REVIEW ARTICLE

Serologic and immunohistochemical prognostic biomarkers of cutaneous malignancies

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Abstract  Biomarkers are important tools in clinical diagnosis and prognostic classification of various cutaneous malignancies. Besides clinical and histopathological aspects (e.g. anatomic site and type of the primary tumour, tumour size and invasion depth, ulceration, vascular invasion), an increasing variety of molecular markers have been identified, providing the possibility of a more detailed diagnostic and prognostic subgrouping of tumour entities, up to even changing existing classification systems. Recently published gene expression or proteomic profiling data relate to new marker molecules involved in skin cancer pathogenesis, which may, after validation by suitable studies, represent future prognostic or predictive biomarkers in cutaneous malignancies. We, here, give an overview on currently known serologic and newer immunohistochemical biomarker molecules in the most common cutaneous malignancies, malignant melanoma, squamous cell carcinoma and cutaneous lymphoma, particularly emphasizing their prognostic and predictive significance.

Keywords  Skin cancer · Biomarker · Prognosis · Serum · Immunohistochemistry

Introduction

Biomarkers play an important role in the diagnosis and prognostic classification of various cancer entities and can moreover be useful in monitoring the patient’s clinical course of disease and response to therapy. In general, biomarkers are proteins, less often carbohydrates or lipids, and their expression profiles are associated with malignant disease. In the majority of cases, the marker molecules are expressed by the tumour cells themselves or by cells of the tumour microenvironment. Thus, most biomarkers can primarily be found in malignant tissues, but after active secretion or passive release at tumour cell destruction also become detectable in body fluids like blood, lymph or urine.

Besides morphological and histopathological biomarkers (anatomic site and type of the primary tumour, tumour size and invasion depth, ulceration, vascular invasion), an increasing variety of molecular markers have been identified, providing the possibility of a more detailed diagnostic and prognostic subgrouping of tumour entities, up to even changing existing classification systems. Recently published gene expression, as well as proteomic profiling data, indicates new candidate molecules involved in skin cancer pathogenesis, which may after further validation represent new markers superior to existing ones. This ongoing process of biomarker identification and validation would result in a rapidly changing molecular view and classification of skin cancers.
Malignant melanoma

Serologic markers of malignant melanoma

Despite numerous therapeutic options, the prognosis of malignant melanoma, once metastasized, is still poor. Thus, the search for reliable methods to detect metastases as early as possible and to identify patients with high risk of disease progression is of major importance. The serological parameters most widely used for the early detection of a tumour relapse or metastasis in the follow-up of melanoma patients are the melanocyte lineage/differentiation antigens S100-beta and melanoma inhibitory activity (MIA) (see Table 1 and Fig. 1). Both proteins are with high, but not exclusive, specificity expressed by melanoma cells and thus correlate with the patient’s tumour load.

The S100 protein is a 21-kDa thermo-labile acidic dimeric protein, which was originally isolated from the central nervous system (CNS) [49]. It consists of two subunits, alpha and beta, in the combinations alpha/alpha, alpha/beta and beta/beta. S100 affects the assembly and disassembly of microtubules and also interacts in a calcium-dependent manner with the p53 tumour suppressor gene. The beta subunit is expressed in cells of the central nervous system as well as in cells of the melanocytic lineage. Therefore, S100-beta measured in the cerebrospinal fluid was known as a marker of CNS damage [59], years before S100-beta was shown to be a useful serum marker in melanoma [30]. MIA was originally detected in melanoma cell culture supernatant [9] and was shown to exert an important role in cell–matrix interaction and metastasis [8].

Serum S100-beta has been shown to be superior compared to MIA, as an early indicator of tumour progression, relapse or metastasis [18, 43], and its distribution as a serum biomarker of melanoma, therefore, is the broadest [36]. Both markers have been shown to be useful prognostic markers in melanoma patients with distant metastases (stage IV, classification system of the American Joint Committee on Cancer, AJCC, see

