Serum markers of cutaneous melanoma

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1. ABSTACT

Malignant melanoma currently accounts for approximately 1%, of all cancer deaths. The incidence of cutaneous melanoma is rising worldwide. The treatment of early-stage melanoma consists primarily of surgical removal of the tumour. The overall 5-years survival rate for malignant melanoma is 81%. Recently, many efforts have been made to analyse the potential significance and the possible relationship of disease progression and circulating markers in malignant melanoma. Several serum biomarkers appear to hold significant potential both as prognostic indicators and as targets for future therapeutic agents. The application of these markers in clinical practice possibly holds the key to significant advances in melanoma. This review summarizes the principal characteristics of serum markers of melanoma. Serum lactate dehydrogenase (ldh), protein S-100β, melanoma-inhibiting activity (MIA) may correlate with melanoma progression. Tenascin-c, Hyaluronan, Laminin-1 and type VI Collagen are involved in melanoma development and extracellular matrix remodelling during melanoma progression.

2. INTRODUCTION

The incidence of cutaneous melanoma is increasing worldwide, but the median tumor thickness as the main prognostic factor concerning morbidity and mortality is decreasing significantly (1). In the past several decades, its incidence has increased more rapidly than that of any other cancer. In Europe, cutaneous melanoma represents 1-2% of all malignant tumors. In fact, the incidence of malignant melanoma varies from 3–5/100000/year in Mediterranean countries to 12–20/100 000 in Nordic countries (2). Incidence rates in the European countries are still lower respect other states, as Australia, New Zealand, Asian and the southern states of the United States (3-8), but showed threefold to fivefold increases during the last decades. The majority of the epidemiologic evidence is drawn from observations of these populations, and most of the public education efforts have addressed these populations since they are at higher risk of developing melanoma. The treatment of early-stage melanoma consist primarily of surgical removal of the tumour. The overall 5-year survival rate for malignant

melanoma is 81% but survival depends on the depth of invasion, the anatomic location, the presence of ulceration, the patient's age and sex, histological factors and clinical subtype (9-11). Besides morphological and histopathologic biomarkers (anatomic site and type of the primary tumor, tumor size and invasion depth, ulceration, vascular invasion, and mithotic index), an increasing variety of molecular markers have been identified that provide the possibility of a more detailed diagnostic and prognostic categorization. Early diagnosis is the key to improved patient survival but is frequently delayed because of the absence of appropriate markers (12). Use of molecular markers should therefore give additional information.

3. MARKERS

3.1. S100β

S100 proteins belong to the S100/calmodulin/parvalbumin/troponin C superfamily (13,14). The components of this family are characterized by calcium binding motifs that are responsible for their effects on cellular functions, including contraction, motility, growth, differentiation, cell cycle progression, transcription and secretion (15, 16). They are also involved in tumorigenesis and the evolution of metastasis (17, 18).

The serum level of S100 seems to be correlated with the extension of the tumor and, therefore, with the degree of malignancy. The connection between S100 proteins and the extension of the disease can be explained through the action of these molecules in the tumorigenesis. In fact, S100 inhibits calcium-dependent phosphporylation of p53 by protein kinase C *in vitro*. This can lead to suppression of regulation of apoptosis mediated by p53 resulting in uncontrolled tumor growth (19, 20). Elevated serum level of S100 proteins have been found in several type of tumors such as astrocytoma, glioblastoma, Schwannoma and ependymona (21) but the most significant values have been detected in malignant melanoma. In 1980 Gaynor *et al* reported that five of seven cell lines derived from human metastatic melanoma produced S 100 protein (22). Since then various studies supported this result (23-27). A lesser increment of S100 has been also identified in thyroid carcinoma, renal cell carcinoma and in inflammatory conditions or liver and renal diseases (28). In the multigene family of these 24 proteins, some of them, can be also expressed in non-small cell lung cancer, breast cancer, gastric cancer and lymphoma (15,16). The sensitivity of this marker is considerably influenced by the stage of the tumour. It has been shown a sensitivity of 15% in patients with malignant melanoma stage I or II but it has been also observed an increase of the sensitivity to 60-85% in stage IV malignant melanoma patients. S100 protein is the most significant prognostic markers in malignant melanoma. Acland *et al* (29) showed the uselessness of serum S 100 concentrations in predicting micrometastasis but various studies showed S100 is an independent prognostic marker in patients with regional lymph node metastasis and distant metastasis (30). Schoultz *et al* (31) and Karnell *et al* (32) showed that over the cut-off of 0.6µg/l the relative hazard of death increased 5-fold. They also demonstrated that serum S100 was prognostically superior to urine metabolities. Other studies reported the superiority of S100 in confront to other markers like NSE. Wibe *et al* in 1990 and in 1992 proposed NSE as a prognostic factor in metastatic melanoma but it was found a better sensitivity of S100 correlated with the clinical stage of the tumor (33,34).

