MATRIX METALLOPROTEINSES IN DEVELOPMENT AND PROGRESSION OF SKIN MALIGNANT MELANOMA

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ABSTRACT

Cutaneous malignant melanoma is one of the most life threatening skin cancers. It derives from skin melanocytes and has an unpredictable clinical behavior – it may remain silent for years or it can metastasize aggressively. Melanoma progression and metastases comprise several processes which require differential expression and regulation of several adhesion molecules and proteinases involved in extracellular matrix (ECM) proteolysis and cell-cell and cell-matrix contacts. MMPs are family of highly homologous extracellular or intracellular, secreted or membrane-bound, Zn^{2+} dependent neutral endopeptidases, which are capable to degrade virtually all protein components of ECM, basement membrane, clotting factors, cell-cell and cell-matrix adhesion molecules, cell-membrane attached precursors of growth factors, growth factor binding proteins, growth factor receptors, other proteinases and proteinase inhibitors, as well as their own inactive zymogene forms.

In the current report we attempt to summarize our previous results and information from the literature concerning the role of differential expression and activation of particular members of MMP family for development, aggressiveness, metastatic capacity, melanoma progression, survival and therapeutic response.

***Key words**: cutaneous melanoma, matrix metalloproteinases, adhesion molecules, progression*

Introduction

Origin of melanoma

Cutaneous malignant melanoma is one of the most life threatening skin cancers. It derives from skin melanocytes and has an unpredictable clinical behavior – it may remain silent for years or it can metastasize aggressively. Melanocytes are highly differentiated cells specialized to synthesize the natural UV protector known as melanin (65). Melanin is a pigment biopolymer, which is derived from the amino acid tyrosine. The production of melanin is a highly complex oxidative process with number of steps that can either proceed enzymatically or non-enzymatically (71). Melanocytes originate from the embryonal neural crest cells and migrate to the basal cell layer of epidermis and hair follicules (64,40,71). Melanocytes have special organelles, melanosomes, where the melanin is synthesized by the catalytic activity of the key enzyme tyrosinase (40). Melanin is transported to surrounding keratinocytes via dendrites of the melanocytes.

Normally, skin melanocytes are mitotically inactive or can proliferate briefly, due to external signals such as sun exposure; nevertheless follicular melanocytes have cyclical proliferative and melanogenic activity (64). Cutaneous malignant melanoma in 70% of cases arises from normal epidermal melanocytes and the rest of 30% of the cases derive from nevus melanocytes.

The preinvasive melanocytic neoplasm is called melanoma *in situ*. The invasive primary melanomas are divided into four subtypes based on their growth pattern: superficial spreading melanoma (SSM); lentigo maligna melanoma (LMM); nodular melanoma (NM); and acral letiginous melanoma (ALM) (3,23).

Model of melanoma development and progression

Melanoma, like other neoplasms, develops via transformation of normal cells into cancer cells: a multiple step process, which involves a loss of growth control and acquisition of immortality due to uncontrolled proliferation and/or impaired apoptosis (28,48,7). Tumor progression in melanocytic system is accompanied by several genetic and biological events such as genetic instability, impaired expression of cell cycle positive and negative regulators, activation of

oncogenes and loss of function of tumor suppressor genes, acquiring of insensibility for external regulatory factors and contact mediated controls, alterations in adhesion molecules, proteolytic enzymes and their inhibitors and acquisition of angiogenic potential (28,48,45,7).

Clinical and pathological observations have shown that melanoma progression passes through five sequential steps: common nevi (congenital or acquired) with structurally normal melanocytes; dysplasic nevi with structural and architectural atypia; *in situ* and early radial growth phase (RGP) primary melanoma; primary melanoma with advanced vertical growth phase and competence for metastasis; metastatic melanoma (48,45).

Factors involved in invasion and metastasis of melanoma

Melanoma progression and metastases comprise several processes such as local invasion of primary tumor, release of tumor cell from primary lesion, migration trough the extracellular matrix (ECM) and basal membrane (BM), entry in the vascular system and establishment of secondary tumors. All these processes require differential expression and regulation of several adhesion molecules and proteinases involved in ECM proteolysis and cell-cell and cell-matrix contacts (70,38,45).

