Matrix Metalloproteinases in Human Melanoma

Uta B. Hofmann,*† Johan R. Westphal,* Goos N. P. van Muijen,* and Dirk J. Ruiter*

*Department of Pathology, University Hospital, Nijmegen, The Netherlands; †Department of Dermatology, Julius-Maximilians University, Würzburg, Germany

Cutaneous melanoma is a highly invasive and metastatic tumor. Degradation of basement membranes and extracellular matrix is an essential step in melanoma cell migration, invasion, and metastasis formation. Matrix metalloproteinases and their tissue inhibitors play a crucial role in these complex multistep processes. Melanoma cells may express a number of matrix metalloproteinase family members (MMP-1, MMP-2, MMP-9, MMP-13, and MT1-MMP) as well as their tissue inhibitors (TIMP-1, TIMP-2, and TIMP-3). Numerous studies have examined matrix metalloproteinases, their tissue inhibitors, and the molecules that regulate their expression and/or activation in melanoma cell lines *in vitro and in vivo*, and in human melanocytic lesions. Recent results have indicated that adhesion molecules such as CD44 and integrin $\alpha_v\beta_3$ are involved in positioning activated matrix metalloproteinase molecules on the cell surface of invasive tumor cells. In this review we evaluate these novel aspects of the role of matrix metalloproteinases and their tissue inhibitors in melanoma progression. We conclude that the balance between levels of activated matrix metalloproteinases and expression levels of their tissue inhibitors, and the coexpression of activated matrix metalloproteinases and adhesion molecules are important factors in determining melanoma cell invasion, tumor growth, and metastasis formation.

Key words: adhesion molecules/matrix metalloproteinases/melanoma/tissue inhibitor of matrix metalloproteinases/tumor progression. J Invest Dermatol 115:337–344, 2000

Cutaneous melanoma is one of the most frequent malignant tumors in younger age people, and is characterized by its high capacity for invasion and metastasis. The incidence of human cutaneous melanoma is increasing in the U.S.A., Australia, and Europe. The lifetime risk for an American having invasive melanoma was calculated as 1 in 75 by the year 2000 (Rigel *et al*, 1996). Cutaneous melanoma may develop *de novo* from normal melanocytes or from potential precursor lesions, such as atypical dysplastic nevi or congenital nevi (Clark *et al*, 1984; Herlyn *et al*, 1987; Albino *et al*, 1997). Historically, the most important prognostic factors in patients without metastases are the tumor thickness (Breslow, 1970) and, to a lesser extent, the level of invasion (Clark *et al*, 1969). Recently, however, it is becoming clear that a multifactorial model may be required to address melanoma progression more accurately.

Dissemination of tumor cells is the principal cause of mortality in tumor patients. Tumor cell invasion as well as metastasis formation are complex and multistep processes, in which tumor cells detach from the primary tumor, invade surrounding tissue and basement membranes, intravasate into the lymphatic or blood circulation, and, finally, adhere and extravasate in distant organs to form a secondary tumor. Degradation and remodeling of the extracellular matrix (ECM) and basement membranes by proteolytic enzymes are essential steps in these processes. The role of matrix metalloproteinases (MMPs) in invasion and metastasis in many types of tumors has been extensively reviewed (Stetler-Stevenson *et al*, 1993; Coussens and Werb, 1996; Chambers and Matrisian, 1997; Polette and Birembaut, 1998; Curran and Murray, 1999; Kleiner and Stetler-Stevenson, 1999; Westermarck and Kähäri, 1999). Studies employing immunohistochemistry and/or *in situ* hybridization have demonstrated that both tumor cells and stromal cells express MMPs (Basset *et al*, 1990; Poulsom *et al*, 1992; Pyke *et al*, 1992; 1993; Newell *et al*, 1994; Okada *et al*, 1995; Heppner *et al*, 1996; Nielsen *et al*, 1996; Ueno *et al*, 1997). The complex interaction between tumor cells and tumor-surrounding stromal cells, however, is not yet fully understood. Evidence has been provided that either the secretion of tumor-derived soluble factors (i.e., cytokines) (Kataoka *et al*, 1993; Mauviel, 1993; MacDougall and Matrisian, 1995) or direct cell-cell contact (Hewitt and Danø, 1996) plays an important role in the induction of stromal proteases.

Also in melanoma progression, different proteolytic enzyme systems, including the plasminogen activator system (de Vries *et al*, 1994, 1996; Ferrier *et al*, 1998) and the MMP family, play important roles (van den Oord *et al*, 1997; Väisänen *et al*, 1998; Airola *et al*, 1999; Hofmann *et al*, 2000a). Recent data indicate that in particular the balance between MMPs and their tissue inhibitors (TIMPs) may be critical in determining this process. Furthermore, coexpression of activated MMPs with certain adhesion molecules may be essential in positioning the active proteases at the invasive front of the tumor. In this review we evaluate the involvement of adhesion molecules and of the MMP/TIMP balance in melanoma progression, on the basis of studies on melanoma cells *in vitro* and *in vivo* and of expression profiles in human melanocytic lesions.

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Reprint requests to: Dr. Uta B. Hofmann, Department of Dermatology, University Hospital, Josef-Schneider-Str. 2, D-97080 Würzburg, Germany. Email: uta.hofmann@mail.uni-wuerzburg.de

Abbreviations: EMMPRIN, ECM metalloproteinase inducer; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; TIMP, tissue inhibitor of matrix metalloproteinase; uPA, urokinase plasminogen activator.

