available for pharmaceutical intervention.

In melanoma, prognostic biomarkers are needed that would help to refine the risk of progression and assess the outcome [2]. Recent developments have uncovered complex patterns of distinct molecular aberrations underlying the oncogenesis of melanoma [3]. 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 [4]. 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.

prognostic tissue biomarkers

The risk assessment of melanoma is based on AJCC melanoma staging and is described in a separate article in this supplement. It takes into account clinical variables, as well as tissue and

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serum biomarkers. Recent advances demonstrated some progress in better understanding the biological significance of tissue biomarkers.

biological significance of thickness measurement

The biological significance of melanoma thickness is still not clear. In his original report in 1970, Alexander Breslow considered both thickness and the cross-sectional tumour area as equal prognostic variables reflecting the tumour burden. It was a misleading view, since it later became clear that the tumour burden of primary melanoma is not associated with prognosis (for instance, large melanomas are not associated with a poorer outcome than small melanomas with the same thickness). In a large expression study of human primary melanomas, it was found that 23 of 24 genes involved in DNA repair have increased expression correlated with thickness, as did all the examined genes associated with cell cycle (8 genes), protein folding (10 genes), chromatin remodelling (10 genes) and heat shock protein activity (11 genes). In contrast, decreased expression with increasing thickness of primary melanoma was observed for all examined genes involved in serine-type endopeptidase inhibitor activity (15 genes), cell adhesion (15 genes), cell–cell signalling (8 genes) and transcription factor activity (33 of the 36 genes examined) [5].

biological significance of melanoma ulceration

The biological significance of melanoma ulceration is almost completely unknown. The adverse prognostic value of ulceration in melanoma has two possible explanations. It may be a consequence of an intrinsic biological attribute of the tumour that favours its dissemination. Alternatively, ulceration may directly favour dissemination of the tumour, for instance by modifying the local environment. Among the intrinsic properties of melanoma that may favour both ulceration and dissemination are proliferative activity of the tumour and overexpression of c-*myc* [6–8]. Proliferation of the tumour may contact-erode epidermis and favour expansion of the tumour burden. However, recent studies have demonstrated that mitotic activity and tumour ulceration were independently associated with prognosis in localised melanomas [9]. Therefore, it is unlikely that melanoma ulceration is only an indicator of tumour proliferation. Studies of the interactions between melanocytes and keratinocytes reinforce the hypothesis that ulceration directly influences the local environment in a way that may favour melanoma progression [10, 11]. These studies indicate that ulceration may provide melanoma cells with a very effective way to interrupt the keratinocyte-mediated control that prevents melanocyte transformation.

In a recent study of dendritic cell (DC) maturation in the sentinel lymph nodes (SLNs) draining melanoma, it was found that the maximum mature DC density in the SLNs correlated significantly and inversely with ulceration of the primary melanoma ($P = 0.0005$) [12]. It is noteworthy to put this finding parallel to a more pronounced impact of pegylated interferon-α2b (PEG-IFN) on recurrence-free survival in patients with ulcerated melanoma as compared with patients with non-ulcerated melanoma [13]. Therefore, it can be hypothesised that melanoma ulceration is an indicator of decreased production of endogenous IFN-γ that is somewhat palliated by exogenous IFN. This hypothesis will be studied in an EORTC trial comparing PEG-IFN with observation in patients with ulcerated melanoma and/or low lymph node burden.

biological significance of mitotic activity

Genes identified in a validated and reproducible signature prognosticating metastases or death are mainly associated with DNA replication or DNA repair. In DNA replication, genes of two pathways are over-represented: replication origins firing (ROF) genes and the separation of sister-chromatids by securin [5, 14]. Melanomas with poor prognoses are characterised by a global overexpression of ROF-related genes. MCM4 and MCM6 expression is strongly correlated with metastasis-free survival and overall survival [5]. This prognostic value is maintained when age, sex, location of the primary tumour, thickness and ulceration are introduced into the multivariate model. The whole ROF system is locked by geminin, which complexes with CDT1 and CDC6. When CDT1 and CDC6 are released, they can recruit MCMs at the replication origins. When this interaction is altered, for instance when BRCA1-IRIS relieves the geminin–CDC6 interaction, the helicase cascade becomes overactive and leads to replication increase. Securin is encoded by the *hPTTG* gene, which is among the top genes of prognostic signature. Securin has three known activities: it blocks the sister-chromatids separation in stabilising separase, it stimulates angiogenesis and it decreases *p53* transcription [15]. Securin acts as an oncogene, and its expression is observed by immunohistochemical staining in the vertical growth phase but not in the radial growth phase of melanoma [16].