Adhesion molecules

The adhesion molecules intermediates cell-cell and cell-matrix contacts. They are members of four main gene families, known as cadherins, integrins, immunoglobulins and selectins (38,9,49,5).

a) Cadherins

Cadherins comprise a group of large transmembrane proteins. Their extracellular domain forms interlocking bonds (zipper like), whereas their internal domain contacts with actin components of cytoskeleton through the intracellular proteins: α-, β and γ-catenins (70,38,9,5). The superfamily of cadherins mediates Ca^{2+} dependent homophilic cell-cell contacts such as zonula ocludens (tight junction), zonula adherens (*adherens junction*), macula adherens (desmosome), macula communicans (gap-junction or nexus). The large cadherin superfamily consists of more than 80 members subgrouped in several subclasses: classic cadherins, desmogleins, desmocollins, protocadherins, CNRs, Fats, seven-pass transmembrane cadherins, and Ret tyrosine kinase (77). The most widely expressed classical cadherins are epithelial (E), neural (N), placental (P) and vascular endothelial (VE) cadherins (9,5).

E-cadherin is expressed in normal melanocytes were it functions as the main adhesion molecule in the interaction with keratinocytes (38,9). Down-regulation of E-cadherin expression or lack of membrane localization are found in early stages of melanoma and in radial growth phase (RGP), whereas high levels are observed in advanced tumors, vertical growth phase (VGP) and melanoma metastases (12,38,9). Synthesis of N-cadherin has been observed in melanoma, but not in dysplastic nevi; contrariwise P-cadherin is expressed in nevi and RGP, lost in VGP, but may be re-expressed in advanced tumors (38,9). In highly invasive melanomas there are detected elevated levels of VE-cadherin, whereas it is undetectable in the poorly invasive tumor cells {Hendrix, 2001 #683).

b) Integrins

Integrins are a large family of transmembrane glycoproteins with heterodimeric structure, which consists of two non-covalently linked α- and β-subunits. There is variety of different α- and β-subunits (30,31,49). The combinations of those different α- and β-subunits determine the integrins' ligand-binding specificity (30,49). Integrins are dispersed into groups on the basis of the β-subunit structure, whereas the ligand-binding specificity is determined by the combination of α and β subunits. Integrins can mediate cell-cell or cell-ECM contacts (18,31,38,63). Some integrins, such as β1 integrins also called VLA (very-late activation antigens) together with α6β4 and αvβ3 act as cell surface receptors for ECM proteins (collagens, laminin, etc.), whereas others can recognize integral membrane proteins of the immunoglobulin superfamily (ICAM-1, ICAM-2 and VCAM-3) thus intermediating direct cell-cell contacts (38,63).

It has been sown that integrin activation leads to triggering signal pathways influencing variety of gene expression including metalloproteinases and cytokine and thus they affect the cell cycle, differentiation, apoptosis, inflammation, immune response, homeostasis, angiogenesis and other cell processes (18,63).

During melanoma progression expression of integrins varies in a wide range (13,18,31,52,63,74,54).

c) Immunoglobulin gene superfamily

The members of this group are soluble or cell surface proteins which can act as cell surface antigen receptors, adhesion molecules, stimulatory or antigen presenting molecules of the immune system. The cell adhesion molecules from the immunoglobulin gene superfamily have a characteristic structure including repeated domains, similar to those found in immunoglobulins (Ig domains) (27).

The expression of different cell adhesion immunoglobuline superfamily members, such as NCAM, Mel-CAM/MUC18/MCAM, IAM-1, VCAM-1 etc, is found to be changed in step-wise fashion in melanocytic lesion development and to correlate to the melanoma progression (18,52,48,63).

d) Selectins

They are single-chain, transmembrane, Ca^{2+}-dependent, carbohydrate-binding glycoproteins. They are divided in three groups: L (leukocyte)-, P (platelet)- and E (endothelial)-selectins. These selectins mediate adhesion interactions between leukocytes and the endothelium and between leukocytes and platelets (59).

P-selectin is mobilized from platelets by signals during the inflammatory response, such as histamine and thrombin, whereas cytokines such as TNF-α stimulate the consecutive expression of E- and P-selectins. Selectins mediate extravasation of leucocytes. E-selectin binds neutrophils and eosinophils (8,76).

The expression of P- and E-selectins has been detected in intratumor vessels of primary malignant melanoma and associated with shorter disease-free and overall survival (62).

Proteinases

Variety of normal and pathological processes require decomposition of the components of basement membrane (BM) and ECM. Directional and controlled degradation is provided by several groups of proteolytic enzymes including plasminogen activators (PAs), cathepsins and matrix metalloproteinases (MMPs) (4).

a.) Plasminogen activators

The plasminogen activators (PAs) are proteolytic enzymes that convert plasminogen into active plasmin, which can activate other matrix proteinases (MMPs) or can directly degrade the ECM. Plasminogen activators are known to be two types: tissue (t-PA)- and urokinase (u-PA)-type, which differ structurally and functionally. Both enzymes are secreted as inactive pro-enzymes, but are activated via specific fashion: u-PA is activated by binding to its specific receptor (u-PAR) while the activation of t-PA occurs at the cell surface(14). Two specific plasminogen activator inhibitors, PAI-1 and PAI-2 have been described and shown to regulate the activity of PAs (14). u-PA/u-PAR complex is involved in autocrine growth stimulation, signal transduction and in metastatic spread (14).