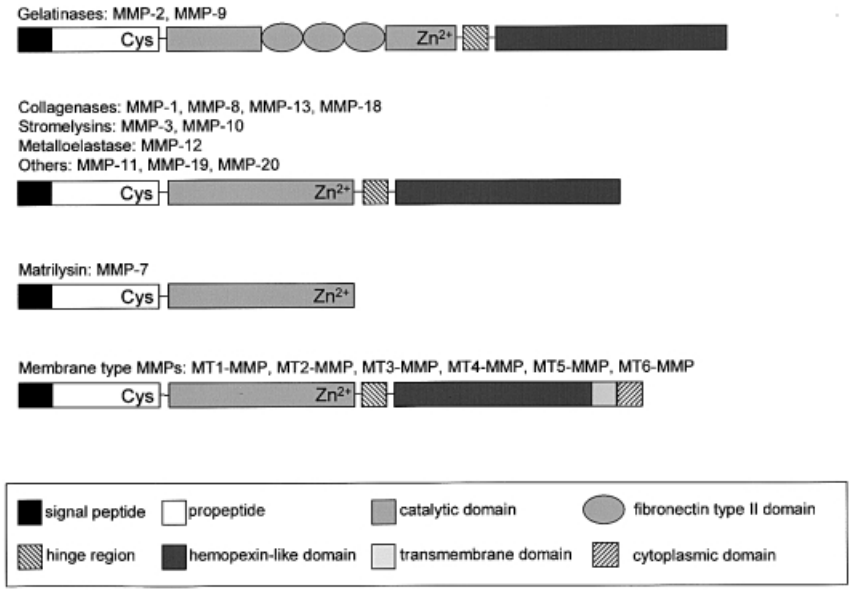

Figure 1. Domain structures of human MMPs. The MMPs are activated by cleavage of the N-terminal propeptide.

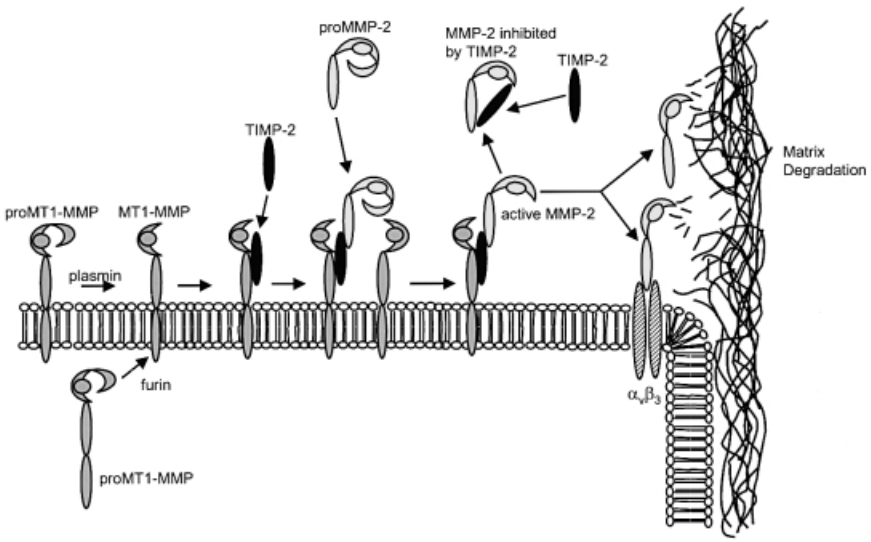

Figure 2. Regulation of cell surface activation and presentation of MMP-2. ProMT1-MMP is activated either intracellularly by furin-like proteinase or extracellularly by proteases such as plasmin. Activated MT1-MMP binds to TIMP-2, thereby generating the MT1-MMP-TIMP-2 complex that serves as a receptor for proMMP-2 on the cell surface. A second MT1-MMP molecule cleaves and activates the bound proMMP-2. Active MMP-2 can remain membrane-bound or be released, and can directly degrade ECM initiating cell migration and invasion. Alternatively, proteolytically active MMP-2 can bind to integrin $\alpha_v\beta_3$. Unbound active MMP-2 can be inhibited by TIMP-2.

MMPS

MMPs belong to a rapidly growing multigene family of zinc-dependent endopeptidases that are involved in the degradation of ECM components in both physiologic and pathologic processes (Woessner *et al*, 1991; Birkedal-Hansen *et al*, 1993; Stetler-Stevenson *et al*, 1993; Polette and Birembaut, 1998; Curran and Murray, 1999; Kleiner and Stetler-Stevenson, 1999; Westermarck and Kähäri, 1999). To date, at least 19 different human MMPs have been cloned and characterized. According to their structure, substrate specificity, and cellular localization, they can be classified into five different subgroups (Birkedal-Hansen *et al*, 1993; Kleiner and Stetler-Stevenson, 1999). In general they contain a signal peptide, an N-terminal propeptide domain, a catalytic domain that includes the highly conserved zinc-binding site, and a hinge region followed by a C-terminal hemopexin-like domain. The two gelatinases MMP-2 and MMP-9 possess an additional fibronectin-type II domain, which is inserted between the catalytic domain and the active site domain. The six membrane-type MMPs (MT1-MMP to MT6-MMP) have a transmembrane domain adjacent to the C-terminal domain and an intracellular domain. MMP-7 (matrilysin) is considerably smaller than the other MMPs as it lacks the hemopexin-like domain and the hinge region (**Fig 1**).

