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Invited Review

Matrix metalloproteinases in squamous cell carcinoma

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Summary. Controlled degradation of extracellular matrix (ECM) is essential in many physiological situations including developmental tissue remodeling, angiogenesis, tissue repair, and normal turnover of ECM. In addition, degradation of matrix components is an important feature of tumor growth, invasion, metastasis, and tumor-induced angiogenesis. Matrix metallo-proteinases (MMPs) are a family of zinc-dependent neutral endopeptidases, which are collectively capable of degrading essentially all ECM components. MMPs apparently play an important role in all the above mentioned aspects of tumor development. In addition, there is recent evidence that MMP activity is required for tumor cell survival. At present, several MMP inhibitors are in clinical trials of malignant tumors of different histogenetic origin. In this review we discuss the current view on the role of MMPs and their inhibitors in development and invasion of squamous cell carcinomas, as a basis for prognostication and therapeutic intervention in these tumors.

Key words: Squamous cell carcinoma, Invasion, Matrix Metalloproteinase

Proteolytic remodeling of extracellular matrix

Degradation of components of extracellular matrix (ECM) is important in many physiological as well as pathological conditions. Interstitial connective tissue and basement membrane can be degraded by four classes of proteolytic enzymes: cystein proteinases, aspartic proteinases, serine proteinases, and metalloproteinases (see Birkedal-Hansen et al., 1993). There is considerable evidence that matrix metalloproteinases (MMPs) play an important role in remodeling of the ECM in physiological situations mentioned above, and that excessive breakdown of ECM by MMPs occurs in many pathological conditions including periodontitis, autoimmune blistering disorders of skin, dermal photoageing, rheumatoid arthritis, osteoarthritis, and chronic ulcerations. In addition, controlled degradation of ECM by MMPs is thought to play an important role in tumor invasion and metastasis (for reviews see Kahari and Saarialho-Kere, 1997, 1999; Johnsen et al., 1998; Shapiro, 1998).

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Matrix rnetalloproteinases (MMPs)

To date, 18 mammalian MMPs have been identified and characterized by cDNA cloning. According to structure and substrate specificity MMPs can be divided into subgroups of collagenases, stromelysins, gelatinases, membrane-type MMPs (MT-MMPs) and other MMPs (Table 1). MMPs have a characteristic multi-domain structure (Fig. 1) consisting of 1) signal peptide that directs secretion of the proenzyme, 2) propeptide, which contains a conserved amino acid sequence (PRCGxPD), which participates in formation of the cystein switch, and which is essential for maintaining the proMMP in latent form, 3) catalytic domain containing the highly conserved Zn^{2+} binding site (HExGHxxGxxHS/T), 4) proline-rich hinge region that links the catalytic domain to 5) hemopexin-like domain, which determines the substrate specificity of MMP. In addition, the catalytic domain of the two gelatinases contains three repeats of the fibronectin type II domain, which allow these enzymes to bind gelatin. MT-MMPs contain a transmembrane domain of 20 hydrophobic amino acids in the C-terminal end of hemopexin domain followed by a 24 amino acid intracellular domain (Sato et al., 1994; Cao et al., 1995).

Collagenases

The human collagenase subfamily consists of three members, collagenase-l (MMP-1), collagenase-2 (MMP-8), and collagenase-3 (MMP-13). These are the principal secreted neutral proteinases capable of degrading native fibrillar collagens of types I, II, III, V, and XI in the extracellular space. All collagenases cleave fibrillar collagens at a specific site between Gly_{775} and

Table 1. Human matrix metalloproteinase family, substrates, exogenous activators and activating capacity