new prognostic tissue biomarkers

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 [17–21]. The initial studies used small arrays and resulted in conflicting results probably due to an insufficient number of replicates and inadequate statistical stringency. Later larger studies finally identified lists of genes that undergo significant and reproducible up or down regulation in melanoma cells [22]. However, although *in vitro* experiments using cell lines are powerful techniques, the results that emerge from these studies should be viewed cautiously. All markers need confirmation in independent data sets and verification in clinically relevant settings. Even data from clinical studies cannot be accepted before they are reproduced. Table 1 summarises a selection of attractive candidate biomarkers from tissue microarrays that need confirmation from independent data sets and/or prospectively collected data sets preferentially from clinical trials.

Table 1. Potential cutaneous melanoma biomarkers detected by immunohistochemical analysis of tissue microarrays

Biomarker	Observation	Ref.
Hsp90	Increased expression in melanomas compared with nevi and in metastatic compared with primary tumours. Correlation with tumour thickness and higher Clark level. No association seen between high expression and survival in the subsets of patients with primary or metastatic tumours.	[23]
RGS1	Correlation with increased tumour thickness, mitotic rate and vascular involvement; reduced RFS and DSS.	[24]
Osteopontin	Correlation with increased tumour thickness, higher Clark level, mitotic index; reduced RFS and DSS; predictive of SLN metastasis and SLN burden.	[25]
HER3	Correlation with increased cell proliferation, tumour progression; reduced survival.	[26]
ING4	Reduced levels associated with melanoma thickness, ulceration and poor DSS and OS.	[27]
ING3	Reduced nuclear expression associated with poor DSS; an independent prognostic factor to predict patient outcome.	[28]
NCOA3	Increased levels predictive of SLN metastasis and associated with poor RFS and DSS.	[29]
MCM4	Increased levels associated with poor DFS and OS.	[5]
MCM6	Increased levels associated with poor DFS and OS.	[5]

DFS, disease-free survival; DSS, disease-specific survival; IHC, immunohistochemistry; OS, overall survival; RFS, relapse-free survival; SLN, sentinel lymph node.

prognostic serum biomarkers

Lactate dehydrogenase. As early as in 1954, increased levels of LDH were detected in serum of melanoma patients [30]; ever since, the value of LDH as a tumour marker for malignant melanoma has been discussed. 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 [31]. Patients with abnormal LDH levels had a significantly decreased survival [32]. 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 [33] and identified, by multiple logistic regression analysis, as the only statistically significant marker for disease progression [33]. LDH has been included in the AJCC staging system, and patients with distant metastases and elevated LDH are considered stage IV M1c [34].

S100B. The best-studied melanoma biomarker is currently S100B. First described in 1980 in cultured melanoma cells [35], S100B has quickly become a well-established and widely used immunohistochemical marker of pigmented skin lesions [36–38]. In 1995, a first study was published evaluating the clinical significance of serum S100B in melanoma [39]. The study assessed 126 patients and found S100B-positive serum from 1.3%, 8.7% and 73.9% of patients with stage I/II, III and IV disease, respectively. Preliminary results of serial measurements of serum S100B demonstrated that its rise was associated with the progression of the disease, and a decline indicated response to treatment.