MMP expression and activation are highly regulated at several levels, and involve both transcriptional and post-transcriptional mechanisms. MMP mRNA expression is primarily regulated by many different factors including cytokines, growth factors, hormones, oncogenes, and tumor promoters (Birkedal-Hansen *et al*, 1993; Westermarck and Kähäri, 1999). At the protein level, biologic activity of MMPs is regulated by their activation state. Most MMPs are secreted from cells in latent forms (proMMPs). Conversion of proMMPs to functionally active forms requires a specific multistep activation process involving the proteolytic removal of part of the molecule (Nagase, 1997). For most MMPs proteolytic activation is initiated in the extracellular space by serine proteases such as plasmin and urokinase plasminogen activator (uPA), or by other members of the MMP family (Nagase, 1997). On the cell surface, MT-MMPs have been identified as potent physiologic activators of some MMPs (Sato *et al*, 1994, 1996). MT1-MMP can form a complex with TIMP-2, which then serves as a receptor for proMMP-2. A second unbound MT1-MMP molecule adjacent to this complex then converts proMMP-2 into its active conformation (Strongin *et al*, 1995; Butler *et al*, 1998; Nagase, 1998). Proteolytically active MMPs may be localized on the cell surface by binding to membrane molecules, which leads to a more directed ECM degradation. Furthermore, functionally

active MMP-2 can also bind to integrin $\alpha_v\beta_3$ (Brooks *et al*, 1996), and similarly, proteolytically active MMP-9 can associate with CD44 (Yu and Stamenkovic, 1999).

Finally, MMP activity is modulated by a family of physiologically occurring TIMPs. To date, four different structurally related members (TIMP-1 to TIMP-4) have been characterized that inhibit acitive MMPs by binding to their catalytic domain (Gomez *et al*, 1997). In addition, TIMP-1 and TIMP-2 regulate activation of some proMMPs by binding to the COOH-terminal hemopexin-like domain (Gomez *et al*, 1997). TIMP-1 inhibits activation of proMMP-9, whereas TIMP-2 binds and regulates activation of proMMP-2 (Ward *et al*, 1991; Goldberg *et al*, 1992; Gomez *et al*, 1997). At low concentrations, TIMP-2 promotes the formation of a complex with proMMP-2 and MT1-MMP on the cell surface, leading to activation of MMP-2. Hence, low concentrations of TIMP-2 promote processing of MMP-2 to its proteolytically active form, but high concentrations of TIMP-2 inhibit MMP-2 activation. The balance between levels of activated MMP and free inhibitors appears to be critical for MMP activity (Nagase, 1997; Butler *et al*, 1998). The regulation of MMP-2 activation and cell surface presentation is illustrated in **Fig 2**.

MMPS IN MELANOMA CELL MIGRATION AND INVASION *IN VITRO*

The expression pattern of various MMPs and TIMPs, and their contribution to melanoma invasive behavior, has been studied in several human and mouse melanoma cell lines. Increased expression of MMP-1, MMP-2, and MMP-9 was shown to correlate with an invasive phenotype using different matrix degradation assays (Montgomery *et al*, 1993; MacDougall *et al*, 1995; Mueller, 1996; Durko *et al*, 1997). In particular, increased expression of MMP-2 and the presence of functionally active MMP-2 have been associated with melanoma progression. Active MMP-2 was only observed in highly invasive cell lines, and was absent in non or poorly invasive cell lines (Hofmann *et al*, 1999; Kurschat *et al*, 1999). MMP-2 activity directly modulates melanoma cell adhesion and spreading onto ECM components, suggesting that MMP-2 may also facilitate cell migration and invasion (Ray and Stetler-Stevenson, 1995). When localized to invadopodia, overexpression of MT1-MMP resulted in activation of MMP-2 on the cell surface of melanoma cells and was required for ECM degradation (Nakahara *et al*, 1997).

Next to the dominant role of MMP-2 and MT1-MMP in melanoma progression, the role of MMP-1 in collagen type I and IV degradation and melanoma cell invasion through Matrigel was described by Durko *et al* (1997) using a metastasis-derived melanoma cell line in which expression of MMP-1 was suppressed by transfection with antisense MMP-1.

MMP-9, finally, was expressed by or could be induced only in cell lines derived from advanced primary melanomas, and was absent in cell lines derived from early stage primary lesions (MacDougall *et al*, 1995). In a recent study by the same authors, MMP-9 production induced by treatment of cell lines derived from advanced stage lesions with either interleukin-1β (IL-1β) or tumor necrosis factor α (TNF-α) was accompanied by loss of TIMP-1 production. Conversely, less aggressive cell lines were unable to produce MMP-9 after identical cytokine treatment, whereas expression of TIMP-1 was increased (MacDougall *et al*, 1999). These results suggest a switch in the MMP expression profile during tumor progression, involving not only emergence of MMP expression but also downregulation of MMP expression (MacDougall *et al*, 1999). The latter observation is illustrative for the intriguing concept that the proteolytic balance between MMPs and TIMPs determines melanoma progression (Henriet *et al*, 1999; MacDougall *et al*, 1999). Accordingly, overexpression of TIMP-1, TIMP-2, and TIMP-3 using recombinant TIMPs or transfecting cells with cDNA or adenovirus-mediated transfer of TIMP genes has been shown to inhibit tumor-mediated proteolysis and melanoma cell invasion (Schultz *et al*, 1988; Albini *et al*, 1991; DeClerck *et al*, 1992; Khokha *et al*, 1992a; Montgomery *et al*, 1993, 1994; Ahonen *et al*, 1998; Valente *et al*, 1998). Interestingly, Ahonen *et al* (1998) reported that overexpression of TIMP-3 induced apoptosis of melanoma cells, indicating that TIMP-3 may inhibit melanoma growth on different levels.

MMPS IN MELANOMA TUMOR GROWTH AND METASTASIS FORMATION *IN VIVO*

Various animal models are used to study the involvement of MMPs and TIMPs in the metastatic process. In a xenograft model, increased expression of functionally active MMP-2 correlated with the metastatic capacity of different melanoma cell lines *in vivo*, indicating that activated MMP-2 may be required for melanoma progression (Hofmann *et al*, 1999). In cell lines that constitutively expressed MMP-9, an enhancement of lung colonization was observed (MacDougall *et al*, 1999). Accordingly, in MMP-9-deficient mice experimental metastasis formation was suppressed (Itoh *et al*, 1999). Evidence has been provided that not the melanoma cells themselves, but the tumor-surrounding host cells secrete MMP-9 *in vivo*, indicating that host-derived MMP-9 plays an important role in melanoma metastasis formation (Hofmann *et al*, 1999; Itoh *et al*, 1999).