3.2. Osteopontin (OPN)

Osteopontin (OPN), is an RGD-containing non-collagenous, sialic acid-rich phosphoglycoprotein. It is a member of the extracellular matrix (ECM) protein family (35,36). It has an Nterminal signal sequence and a highly acidic region consisting of nine consecutive aspartic acid residues (37). It binds with several integrins and CD44 variants in an RGD sequencedependent and -independent manner (38). It is produced by a number of cell types, involved in cell migration, survival and immunity. This protein is also involved in normal tissue remodeling processes such as bone resorption, angiogenesis, wound healing, and tissue injury as well as certain diseases such as tumorigenesis, restenosis, atherosclerosis and autoimmune diseases. After its binding to integrin receptors, OPN initiates signal transduction through the activation of kinases and transcription factors capable of regulating cell growth.(39,40) Several mechanisms could explain the potential role of OPN in cancer. In fact, OPN not only induces cell growth and cell survival, but it also regulates cell migration.(41-46) The former is due to the inhibition of apoptosis, the latter is related to the activaton of matrix metalloproteasis (MMPs), particularly MMP2 and MMP9. These are important enzymes capable of extracellular matrix degradation. Moreover, elevated levels of OPN have been correlated with the activation and up-regulation of key cell-cycle modulatory molecules such as Ras, protein kinase C PI3-K/Akt(47,48). OPN expression is upregulated in several cancers and is reported to associate with tumor progression and metastasis. Several studies have demonstrated the potential role of OPN as a prognostic marker of several cancer types including melanoma. High level of OPN expression has been found in a number of cancer types including breast (49), prostate (50,51), colon (52), head and neck (53), and hepatic cancers (54). Rangel *et al* have found that osteopontin expression was associated with increased tumor thickness, Clark level and mitotic index (55). Furthemore, Kaplan-Meier analysis demonstrated an association between osteopontin expression and reduced recurrence free survival. This study supports previous encouraging microarray data, confirming the need of further clinical investigations to better characterize the role of OPN as a biomarker of disease progression.

3.3. Melanoma inhibitory activity (MIA)

MIA is a soluble protein secreted by melanoma cells and malignant condrocytes (56). It has been isolated and characterized in the supernatant of melanoma cell-culture (57,58). Further studies have demonstrated its role in melanoma cells growth, progression and invasion (59,60). Of importance, MIA is highly expressed in several melanoma cell lines and in melanoma tissues. It was further demonstrated that MIA interferes with cell-to cell contact between melanoma cells and extracellular matrix, inducing melanoma cells migration and metastatization.

Clinically, the first report demonstrated that 100% of patients with stage III and IV had enhanced levels of MIA in the serum. A larger study has confirmed these data, in fact, the levels of MIA positively correlated with disease stage with elevated concentration found in 89.5% of patients in stage IV(61-63). Moreover, other investigations have reported a strong correlation between serum levels of MIA and metastasis (64,65). Of note, the expression of MIA was significantly higher in metastatic cells as compared with melanoma primary cells (66-68). Several studies on the association of MIA with other progression markers, such as S100B and LDH are still under investigation.

3.4. Lactate Dehydrogenase (LDH)

The enzyme Lactate dehydrogenase is found in the cells of almost body tissues. LDH is encoded by two genes, LDH-A (the M subunit-muscle type) and LDH-B (the H subunit- heart type), which give rise to two polypeptide chains that form five isoenzymes depending on the combination of the subunits H and M. The five LDH isoenzymes are LDH1 (in the hearth), LDH 2 (in the reticuloendhotelial system), LDH 3 (in the lungs), LDH 4 (in the kidneys) and LDH 5 (in the liver and striated muscle). The mayor role of LDH in normal cellular homeostasis is to enhance the catalysis of pyruvate to lactate to produce adenosine triphosphate (ATP). LDH-5 is the most efficient isotype in this catalysis (69). LDH can be elevated in various conditions such as myocardial infarction, hemolysis and hepatitis.

Since LDH is involved in the cellular production of energy , its overexpression is correlated to anaerobic metabolism within tumour cells and reduced cellular dependence of oxygen (70). Unlike normal cells that produce the majority of ATP from glucose through oxidative phosphorilation many cancer cells produce ATP converting glucose to lactate due to hypoxic state in which many cancer cell exist. LDH A (also known as LDH-5 in its tetrameric form) is upregulated in the hypoxic environment. Since LDH is not a secreted enzyme, its elevated concentration in patients with advanced melanoma is correlated to tumoural cells necrosis. Previous studies have shown that elevated levels of LDH in melanoma patients are associated with adverse prognosis (71-74). Patients with abnormal LDH levels have a decreased survival (75) and can show liver metastasis. LDH is a marker of the degree of malignancy in melanoma (76) but increasing LDH concentration does not necessarily indicate liver involvement in progression of melanoma.