In a subsequent study of 643 melanoma patients, overall survival was strongly associated with serum concentrations of S100B [40]. The observed death ratio was markedly increased with increasing concentrations of S100B ($P < 0.001$). A five-fold increase in relative hazard was indicated by a value of S100B of >0.6 μg/l ($P < 0.001$), and when this cut-off level was used, S100B had additional prognostic value independent of clinical stage ($P < 0.001$). 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 [41–43]. This was validated in a study of 1339 serum samples from 412 melanoma patients [44]. Statistically significant differences for stage I/II compared with III, I/II compared with IV and III compared with IV ($P < 0.001$) were observed. The estimated overall survival time for patients with S100B values of <0.2 μg/l was significantly longer ($P < 0.001$) compared with that for patients with elevated S100B levels (≥0.2 μg/l); this result was independent of disease stage (I–IV). Similarly, in another study of 214 melanoma patients, rising concentrations of serum S100B preceded the conventional detection of melanoma progression by 5–23 weeks [45]. Analysis of S100B in 103 patients from phase II adjuvant IFN trial E2696 showed that a concentration of ≥0.08 μg/l is an independent prognostic marker for adverse relapse-free survival at baseline (HR = 1.96; $P = 0.0273$) and at 1 year of follow-up (HR = 4.3; $P < 0.001$) [46]. Preliminary multivariate data analysis adjusting for significant prognosis factors (ulceration and lymph node status) and treatment from 880 patients in phase III trial E1694 indicates that lower S100B concentrations (<0.15 μg/l) at baseline and during follow-up are associated with significantly better overall survival (J. M. Kirkwood, personal communication).

Swiss and German guidelines recommend determination of S100B in serum of patients with Breslow >1 mm lesions every 3–6 months [47–49]. 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.

Melanoma-inhibiting activity. Melanoma-inhibiting activity (MIA) was identified in the early 1990s as a soluble 11 kDa protein with growth-inhibiting activities secreted from malignant melanoma cells [50–52]. 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 [53]. The first study published on MIA reported enhanced MIA serum concentrations in 13% and 23%

of patients with stage I and II disease, respectively, and in 100% of patients with stage III or IV disease. By analysing the serum of 350 patients with a history of stage I or II melanoma during each follow-up, development of MIA positivity was detected in 32 patients [54]. By the time of the serum analysis, 15 of the MIA-positive patients had developed metastases, and one was diagnosed with metastatic disease 6 months later. In contrast, none of the patients with normal MIA serum concentrations developed metastases during the follow-up period of 6–12 months. A large German study of >830 blood samples from 326 melanoma patients with 9.8 ng/ml cut-off of MIA detected increased MIA concentrations in 5.6% of patients at stage I/II but in 60% and 89.5% of patients at stage III and IV, respectively [55]. Patients at stage III/IV with MIA concentrations below the cut-off had been previously operated on for metastatic disease or received irradiation or chemotherapy before the analysis. None of these patients developed further metastasis during follow-up, similar to patients at stage I or II without increased MIA concentrations. A significant rise in MIA concentration was associated with metastasis detected at the time of analysis or after 2–6 months. However, in a subsequent study that evaluated the combination of S100B, MIA and LDH markers in 373 melanoma patients [284 patients with *in situ*, stage I and II melanoma, and 89 patients with stage III or IV, International Union Against Cancer (UICC) staging], MIA had lower sensitivity compared with S100B, and lower specificity compared with both S100B and LDH [56]. The investigators concluded that S100B is a more reliable tumour marker than MIA, albumin or LDH in peripheral blood of patients with newly developed melanoma metastases. An additional study assessed sensitivity and specificity of S100B and MIA in 96 patients with advanced melanoma and no evidence of disease (NED), and in 86 patients with metastatic melanoma, and found abnormal levels of S100B and MIA in 1.1% and 3.2% of NED patients, respectively, and in 59.3% and 54.6% of patients with active melanoma ($P < 0.001$), respectively [57]. Using both tumour markers simultaneously, the sensitivity increased to 69.8% with specificity 96.8%. In a most recent prospective study, four tumour markers—L-DOPA/tyrosine ratio, S100B, MIA, LDH and their various combinations—were evaluated in 170 stage I–IV melanoma patients [58]. All markers except LDH were elevated in stage IV compared with other stages. S100B and MIA highly correlated, especially in stage IV ($P < 0.001$). The combination of L-DOPA/tyrosine ratio with S100B displayed the highest sensitivity/specificity (73%/70%) to confirm stage III/IV or stage IV alone (69%/75%). However, only the L-DOPA/tyrosine ratio significantly increased ($P = 0.001$) during progression from stage I to III to higher stages. In contrast, S100B, MIA and LDH, but not the L-DOPA/tyrosine ratio, responded to progression towards death in stage IV. All markers exhibited a prognostic value in deceased patients; S100B and MIA were the best predictors for survival time by Cox proportional-hazard regression. Some of these discrepancies could be attributed to heterogeneity of the patient groups, differences in test kits and differences in diagnostic procedures employed for patient follow-up such as lymph node ultrasound, CT and/or PET CT.