During melanoma development t-PA is produced in considerable amounts, but it is believed that u-PA rather than t-PA plays a major role in melanoma metastasis (14).

b.) Cathepsins

Cathepsins belong to a large family, which consists of cysteine or aspartyl proteases, normally found in lysosomes, but released in high levels by many types of cancer cells. They are inhibited by two groups of endogenous inhibitors stefins (e.g. stefin A, and B) and cystatins (e.g. cystatin C) (26,43).

The high levels and activity of cathepsins A, B, D and H, as well as of their inhibitors, stefin A, B and cystatin C in sera of patients with metastatic melanoma are associated with shorter disease-free and overall survival and with a lack of response to administrated chemoimmunotherapy (42,26,43).

c.) Matrix metaloproteinases

MMPs are family of highly homologous extracellular or intracellular, secreted or membrane-bound, Zn^{2+} dependent neutral endopeptidases, which are also known as matrixins. (75,50). They generally degrade virtually all protein components of the extracellular matrix (ECM), basal lamina, clotting factors, cell-cell and cell-matrix adhesion molecules, cell-membrane attached precursors of growth factors, growth factor binding proteins, growth factor receptors, other proteinases and proteinase inhibitors, as well as their own inactive zymogene forms (37,19,73,53,57).. MMPs are naturally inhibited by specific inhibitors called tissue inhibitors of metalloproteinases, TIMPs (37,73,53,50), but also can be inhibited by metal chelators. MMPs are secreted as pro-enzymes (zymogens) which require extracellular activation and act in physiological pH (58,37,73,53,50).

Currently there are known 23 MMPs in humans, which differ in substrate specificity, regulation and potential interactions with others MMPs and TIMPs (37,73,57). On the basis of their substrate specificity the MMPs can be divided into five groups: collagenases (MMP-1, -8 and -13), stromelysins (MMP-3, -10 and -11), gelatinases (MMP-2 and -9), matrilysins (MMP-7 and -26), and membrane-type matrix metalloproteinases (MT-MMPs) (75,53,50).

The MMP activity is very strictly controlled at the level of gene transcription, latent zymogen activation, interaction with specific ECM components and inhibition by endogeneous inhibitors (44,47,75,50,11).

Cleaving the cysteine residue in the propeptide domain activates the latent zymogens. MMP proenzymes may be activated and by a variety of other enzymes such as trypsine 2, catepsines G, B and L, PMN elastase, plasminogen activators etc. Activated MMPs have capability for auto-activation and activation of other proMMPs (37). An important role in activation of secreted proMMPs have the MT-MMPs (37,2).

MMPs participate directly in a number of physiological processes such as wound healing, regeneration, endometrial cycling, neovascularization and the embryonic stages of organ development (75,53,72). They also can modify indirectly the cellular and tissue behavior by cleaving different proteins into fragments, which changes their biological activity, they may be extracellular proteins, growth factors (releasing them from their binding proteins and cell-membrane-bound precursor forms), signal and receptor molecules (53). Cleaving of cell adhesion receptors or ligands of cell surface receptors can affect the multiple intracellular signaling cascades and thus may change cell responses. Soluble MMPs may participate in integrin-mediated signaling cascades being ligands for the integrins (69).

TIMPs inhibit all MMPs and several zymogens by connecting to the conserved Zn-binding sequence (2). The major factor involved in regulation of ECM degradation in order to maintain the normal functions of tissues is the local balance between MMPs and TIMPs. Disturbance of this balance is observed in different pathological conditions such as rheumatoid arthritis, osteoarthritis, atherosclerotic plague rupture, peridontitis, dermal photoaging, chronic ulceration, COPD, Bronchial asthma and cancer (73,72,50,10,15).

MMPs synthesized by cancer cells and adjacent stromal cells contribute to every step of cancer development where ECM proteolysis is required: growth, invasion, metastasis, penetration the basal lamina, infiltration of lymphatic or blood vessel, cancer cell survival and neovascularization (19,73,2,72,57) (21,22).