Enzyme	Structure	Substrates	Activated by	Activator of
Collagenases				
Collagenase-1 (MMP-1)		FIB COL I, II, III,(III>>I)VII, VIII, X AGR, serpins, α2M	MMP-3, -10, plasmin kallikrein, chymase	MMP-2
Collagenase-2 (MMP-8)		FIB COL I, II, III, (I>>III), AGR serpins, α2M	MMP-3, -10, plasmin	ND
Collagenase-3 (MMP-13)		FIB COL I, II, III, (II>>I) IV, IX, X, XIV GEL, FN, LN, TN, AGR, serpins	MMP-2, -3, -10, -14, -15 plasmin	MMP-2, -9
Stromelysins				
Stromelysin-1 (MMP-3)		Type IV COL, FN, AGR, nidogen	plasmin, kallikrein, chymase	MMP-1, -7, -8, -9, -13
Stromelysin-2 (MMP-10)		as MMP-3	tryptase, elastase, cathepsin G	MMP-1, -8, -13
Stromelysin-3 (MMP-11)		α1-proteinase inhibitor	furin	ND
Metalloelastase (MMP-12)		COL IV, GEL, FN, LN, VN, α1-antitrypsin, EL	ND	ND
Matrilysin (MMP-7)		as MMP-3, EL, entactin, TN	MMP-3, plasmin	MMP-2
Gelatinases				
Gelatinase-A, 72 kDa (MMP-2)		GEL, COL I, IV,V, VII, X, FN, TN, α2M	MMP-1, -7, -13, -14, -15, -16	MMP-9, -13
Gelatinase-B, 92 kDa (MMP-9)		GEL, COL I, IV,V,VII, XI XIV,EL, α2M	MMP-2, -3, -13, plasmin	ND
Membrane-type MMPs				
MT1-MMP (MMP-14)		COL I, II, III, GEL, FN, LN, VN, AGR TN, nidogen, perlecan	Plasmin, furin	MMP-2, -13
MT2-MMP (MMP-15)		FN, LN, AGR, TN, nidogen, perlecan	ND	MMP-2, -13
MT3-MMP (MMP-16)		COL III, FN, GEL, casein	ND	MMP-2
MT4-MMP (MMP-17)		ND	ND	ND
Other MMPs				
MMP-19		ND	trypsin	ND
Enamelysin (MMP-20)		amelogenin	ND	ND

Modified from Murphy and Knäuper 1997 ; Kähäri and Saarialho-Kere, 1997. FIB, fibrillar, COL, collagen; AGR, aggregan; GEL, gelatin; FN, fibronectin, LN, laminin; TN, tenascin; α2M, α2-macroglobulin; EL, elastin; ND, not determined. See Fig 1. for details of structure.

Leu/Ile_{776} of the α-chains, resulting in generation of N-terminal 3/4 and C-terminal 1/4 fragments, which rapidly denature to gelatin in body temperature and are further degraded by other MMPs, e.g. gelatinases. MMP-1 preferentially degrades type III collagen and MMP-8 prefers type I collagen. MMP-13 cleaves type II collagen 6-fold more effectively than type I and III collagens and displays 40-fold stronger gelatinolytic activity than MMP-1 and MMP-8 (Knäuper et al., 1996a; Mitchell et al., 1996). In addition to the classical cleavage site, both human and mouse MMP-13 cleave type I collagen in N-terminal non-helical telopeptide (Liu et al., 1995; Krane et al., 1996). N-telopeptidase activity appears to be sufficient for resorption of type I collagen during fetal development and early postnatal life of mice, whereas triple helicase activity is necessary during intense tissue resorption, e.g. involution of postpartum uterus and also in normal turnover of collagen in murine skin (Liu et al., 1995).

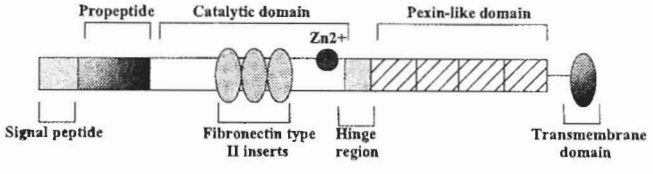

Fig. 1. Prototype of MMPs (modified from Birkedal-Hansen et al., 1993).

Human MMP-1 was the first MMP, the primary structure of which was determined by cDNA cloning (Goldberg et al., 1986). Latent MMP-1 is secreted as a major 52 kDa or a minor glycosylated 57 kDa form, which are proteolytically activated by propeptide cleavage to 42 and 47 kDa forms, respectively (see Nagase, 1997). MMP-1 is secreted by various types of cells including fibroblasts, keratinocytes, endothelial cells, macrophages, hepatocytes, chondrocytes, osteoblasts, and tumor cells (Birkedal-Hansen et al., 1993).

MMP-8 is synthesized by polymorphonuclear

leucocytes during their maturation in bone marrow, stored in their intracellular granules, and released in response to external stimuli as 55 kDa and glycosylated 75 kDa forms (Hasty et al., 1986, 1990). In addition, MMP-8 is expressed by chondrocytes, rheumatoid synovial fibroblasts, endothelial cells, in osteoarthritic cartilage, and melanoma cells (Cole et al., 1996; Hanemaaijer et al., 1997; Shlopov et al., 1997; Giambernardi et al., 1998). The substrate specificity of MMP-8 is similar to that of MMP-1 (Hasty et al., 1987; Table 1). Recently, the cDNA of mouse MMP-8 was cloned and was shown to be expressed in postpartum uterus (Balbín et al., 1998).