individual investigational serum biomarkers

Tumour-associated antigen 90 immune complex. Tumour-associated antigen 90 immune complex (TA90IC) was compared with MIA protein and S100B protein in stage III melanoma patients undergoing adjuvant vaccine immunotherapy [10]. The serum of 75 patients representing three prognostic cohorts was analysed for the tumour markers before initiation of immunotherapy and at six follow-up time points. At least one marker became elevated before 41 of 51 (80%) recurrences. TA90IC was the earliest elevated marker in 29 (57%) recurrences. 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 elevation of MIA in patients who developed recurrence. Additional studies in populations not receiving vaccines will further clarify the clinical utility of these assays.

YKL-40. YKL-40 is a heparin- and chitin-binding lectin secreted by activated neutrophils and macrophages during the late stages of differentiation, and 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.

In one study, YKL-40 was measured in serial serum samples from 110 patients with metastatic melanoma obtained immediately before and during treatment, and from 245 healthy subjects [59]. Pre-treatment serum levels of YKL-40 was increased in 45% of the patients and correlated with the site of metastases ($P = 0.03$) and poor performance status ($P = 0.002$). Multivariate Cox 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 combination of YKL-40 and LDH had a stronger prognostic impact than the AJCC stage IV classification. YKL-40 was also evaluated by the same investigators in serial serum samples from 234 patients with AJCC stage I and II melanoma collected at the time of diagnosis and during routine median follow-up of 66 months [60]. Serum YKL-40 was an independent prognostic factor of relapse-free survival ($P = 0.03$) and overall survival ($P = 0.002$). The serum level of YKL-40 (dichotomised as normal or elevated) at the time of diagnosis was also an independent prognostic factor for overall survival ($P = 0.001$). These findings should be validated in an independent study. The use of serum YKL-40 has not received Food and Drug Administration approval for use as a biomarker for cancer or any other disease [61].

complex-signature serum biomarkers

Proteomic and bioinformatic approaches were shown to be able to dissect the serum proteome and identify signature patient

biomarkers indicative of cancers of different origin. A study of serum samples from patients with stage I or IV melanoma analysed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF) utilising protein chip technology and artificial neural networks (ANNs) correctly identified the disease stage in 84 of 96 (88%) samples [62]. Forty-four of 55 (80%) stage III serum samples were correctly assigned as progressors or non-progressors using random sample cross-validation statistical methodologies. Twenty-three of 28 (82%) stage III progressors were correctly identified by MALDI-ToF combined with ANN, whereas only 6 of 28 could be detected by using the S100B marker. These findings need to be validated.

response-predictive biomarkers

Very few data are available concerning the predictive value of biomarkers in response to melanoma therapy. Data from phase III studies have pointed out the potential correlation between melanoma ulceration and response to PEG-IFN [13], serum LDH and response to oblimersen [63], auto-antibodies and response to high-dose IFN [64].

A large number of clinical trials are evaluating several novel therapeutic targets such as activated pathways. However, in many cases, it is still unclear whether the presence of genetic alteration predicts the final outcome of a therapeutic intervention. In mucosal melanomas, an activating mutation of c-*kit* seems to predict sensitivity to the kinase inhibitor imatinib [65]. A recent report suggested that the most common B-Raf mutation, V600E, is necessary for a remission in treatment using the MEK kinase inhibitor AZD6244 [66].

conclusion

After several years of investigation using high-throughput technologies such as DNA and RNA microarrays that provide thousands of data points within one experiment, it still needs to be established whether these techniques are useful to identify new tumour markers for melanoma progression, clinical outcome and the selection of optimal treatment strategies [67]. However, these techniques have already enhanced the discovery of new pathways associated with melanoma progression.

Due to its high prognostic significance, coupled with its easy, widely distributed detection methodology, serum LDH is the only serum molecular marker that has been included in the current melanoma AJCC staging system. Moreover, it serves as a stratification parameter in clinical trials.

Careful research in the field of biomarkers in melanoma is essential to achieve a proper therapeutically predictive classification of human melanomas. Since the overall treatment results are frustrating, major efforts are necessary to identify patient populations that will profit from therapeutic interventions.

conflict of interest

The authors declare no conflict of interest.

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