In agreement with *in vitro* data, the proteolytic balance between the production of MMPs and TIMPs seems to determine the metastatic capacity of melanoma cells *in vivo* as well. Thus, overexpression of TIMPs has been shown to reduce tumor growth and metastasis formation (Khokha *et al*, 1992b; Khokha, 1994; Montgomery *et al*, 1994; Imren *et al*, 1996; Ahonen *et al*, 1998; Valente *et al*, 1998). Overexpression of TIMP-1 in B16-F10 murine melanoma cells reduced tumor growth and their metastatic potential in both chick embryos and mice (Khokha *et al*, 1992b; Khokha, 1994). Comparable reduction of tumor growth has been described in B16-F10 cells overexpressing TIMP-2 as well (Valente *et al*, 1998). In this study TIMP-2 cDNA transfected melanoma cells formed smaller tumors with reduced blood vessel formation when grown in Matrigel. Recombinant TIMP-2 has proven effective in inhibiting tumor cell mediated proteolysis, tumor growth, and lung colonization (Schultz *et al*, 1988; DeClerck *et al*, 1992). Interestingly, in immunodeficient mice overexpression of TIMP-2 in a highly metastatic human melanoma cell line markedly reduced subcutaneous tumor growth, whereas spontaneous metastasis to the lung or lymph nodes was not prevented (Montgomery *et al*, 1994). These findings suggest either that this cell line may recruit other molecules such as plasmin and cathepsins to initiate tumor dissemination or that the lack of a tumor-induced host response in immunodeficient mice may promote metastasis formation. Furthermore, these data suggest the suppression of tumor growth and metastasis formation may ultimately be directed by elements of the host-tumor environment.

In contrast to the apoptosis-inducing capacities of TIMP-3 *in vitro*, overexpression of TIMP-2 protects melanoma cells from apoptosis *in vivo*, indicating a possible role for TIMP-2 in cell survival, another relevant aspect of tumor growth and metastasis formation (Valente *et al*, 1998). The interesting and apparently conflicting effects of TIMP-2 and TIMP-3 on melanoma cell survival definitely warrant further studies.

The function of TIMP-3 and TIMP-4 in tumor growth and metastasis formation in human melanoma has not been studied thus far.

Taken together, these results provide further evidence for the notion that the balance between levels of activated MMP and expression of TIMPs plays a crucial role in melanoma cell invasion, tumor growth, and metastasis formation. Additionally, some studies suggest that TIMPs are multifunctional molecules, with apparently paradoxical effects on tumor progression. Beside their MMP inhibitory functions, TIMP-1 and TIMP-2 may have growth-factor-like properties (Gomez *et al*, 1997). Several reports have demonstrated growth-promoting activities of TIMP-1 and TIMP-

2 on keratinocytes, fibroblasts, and some tumor cell types (Bertaux *et al*, 1991; Hayakawa *et al*, 1992, 1994; Kikuchi *et al*, 1997). A growth-stimulating effect of TIMP-2 on human melanoma cells has also been described (Nemeth *et al*, 1996). This stimulatory response is concentration dependent and requires the presence of insulin as a cofactor.

REGULATION OF MMP EXPRESSION AND/OR ACTIVATION IN MELANOMA CELLS

Numerous factors are involved in the regulation of MMP expression and activity as well as in the localization of the active molecules. As described previously, IL-8-transfected melanoma cells displayed upregulation in MMP-2 mRNA and collagenase activity and increased tumorigenic and metastatic potential in nude mice (Luca *et al*, 1997). As stated before, IL-1β and TNF-α can upregulate MMP-9 expression (MacDougall *et al*, 1999).

In another study, treatment of human melanoma cells with anti-CD44 monoclonal antibodies upregulated MMP-2 production (Takahashi *et al*, 1999). Various studies have shown that perturbation of various integrins, including $\alpha_v\beta_3$ and $\alpha_2\beta_1$ on metastatic melanoma cell lines, leads to upregulation of MMP-2, MT1-MMP, and TIMP-2 associated with increased invasive ability *in vitro* (Seftor *et al*, 1992, 1993; Bafetti *et al*, 1998; Kurschat *et al*, 1999).

A third important factor controlling MMP activity is reciprocal interactions between MMP family members. Activation of MMP-2 could be inhibited by antibodies against MT1-MMP, by addition of recombinant TIMP-2, and by inhibition of MT1-MMP cleavage, supporting the important role for MT1-MMP as an activator for proMMP-2 in malignant melanoma (Kurschat *et al*, 1999). Addition of TIMP-2 reduced the proteolytic capacity as well as integrin-dependent adhesion and spreading of melanoma cells *in vitro* (Ray and Stetler-Stevenson, 1995). Conflicting data have been reported regarding the effect of TIMP-2 expression levels on MMP-2 activation. Whereas Kurschat *et al* (1999) showed that the presence of active MMP-2 in highly invasive melanoma cell lines was accompanied by significantly lower amounts of TIMP-2, in other studies MMP-2 activation could not be linked to different expression levels of TIMP-2 (Stanton *et al*, 1998; Airola *et al*, 1999; Hofmann *et al*, 1999), but correlated well with the appearance of the 45 kDa processing form of MT1-MMP (Stanton *et al*, 1998; Hofmann *et al*, 2000a). Processing of MT1-MMP to a 45 kDa form may represent an end-point in the activation process of MMP-2 (Stanton *et al*, 1998). In agreement with these findings, proteolytic processing of MT1-MMP to the inactive 43 kDa form correlates with MMP-2 activation in fibrosarcoma cells (Lehti *et al*, 1998), which may be the functional equivalent of the 45 kDa form described before. It remains elusive whether during activation MMP-2 releases itself from the MT1-MMP complex by cleaving MT1-MMP to the inactive 43 kDa form, or whether MT1-MMP inactivates itself. Recently, however, it has been demonstrated that MT1-MMP activity enhanced melanoma cell invasion and induced cleavage of MT1-MMP to an inactive 43 kDa cell surface form and a soluble 20 kDa fragment (Lehti *et al*, 2000). In this study the gelatinase specific inhibitory peptide did not prevent MT1-MMP processing, suggesting that MMP-2 is not involved in MT1-MMP inactivation. These results suggest a negative regulation mechanism of MT1-MMP activity at the site of high focal activities (Lehti *et al*, 2000).