Table 1 Serologic markers of malignant melanoma

| Serologic marker                                      | Selected literature |
|-------------------------------------------------------|---------------------|
| Melanocyte lineage/differentiation antigens            |                     |
| S100-beta                                             | [30, 71, 33, 18, 43, 28] |
| MIA (melanoma inhibitory activity)                    | [9, 8, 10, 74, 28] |
| Tyrosinase                                            | [2]                 |
| 5-S-CysteinylDopa                                      | [91, 34]            |
| L-Dopa/L-tyrosine                                      | [75]                |
| Proangiogenic factors                                  |                     |
| VEGF (vascular endothelial growth factor)             | [83, 56, 12]        |
| BFGF (basic fibroblast growth factor)                 | [83, 12]            |
| IL-8 (Interleukin-8)                                   | [68, 83, 12]        |
| Molecules involved in cell adhesion and motility       |                     |
| sICAM-1 (soluble intracellular adhesion molecule 1)   | [34, 87, 94]        |
| sVCAM (soluble vascular cell adhesion molecule 1)     | [26, 87]            |
| Matrix metalloproteinases (MMP)-1 and 9               | [63, 53]            |
| Cytokines and cytokine receptors                       |                     |
| IL-6 (Interleukin-6)                                   | [50, 73]            |
| IL-10 (Interleukin-10)                                | [21, 51]            |
| sIL-2R (soluble interleukin-2-receptor)               | [11, 58]            |
| HLA molecules                                         |                     |
| sHLA-DR (soluble HLA-DR)                              | [62]                |
| sHLA-class-I (soluble HLA-class I)                    | [89]                |
| Others                                                |                     |
| LDH (lactate dehydrogenase)                           | [72, 18, 6]         |
| CRP (C-reactive protein)                              | [19]                |
| Albumin                                               | [72]                |
| TuM2-PK (Tumour pyruvate kinase type M2)              | [81]                |
| sFas/CD95                                              | [84]                |
| YKL-40                                                | [69, 70]            |
| CYT-MAA (cytoplasmic melanoma-associated antigen)     | [85]                |
| HMW-MAA (high-molecular-weight melanoma-associated antigen) | [85]            |
A variety of other molecules of peripheral blood have been described as markers of tumour load and disease progression in melanoma. These biomarkers are derived from different fields like melanocytic differentiation (e.g. tyrosinase; see Fig. 1), tumour angiogenesis (e.g. VEGF, bFGF, IL-8), cell adhesion and motility (e.g. ICAM-1, MMPs), cytokines and their receptors (e.g. IL-6, IL-10), antigen presentation (e.g. HLA molecules), tumour cell metabolism (e.g. TuM2-PK), apoptosis (e.g. Fas/CD95) and many others (see Table 1). However, neither one of these markers could be confirmed to be superior to S100-beta or LDH in reflecting the prognosis of patients in advanced disease stages, nor could any marker be shown to be of strong prognostic relevance in early stage tumour-free patients.

The serum proteomic profiling is an innovative approach to identify new, potentially better serological biomarkers in melanoma. This methodology offers the possibility of screening the whole serum proteome for markers, which match different criteria like prognostic significance, prediction of therapy response, etc. Using this technology, marker proteins from thematic fields, different from the above-mentioned ones, might be found and thereafter validated for their clinical use. The first promising results have been obtained and are currently tested in large sets of serum samples [46].

Immunohistochemical markers of malignant melanoma

Cutaneous malignant melanoma regularly develops from the radial to the vertical growth phase and thereafter to metastatic disease. The variability of this clinical course is only partially explained by morphological and histopathological parameters like primary tumour localization, patient gender and age, mitotic rate, tumour thickness and ulceration. There is a need to identify molecular variables, which help to assign patients to specific risk groups. The number of modalities for diagnosing and subclassifying malignant melanomas is rapidly increasing and includes immunohistochemistry of tissue sections and microarrays, gene expression profiling, comparative genomic hybridization and mutational analysis. These methodologies promise to improve our prognostic classification systems, as well as our diagnostic and therapeutic potential.

For diagnostic purposes, a small panel of melanocytic lineage markers, e.g. S100, MART-1/MelanA and gp100/HMB45, is sufficient to distinguish melanoma from non-melanocytic cancers. For the differentiation between benign and malignant melanocytic lesions, a
A review of immunohistochemical markers is given in ref. [44]. The present review focuses on newer markers with potential prognostic impact for the disease. For this purpose, the situation is more complex. The transformation from benign melanocytes to metastatic melanoma is the result of a compilation of genetic alterations crucial to cell division, differentiation, anti-apoptosis, invasion, angiogenesis and sustenance in a microenvironment distant from the point of origin of the cell. Several marker molecules involved in these genetic alterations have been identified, and their expression in primary melanoma has been studied and correlated with the prognosis. Table 2 gives a current overview on already identified biomarkers, whose abnormal expression is associated with the patient’s prognosis. It may be expected that the most detailed prognostic classification will result not from one, but rather from a panel of multiple biomarkers from this list.