3.5. Serum Hyaluronan

Hyaluronic acid (HA) is an unbranched, negatively charged polysaccharide with a high molecular weight. In the extracellular region, hyaluronan interacts with proteoglycans to form macromolecular complexes. It is an important component of connective tissue and it seems to play an important role in various phases of the metastasis (77,78). In fact, it has been shown that HA-rich matrices surrounding cancer frequently lead to metastasis (79,80). Serum hyaluronan levels are significantly increased in various type of tumours such as multiple myeloma, malignant lymphoma, in metastatic mesothelioma and in metastatic breast cancer (81-84). Hyaluronan has a role in tumour development, in rapid proliferation and in tumour invasion. Particularly Burchardt *et al* (85) found an increment of serum hyaluronan levels in malignant melanoma patients in stage I, II as well as in stage IV. In melanoma it was also demonstrated a strong correlation between hyaluronic acid and the expression of CD44 on the tumour cell surface. The isoform $CD44_s$ represents the major cellular receptor for the hyaluronic acid (86-89) and its expression is associated with tumor growth. Bartolazzi *et al.* (90) noted that the expression of HA-binding of wild-type CD44 isoform encouraged the development of melanoma, while this became impossible in presence of a CD44 mutant incapable of binding HA. Therefore we can suppose that an increment of hyaluronic acid could be considered a prognostic indicator.

3.6. Serum Laminin-1

Laminins, large trimeric molecules, composed of one α, one β, and one χ chain, are found in basement membrane extracellular matrices (91). The fifteen different laminin isoforms described to date are assembled from five different α, three different β, and three different χ chains. Laminin-1 belongs to a family of large glycoproteins wich form disulphide- bridged heterotrimers (92). Laminin 1 is matrix component. In addition to being a major structural component of the basement membrane, laminins function in cell proliferation, adhesion, differentiation and migration. Increased laminin 1 expression was also detected in melanoma cell lines with increased metasttic potential. Laminin 1 promotes human melanoma cell proliferation (93). Laminin 1 was elevated in the stage IV melanoma patients with metastases only (94).

3.7. Tenascin C

Tenascin C (Tn-C) belongs to the family of tenascin glycoprotein of the extracellular matrix. The tenascin C is a hexameric disulphride linked extracellular matrix glycoprotein (95). The expression of tenascin show an oncofetal predominance in fact during embryonic development it is expressed in the mesenchyme of developing organs. Tenascins can be re-expressed in the adult during normal states such as wound healing, nerve regeneration, and tissue involution, and in pathological states including vascular disease, tumorigenesis, and metastasis (96-98). Tenascin-C play an important role in cell adhesion, spreading and cellular proliferation. Tenascin may directly bind the epidermal growth factor receptor (EGFR), activating the EGFR kinase cascade and subsequently increasing the proliferative and migratory activity of tumour cells (99). Deposition of tenascin C is associated with antiadhesive or motility promoting behavior of neoplasia. Tenascin C expression has been reported in melanoma cell lines (100). Tn-C expression is moderately enhanced in benign and dysplastic melanocytic tumours and the expression is greatly enhanced in malignant melanomas and melanoma metastases (101). Tn-C is secreted by melanoma cells and has been found in the sera of melanoma patients, with the highest levels in advanced cases (102).

3.8. Serum collagen type VI

Collagen type VI is a ubiquitous matrix protein found in association with collagen types I and III. It forms microfibrils in the papillary dermis surrounding interstitial collagen and elastic fibres (103). High expression levels of type VI collagen have been described in chronic fibrous conditions (104), but also in tumours such as melanomas or gliomas (105). Burchardt *et al.* found an increase in type VI collagen levels in stage I, II (5.3 ± 3.0 ng/ml) and stage IV melanoma patients (4.8 ± 1.2 ng/ml) in confront to normal values. Besides collagen type VI expression in melanoma cell lines ,derived from metastasis and primary tumours, has been detected in contrast to primary melanocytes (106). Particularly, a more aggressive tumour growth has been noted in tumours expressing collagen type VI (105; 107). It has been suggested that type VI collagen expression and deposition, provide an early scaffold for subsequent angiogenesis in invasive melanomas (108). Four integrin receptors ($\alpha 1 \beta 1$, $\alpha 2 \beta 1$, $\alpha 10 \beta 1$ and $\alpha 11 \beta 1$) and NG2 (109) were recognized as receptors of collagen type VI. In advanced melanomas the increase of some constituents of these receptors ($\alpha 1$-, $\alpha 2$- and $\alpha 11$-integrin mRNA) were reported to be associated with a worse prognosis (110). The NG2 has been also found to augment the growth and metastatic properties of melanoma cells (111).

4. CONCLUSION

The traditional assays used for the measurement of tumor markers have been ELISA-type assays for serum markers and immunohistochemistry for tissue markers. Although these individual markers may be less sensitive and specific than existing markers. There have been many predictions of survival proposed according to different stages. Larger patients populations may be needed for more detailed analysis .For melanomas with regional metastases, number of limphnodes positive for melanomas and presence of extranodal disease are the most important prognostic factors or survival. (AC). Further studies are needed to determine the biologic behaviours and outcomes of melanoma

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