MMP-1 (collagenase-1) is a member of the subclass of collagenases which are neutral proteinases capable to degrade fibrillar collagens (type I, II, III and V) (6). It has been shown tat MMP-1 is expressed in various types of cells in culture and *in vitro* (6), as well as the increased expression of this MMP has been associated with chronic cutaneous wounds (60). In human

malignant melanoma, the enhanced expression of MMP-1 has been found to correlate with tumor invasion capacity and melanoma progression (67,1,34). Recently, we reported that high MMP-1 expression in biopsies of patients with metastatic melanoma was associated with sorter disease-free survival (56) and interestingly with better response to the applied combined DOBC-INF- chemoimmunotherapy (55).

Stromelysin-1 (MMP-3) is one of the closely related to collagenases enzymes with respect to structure and substrate specificity. Due to its broad substrate specificity, MMP-3 degrades the components of ECM and basement membrane (proteoglycan, fibronectin, laminin, elastin, gelatin, and some types of collagens). It is able to cleave the net-work-forming collagens (type IV, VII and X) and in less extend the fibrillar collagens (type III, V and XI), having preference to the type IV collagen that form the basal membrane (24,75). In addition, MMP-3 is also involved in activation of some other members of the MMP family, including MMP-1 (39,29). Normally, MMP-3 is expressed in keratinocytes and fibroblasts (39). Increased expression of MMP-1 was shown to correlate with progression of melanocytic lesions (33,36) and with sorter disease-free survival (56).

Gelatinases (MMP-2 and -9) digest type IV collagen, which is an important component of basement membrane. Thus gelatinase-1 (MMP-2) and gelatinase-2 (MMP-9) promote invasion and metastasis of the tumor cells (46,20,17,16). MMP-2 is regularly expressed by most of the cells, whereas, MMP-9 is expressed only by polymorphonuclear leukocytes and its expression is induced in normal keratinized cells by several factors (12-O-tetradecanoylphorbol-13-acetate [TPA], growth factor, cytokines and so forth) (61,73). MMP-9 was associated with microvessel density (MVD) and VEGF expression, and was shown to act as a controller of the tumor neovascularization (16).

Increased expression of MMP-2, MMP-9, as well as the presence of functionally active MMP-2 were shown to correlate with an invasive phenotype and metastatic capacity of melanoma cell lines and with tumor progression (68,66,1,32,35,36). Overexpression of MMP-2 in primary melanoma has been shown to correlate with later haematogeneous metastasis and increased risk of dying from melanoma indicating that the expression of this proteinase might be a good prognostic factor in primary melanoma (67,66). In addition, the expression of MT1-MMP and TIMP-2 and activation of MMP-2 has been correlated with tumor progression both in the xenograph model and in human melanoma lesions (67,66).

The role of collagenase3 (MMP-13) has also been studied in metastatic melanoma. This MMP has exceptionally wide substrate specificity and in addition to the native fibrillar collagens (Type I, II and II) it is able to degrade type IV,X and XIV collagens, tenascin, fibronectin, fibrillin, perlecan and the aggrecan core protein (36). We previously found that MMP-13 was associated with higher proliferative activity of malignant melanoma, but not with the prognosis of the patients (55,56).

Besides the secreted MMPs, one of the membrane-associated MMPs (MT1-MMP) has been extensively studied in melanoma cells and lesions: it as been found that overexpression of MT1-MMP results in activation of MMP-2 on the cell surface of melanoma cells and is required for degradation of ECM proteins (51). Moreover, as we mentioned above, the expression of MT1-MMP is associated with tumor progression in human melanoma lesions (67,66), and the low MT1-MMP expression in sinonasal and oral malignant melanoma correlated with greater overall survival of the patients (41).

Recently, the expression levels of members of new classes matrix proteinases, ADAMs (a disintegrin and metalloproteinase, adamalysin) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) have been studied on mRNA level in multiple mormal fibroblasts and metastatic melanoma cell lines and in the isogenic normal tissue and tumor samples. Three of the ADAM members, ADAM-10, ADAM-12 and ADAM-15 showed more than a twofold increase in melanoma tissues compared to the normal tissue samples(25). These metalloproteinases are involved in the processing and shedding of many membrane-bound bioactive molecules, such as TNF-α, EGF, Notch, E-caderin, ErbB ligands etc, and in digestion of ECM proteins, and thus can be implicated in cancer progression (25).

Conclusions

Development, progression and metastasizing of human skin melanoma require a directional and controlled degradation of basement membrane and extracellular matrix protein. These proteolytic processes are accomplished by variety of proteinases. During different phases of progression of melanocityc lesions the particular members of MMP superfamily are differentially expressed and activated, which prove the pivotal role of those matrix proteinases and. provides possibility for targeting proper MMPs for therapeutic intervention in combination with the conventional chemotherapy.

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