Table I. Matrix metalloproteinases and tissue inhibitor matrix metalloproteinases in human melanocytic lesions[a]

References	Type of lesions	Techniques	Components	Major findings
Woolley and Grafton, 1980	14 MM	IHC	MMP-1	Five out of 14 cases showed immunoreactivity of dermal collagen and stromal cells adjacent to MM. No significant staining of tumor cells. Normal skin was almost negative.
Airola *et al*, 1999	3 LM 4 MIS 11 ePM 13 aPM	ISH	MMP-1 MMP-13 TIMP-1 TIMP-3	No expression of MMP-1 and MMP-13 mRNAs in LM and MIS; detection of MMP-1 at the edges of tumors, while MMP-13 was detected more centrally within the tumor; increased expression of MMP-1, MMP-13, TIMP-1 and TIMP-3 during melanoma progression
	2-4 MIS 4-8 ePM 5-8 aPM	IHC	MT1-MMP TIMP-2 MMP-2	TIMP-2 was expressed in almost all PM; increased expression of MT1-MMP in aPM; MMP-2 was detected mainly in stromal cells bordering the tumors.
Väisänen *et al*, 1996	34 NN, 14 AN 12 LM, 9 MIS 20 PM 29 MM	IHC	MMP-2	Strong MMP-2 expression was observed in a few benign lesions and was clearly increased with architectural disorder, atypia and progression to melanoma. In PM and MM expression of MMP-2 was correlated with later hematogeneous metastasis.
Väisänen *et al*, 1998	50 PM	IHC	MMP-2	MMP-2 expression in 64% of PM; MMP-2 positivity was increased in male patients; 10-year survival rate of MMP-2 positive male patients was significantly decreased. Overexpression of MMP-2 indicated a 4.5 fold relative risk of dying from melanoma.
Hofmann *et al*, 2000b	9 NN, 6AN 10 MIS 9 ePM 6 aPM 20 MM	IHC RT-PCR, Zymography	MMP-2, MT1-MMP TIMP-2	No expression of MMP-2 in nevi and MIS; increased expression of MT1-MMP from benign to malignant lesions; ubiquitous expression of TIMP-2; colocalization of MMP-2 with MT1-MMP and TIMP-2; active MMP-2 is present in melanoma metastases. Increased expression of MMP-2 and MT1-MMP in melanocytic tumor progression.
van den Oord *et al*, 1997	33 nevi (including NN, AN, BN, SN) 4 LM, 4 MIS 15 ePM, 13 aPM 5 MM	IHC	MMP-9, EMMPRIN	No expression of MMP-9 and EMMPRIN in nevi and lentigo maligna; MMP-9 and EMMPRIN were variably expressed in the radial growth phase of PM and decreased expressed in the vertical growth phase; no expression in MM. Expression of MMP-9 and EMMPRIN was associated with early stage of melanoma progression.
Wagner *et al*, 1992	5 PM	Northern blot	MMP-11	Expression of MMP-11 mRNA was either not detectable or extremely weak.
Thewes *et al*, 1999	28 nevi 12 melanoma	IHC	MMP-11	No expression of MMP-11 protein in nevi and malignant melanoma.

[a]NN, common nevi; AN, atypical nevi; BN, blue nevi; SN, Spitz nevi; LM, lentigo maligna; MIS, melanoma *in situ*; ePM, early primary melanoma (tumor thickness < 1.5 mm); aPM, advanced primary melanoma (tumor thickness > 1.5 mm); MM, melanoma metastases. IHC, immunohistochemistry; ISH, in situ hybridization; RT-PCR, reverse transcription PCR.

Not only the presence of active MMP, but also the cellular localization of the active form plays a crucial role in cell invasion. Integrin $\alpha_v\beta_3$ can bind and position proteolytically active MMP-2 on the cell surface of melanoma cells (Brooks *et al*, 1996). We have recently shown, in a melanoma xenograft model consisting of the $\alpha_v\beta_3$ negative cell line BLM, its β_3-transfected counterpart, and xenografts derived from these cells, that MT1-MMP and $\alpha_v\beta_3$ are colocalized on the cell membrane of the melanoma cells. Expression of $\alpha_v\beta_3$ cell lines and xenografts was accompanied by an accumulation of cell-surface-bound active MT1-MMP and active MMP-2, suggesting a role of MT1-MMP in activating MMP-2 bound to $\alpha_v\beta_3$ (Hofmann *et al*, 2000b). Similarly, the cell surface hyaluron receptor CD44 promotes cell-mediated collagen IV degradation and tumor cell invasion by anchoring proteolytic active MMP-9 on the cell surface of human melanoma cells (Yu and Stamenkovic, 1999). Taken together these results indicate that the collaboration between cell adhesion molecules such as integrins and CD44, and MMPs at the cell surface, may result in highly localized and tightly regulated pericellular degradation of the ECM (Murphy and Gavrilovic, 1999).