In a recent retrospective study, frozen tissue samples from primary melanomas with long-term clinical

| Table 2 Immunohistochemical markers of malignant melanoma associated with prognosis | Association with poor prognosis | Selected literature |
|---------------------------------|-------------------------------|-------------------|
| Melanocyte lineage/Differentiation antigens | gp100/HMB45 | Increased expression | [52] |
| Tumour suppressors/oncogenes/signal transducers | p16 INK4a | Decreased expression | [47, 3] |
|   | PTEN | Decreased expression | [48] |
|   | pRb (retinoblastoma protein) | Inactivation due to protein phosphorylation | [65] |
| EGFR (epidermal growth factor receptor) | Increased expression | [80] |
| p-Akt (activated serine-threonine protein kinase B) | Increased expression | [17] |
| c-Kit | Expression | [35, 82] |
| c-myc | Increased expression | [42] |
| AP-2 (activator protein-2alpha) transcription factor | Loss of nuclear AP-2 expression | [7] |
| HDM2 (human homologue of murine mdm2) | Increased expression | [61] |
| bcl-6 | Expression | [3] |
| Cell cycle associated proteins | Ki67 (detected by Mib1) | Increased expression | [29, 3, 57] |
|   | Cyclin A, B, D, E | Increased expression | [24, 25] |
|   | p21cip1 | Decreased expression | [3] |
| Geminin | Increased expression | [92] |
| PCNA (proliferating cell nuclear antigen) | Increased expression | [92] |
| Regulators of apoptosis | bcl-2 | Increased expression | [78] |
|   | bax | Decreased expression | [23] |
|   | Bak | Decreased expression | [23] |
| APAF-1 (Apoptotic protease activating factor-1) | Decreased expression | [27] |
| Surviving | Increased expression | [78] |
| Molecules involved in angiogenesis | LYVE-1 (lymphatic vascular endothelial hyaluronan receptor-1) | Increased expression | [16] |
| PTN (pleiotrophin) | Increased expression | [93] |
| Molecules involved in cell adhesion and motility | P-Cadherin | Strong cytoplasmic expression | [5] |
|   | E-Cadherin | Decreased expression | [4] |
|   | Beta-catenin | Loss of nuclear staining | [5] |
|   | Integrins beta1 and beta3 | Increased expression | [66] |
|   | MMPs (matrix metalloproteinases) | Increased expression | [63] |
| Dysadherin | Increased expression | [54] |
| CEACAM1 (carcinoembryonic-antigen-related cell-adhesion molecule 1) | Increased expression | [79] |
| Osteonectin (also termed BM40 or SPARC (secreted protein, acidic and rich in cysteine)) | Increased expression | [45] |
| Others | TA (telomerase activity) | Increased expression | [13] |
|   | Melastatin | Decreased expression | [22] |
|   | ALCAM/CD166 | Increased expression | [77] |
|   | (Activated leukocyte cell adhesion molecule) | Increased expression | [67] |
|   | CXCR4 receptor | Increased expression | [67] |
|   | Metallothionein | Increased expression | [88] |
Squamous cell carcinoma of the skin

While primary cutaneous squamous cell carcinomas (SCC) are usually easily treatable, they have the potential to recur locally and even metastasize, then leading to a significant morbidity and mortality. Therefore, it is important to identify those tumours that are more aggressive and require closer follow-up and additional treatments, such as lymph adenectomy or radiation therapy. Established prognostic factors include anatomic site of primary, tumour size, depth of invasion, rapid growth, grade of differentiation, perineural invasion, history of previous treatment, host immunosuppression, and etiologic factors such as burn scars, radiation and chronic ulceration. The histological subtypes of SCC have also been considered as a factor in determining the prognosis [60].

Only few molecular markers are known to be associated with progression or prognosis of cutaneous SCC. In the following, we give an overview on some recently described proteins whose abnormal expression contributes to a malignant phenotype in this cancer entity. STAT3, a member of the signal transducer and activator of transcription (STAT) family of transcription factors is a known regulator of cell motility. The expression of phosphorylated STAT3 (p-STAT3) was described to be stronger in poorly differentiated than in well-differentiated SCCs. Moreover, the percentage of tumour cells expressing p-STAT3 correlated with the depth of tumour invasion and with metastasis formation [76].