MMPS IN HUMAN MELANOCYTIC TUMORS

Different proteolytic enzymes, including the plasminogen activator system, aspartyl proteases, cystein proteases, and MMPs, have been identified in human cutaneous melanoma progression (de Vries *et al*, 1996; Ferrier *et al*, 1998). Regarding the MMPs, expression levels of several components may increase, including MMP-1 (Airola *et al*, 1999), MMP-2 (Väisänen *et al*, 1996, 1998; Hofmann *et al*, 2000a), MT1-MMP (Airola *et al*, 1999; Hofmann *et al*, 2000a), MMP-9 (van den Oord *et al*, 1997), MMP-13 (Airola *et al*, 1999), as well as TIMP-1 and TIMP-3 (Airola *et al*, 1999) (**Table I**). Using several techniques most of these enzymes were detected in melanoma cells as well as in tumor-surrounding stromal cells (Woolley and Grafton, 1980; Airola *et al*, 1999; Hofmann *et al*, 2000a), strongly indicating that there are important reciprocal interactions between tumor cells and stromal cells. To date, only a few studies have been published on the distribution of MMPs in human melanocytic lesions comprising the consecutive stages of melanocytic tumor progression, including common nevi, atypical or dysplastic nevi, melanoma *in situ*, primary melanoma, and melanoma metastasis (Väisänen *et al*, 1996; van den Oord *et al*, 1997; Hofmann *et al*, 2000a). Väisänen *et al* performed two immunohistochemical studies on paraffin-embedded human cutaneous melanocytic tumors (Väisänen *et al*, 1996, 1998). In the first study on 118 cases expression of MMP-2 was found in both benign and malignant lesions. Expression of MMP-2, however, increased notably with architectural disorder, atypia, and progression to melanoma. Furthermore, increased MMP-2 expression correlated with hematogeneous metastasis (Väisänen *et al*, 1996). In a later study on 50 cases of primary cutaneous melanomas the same authors reported a correlation of MMP-2 expression with human melanoma prognosis. A correlation between high expression of MMP-2 and low survival rate was shown, which was independent of Clark and Breslow microstage. Remarkably, there was a distinct predominance of MMP-2 positivity in male patients, followed by an unfavorable prognosis due to hematogeneous metastasis (Väisänen *et al*, 1998). Similar results were described in other studies (Väisänen *et al*, 1996; Hofmann *et al*, 2000a), thereby establishing the importance of MMP-2 in melanoma progression.

A relation between tumor progression and MT1-MMP expression was described as well. Expression of MT1-MMP protein increased in advanced primary melanoma and melanoma metastases, and was associated with melanoma progression (Airola *et al*, 1999; Hofmann *et al*, 2000a). Induction of MMP-1 and MMP-13 mRNA expression as well as their tissue inhibitors TIMP-1 and TIMP-3 has been described as a late event in melanocytic tumor progression, and was correlated with the level of invasion of cutaneous melanoma in three cases of lentigo maligna and 28 cases of Clark level I-V melanomas (Airola *et al*, 1999). An inverse relationship between MMP expression and melanoma progression was found for MMP-9. In contrast to the *in vitro* data mentioned before (MacDougall *et al*, 1995, 1999), another immunohistochemical study on 33 benign and 41 malignant melanocytic lesions