E-Cadherin is a Ca(2+)-dependent, intercellular adhesion molecule that is specifically expressed in epithelial cells and tissues and functions by maintaining intercellular connections. In some types of carcinomas, E-cadherin expression of tumour cells is decreased in association with metastasis. In cutaneous SCC, a decreased expression of E-cadherin in the primary lesion is correlated with the development of regional lymph node metastasis [41]. Additionally, a decreased expression is more often associated with well-differentiated tumour cells expressing p-STAT3 correlated with the depth of tumour invasion and with metastasis formation [76].

Another marker to distinguish between well-differentiated and poorly differentiated SCC is Ets-1 [37]. Ets-1 is a transcription factor regulating the expression of various genes including matrix metalloproteinases (MMPs). Therefore, Ets-1 might be important in the pathogenesis of invasive SCC. MMP-12 was found to be expressed by tumour cells in squamous cell carcinoma of the vulva. Its expression correlates with invasiveness, while that of macrophages predict a better clinical outcome [38]. A cell surface marker, CD44, is a glycoprotein widely distributed in the extracellular matrix. CD44 isoforms, which arise from alternative mRNA splicing, were found to be implicated in the formation of tumour metastasis. In a study by Rodriguez-Rodriguez et al., it was shown that lymph node metastases of cutaneous SCC of the vulva were immunoreactive for CD44–9v [64]. Also, CD44–10v expression was present in 78% of tumours compared to only 56% of normal epithelium. CD44–10v membrane expression, but not cytoplasmic expression, correlates with disease recurrence [64]. In ocular squamous cell carcinomas, over-expression of CD44–6v is correlated with tumour progression and metastasis [55].

Cutaneous T-cell lymphomas

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of cutaneous non-Hodgkin’s lymphomas.
In this cutaneous malignancy, the tumour cells home to and persist in the skin, producing a broad spectrum of clinical entities. The prognosis of CTCL depends on histologic and molecular aspects. The new WHO/EORTC classification for cutaneous lymphomas comprises mature T-cell and natural killer (NK)-cell lymphomas, mature B-cell lymphomas and immature haematopoietic malignancies. Marker proteins for the diagnosis of CTCL include, for example, CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD16/56, CD19, CD25, CD45, CD45RA and CD45RO [90].

The probability of survival in CTCL can be accurately predicted by a formula based on the clinical CTCL-Severity-Index (CTCL-SI) [20], which evaluates the involvement of the skin, lymph nodes, blood and visceral organs [40]. Besides clinical and morphological parameters, several molecules have been investigated in CTCL that are involved in general cellular signalling processes, regulation of cellular proliferation and apoptosis, like Jun, Myc, c-myc, p53, STATs, bcl-2, Fas/CD95 and SOCS-3, or contribute to the putative immunopathology of the disease such as expression of inhibitory MHC receptors (ILT2/CD85j), NK receptors (p140/KIR3DL2) and dendritic cell defects (CD40). The abnormal expression of these molecules could be relevant for the prognosis of CTCL, as it has been shown for other tumour entities [39].

With regard to serological biomarkers in CTCL, it has been shown that the serum concentrations of the soluble alpha-chain of the interleukin-2 receptor (sIL-2R) as well as lactate dehydrogenase (LDH) strongly correlate with lymph node size, but only sIL-2R significantly correlates with the severity of skin manifestations in erythrodermic patients [86]. Moreover, sIL-2R was demonstrated to be produced at a relatively low rate by tissue-based lymphoma cells, whereas large-cell transformation in CTCL results in a marked increase in the sIL-2R production in some patients [86]. In addition to sIL-2R, neopterin and beta2-microglobulin have been shown to be significantly elevated in the serum from patients with Sezary syndrome. Thus, sIL-2R seems to be the most sensitive marker, which is typically increased in Sezary syndrome. Concerning the outcome of the disease, in terms of disease progression versus non-progression, only neopterin showed a significant prognostic value in non-leukemic CTCL patients [31].

Conclusion and future directions

Taken together, molecular markers provide additional and much more detailed information for the prognostic classification of cutaneous malignancies. Currently, this is particularly true for malignant melanoma, but will certainly also affect other entities in due time. In addition to the serological and immunohistochemical biomarkers discussed here, genetic abnormalities have recently been recognized to influence the prognosis of cancer patients in higher extents than previously assumed. With regard to malignant melanoma, a new classification system was proposed combining genetic aberrations with histomorphological changes, resulting in new insights into the pathogenesis of this malignancy [14, 15]. It may be expected that the rapidly increasing knowledge of molecular mechanisms would lead to mainly biomarker-based, rather than morphology-based, classification systems that might facilitate an individualized, molecular-driven cancer therapy.

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