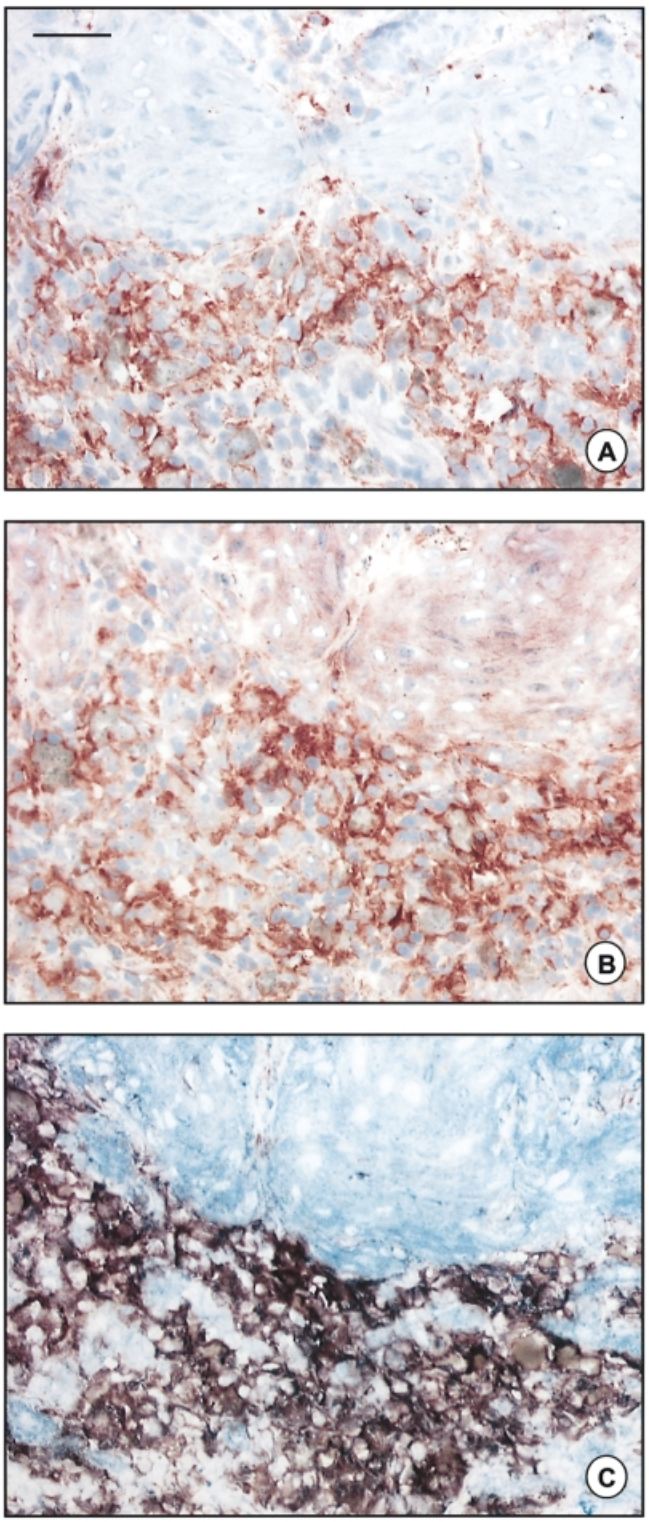

Figure 3. Immunostaining of MMP-2 and MT1-MMP at the tumor-stroma interface at the invasive front of a primary melanoma. (*A*) MMP-2; (*B*) MT1-MMP; (*C*) double staining for MMP-2 (red) and MT1-MMP (blue) of the same lesions. The purple staining represents double labeling of MMP-2 and MT1-MMP; note that all MMP-2 positive cells also expressed MT1-MMP (purple); in this section no counter staining was used. *Scale bar*: 12 μm.

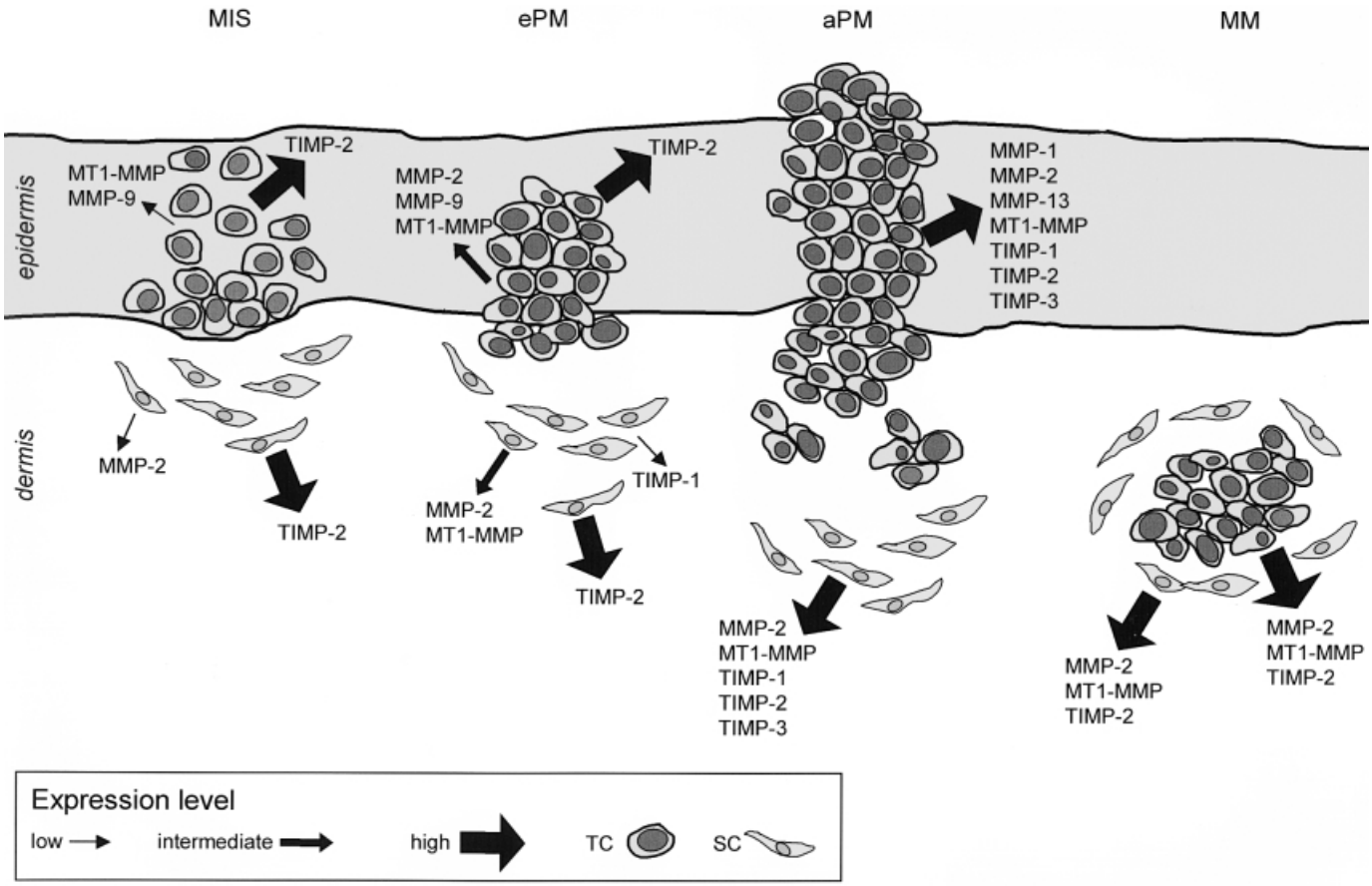

Figure 4. Expression of MMPs and TIMPs in tumor cells (TC) and tumor-surrounding stromal cells (SC) of melanoma *in situ* (MIS), early primary melanoma (ePM) in the horizontal growth phase (< level III), advanced primary melanoma (aPM) in the vertical growth phase (≥ level III), and melanoma metastasis (MM). In tumor cells expression of MMP-1, MMP-2, MMP-13, MT1-MMP, TIMP-1, and TIMP-3 increases during melanoma progression. Additionally, stromal cells surrounding later stage melanoma show increased expression of MMP-2, MT1-MMP, TIMP-1, and TIMP-3, whereas TIMP-2 shows comparable high expression levels in both cell types. Expression of MMP-9 is detectable in tumor cells only in the horizontal growth phase and is absent in the vertical growth phase of primary melanoma and melanoma metastases. Expression of MMP-9 in stromal cells has not yet been described.

revealed that MMP-9 and the ECM metalloproteinase inducer EMMPRIN were exclusively expressed in the horizontal growth phase but absent in the vertical growth phase, suggesting that expression of both factors is an early event in degradation of the ECM and invasion of melanoma cells (van den Oord *et al*, 1997). No expression of stromelysin 3 (MMP-11) mRNA and protein was found in different types of melanocytic nevi and in malignant melanoma, indicating that stromelysin 3 may be not involved in melanoma progression (Wagner *et al*, 1992; Thewes *et al*, 1999).

As already shown in mouse model systems (Hofmann *et al*, 1999), not only expression of MMPs, but rather the presence of functionally active components is required for tumor progression. The importance of proteolytically active MMP-2 in melanoma progression is demonstrated in a study describing the expression and/or activation status of MMP-2 and its activators MT1-MMP and TIMP-2 in 60 fresh human melanocytic lesions comprising all different stages of melanocytic tumor progression (Hofmann *et al*, 2000a). Expression of both MMP-2 and MT1-MMP was clearly increased in primary melanoma and melanoma metastasis, whereas TIMP-2 was strongly expressed in both benign and malignant lesions. MMP-2 and MT1-MMP positive tumor cells were often localized in subepidermal nests of primary melanoma, and/or at the tumor-stroma interface at the invasive front of both primary melanomas and melanoma metastases (**Fig 3**). Double staining experiments demonstrated that all tumor cells expressing MMP-2 also expressed MT1-MMP and TIMP-2. Zymography of melanoma metastases showed that MMP-2 was present in its functionally active form. These results indicate that enhanced expression of MT1-MMP is associated with increased expression and activation of MMP-2, and that both are correlated with malignant progression of melanocytic tumors. In tumor progression an imbalance between MMPs and TIMPs (high levels of MMPs and low levels of TIMPs) has been implicated. In agreement with the *in vitro* data, however, also in human melanoma lesions partly contradictory expression profiles of TIMPs have been described. Increased expression of TIMPs produced by tumor cells and tumor-surrounding stromal cells was associated with melanoma progression (Airola *et al*, 1999). Whether this increased expression of TIMPs may reflect host response to tumor invasion, in an effort to control MMP activity and preserve ECM integrity, is not clear and requires further investigation (Airola *et al*, 1999). Recent studies in other tumor types, however, have shown that high levels of TIMPs have not only antimetastatic effects but also correlate with poor prognosis (see the review by Curran and Murray, 1999). Interestingly, identical biologic and clinical findings have been reported for PAI-1, an inhibitor of the serine proteases uPA and tPA (see the review by Ferrier *et al*, 1998).

In general, a great variety of staining aspects for MMPs can be observed both within the same lesion and within different lesions in the same progression stage, illustrating the strong phenotypical heterogeneity found within one tumor and among comparable tumors. These findings suggest that degradation of the ECM followed by invasion of tumor cells is limited to focal microscopic sites of the tumors.

The fact that expression of MMP-1, MMP-13, TIMP-1, and TIMP-3 (Airola *et al*, 1999), as well as MMP-2 and MT1-MMP

(Väisänen *et al*, 1998; Hofmann *et al*, 2000a), is increased in advanced phases and that expression of MMP-9 and EMMPRIN is limited to early phases of melanoma indicates that different MMPs may be involved in degradation of the ECM during different stages of tumor progression (van den Oord *et al*, 1997).

Both tumor cells and tumor-surrounding stromal cells have the capacity to express MMPs and TIMPs, which may promote melanoma cell invasion and metastasis formation. During melanoma progression increased expression of MMP-2, MT-MMP, TIMP-1, and TIMP-3 was detected not only in tumor cells but also in stromal cells, whereas expression of TIMP-2 in both cell types was comparable in all tumor stages (Airola *et al*, 1999; Hofmann *et al* 2000a). **Figure 4** illustrates the expression profiles of MMPs and TIMPs in tumor cells and stromal cells in human melanoma progression. Further studies are needed to determine whether melanoma cells can stimulate or enhance stromal production of MMPs and TIMPs, and to elucidate the specific contribution of MMPs and TIMPs expressed by stromal cells during melanoma progression.

CLINICAL RELEVANCE AND CONCLUSIONS

This review demonstrates that MMPs and TIMPs play important roles in melanoma progression. In human primary melanoma and melanoma metastases expression of different MMPs and TIMPs are correlated with the stage of melanocytic tumor progression. Beside the possible role of individual MMPs as a marker of melanoma progression, expression of MMP-2 has been found to correlate with unfavorable prognosis (Väisänen *et al*, 1998). To evaluate the prognostic significance for individual MMPs, however, comprehensive studies of the MMP profile in large defined series of patients with known pathologic and clinical data are needed.

In addition, inhibition of MMP activity has been investigated as a new method to control metastatic spread (Curran and Murray, 1999; Kleiner and Stetler-Stevenson, 1999; Stetler-Stevenson, 1999), and consequently several pharmaceutical companies are currently developing low-molecular-weight MMP inhibitors for clinical purposes (Brown and Giavazzi, 1995; Watson *et al*, 1995, 1996, 1999; Giavazzi *et al*, 1998; Steward, 1999). New therapies designed to interfere with specific MMP actions may be useful in the treatment of metastatic melanoma.

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