Human MMP-13, originally cloned from breast carcinoma tissue shows high homology (86% at amino acid level) to rat and mouse interstitial collagenases and only 50% homology to human MMP-1 indicating that these rodent collagenases are counterparts for human MMP-13 instead of MMP-1 (Freije et al., 1994). Furthermore, no rat or murine homologue for human MMP-1 has been found. The exceptionally wide substrate specificity and limited expression distinguishes MMP-13 from other MMPs. In addition to fibrillar collagens and gelatin, MMP-13 degrades type IV, IX, X, and XIV collagens, large tenascin C isoform, fibronectin, laminin, aggrecan core protein and serine proteinase inhibitors (Fosang et al., 1996; Knäuper et al., 1996a, 1997a; Mitchell et al., 1996). Latent MMP-13 is activated *in vitro* by organomercurials (e.g. APMA), trypsin, plasmin, stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), 72-kDa gelatinase (MMP-2), MT1-MMP (MMP-14), and MT2-MMP (MMP-15) (Knäuper et al., 1996a,b; Murphy and Knäuper, 1997; d´Ortho et al., 1997) and its activity is inhibited by tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-3 and less potently by TIMP-2 (Knäuper et al., 1996a, 1997a). MMP-13 also activates latent MMP-2 and 92-kDa gelatinase (MMP-9) (Knäuper et al., 1997b; Murphy and Knäuper, 1997).

Apparently due to its wide substrate specificity, the physiological expression of human MMP-13 is restricted to developing fetal bone, in which it is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts, but not by osteoclasts (Johansson et al., 1997a; Ståhle-Bäckdahl et al., 1997). In contrast, expression of human MMP-13 has been observed in several pathological conditions characterized by excessive degradation of collagenous ECM, i.e. osteoarthritis and rheumatoid arthritis (Mitchell et al., 1996; Reboul et al., 1996; Lindy et al., 1997; Moldovan et al., 1997; Shlopov et al., 1997; Ståhle-Bäckdahl et al., 1997; Tetlow and Woolley et al., 1998), chronic cutaneous ulcers (Vaalamo et al., 1997), intestinal ulcerations (Vaalamo et al., 1998), and chronic periodontal inflammation (Uitto et al., 1998). In addition, MMP-13 expression has been detected in invasive malignant tumors, i.e. breast carcinomas (Freije et al., 1994; Heppner et al., 1996; Uría et al., 1997), squamous cell carcinomas (SCCs) of the head and neck (Johansson et al., 1997c; Airola et al., 1997, Cazorla et al., 1998) and the vulva (Johansson et al., 1999), and chondrosarcomas (Uría et al., 1998).

Stromelysins

Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) are closely related with respect to structure and substrate specificity. MMP-3 and MMP-10 are expressed by fibroblastic cells, and their expression has also been observed in normal and transformed epithelial cells *in vitro* and *in vivo* (Windsor et al., 1993; Saarialho-Kere, 1998). MMP-3 and MMP-10 degrade a wide range of ECM proteins including fibronectin, type IV, V, IX, and X collagens, proteoglycans, gelatin, fibronectin, and laminin (Birkedal-Hansen et al., 1993; Chandler et al., 1997). In addition, MMP-3 cleaves α1-proteinase inhibitor, TNF-α precursor, and myelin basic protein (Chandler et al., 1997) and degrades and inactivates IL-1ß (Ito et al., 1996).

Stromelysin-3 (MMP-11), matrilysin (MMP-7), and metalloelastase (MMP-12) are often included in the stromelysin subgroup, although they are structurally less closely related to MMP-3 and MMP-10 (Birkedal-Hansen et al., 1993). MMP-11 was cloned from human breast cancer cDNA library and it is also expressed in uterus, placenta, and involuting mammary gland (Basset et al., 1990). To date, human MMP-11 has not been shown to degrade any ECM component, but instead it degrades serine proteinase inhibitors, α1-proteinase inhibitor and α1-anti-trypsin (Pei et al., 1994).

Matrilysin (MMP-7) is the smallest MMP due to absence of the hemopexin domain. MMP-7 is expressed by normal glandular epithelial cells, e.g. of the cycling human endometrium, small intestinal crypts, skin, and airways (Saarialho-Kere et al., 1995; Dunsmore et al., 1998). MMP-7 is also expressed by malignant epithelial cells in tumors of the gastrointestinal tract, prostate, and breast (Karelina et al., 1994; Wilson and Matrisian, 1996). In addition to a wide range of ECM components (fibronectin, laminin, nidogen, type IV collagen, and proteoglycans), MMP-7 cleaves ß4 integrin (von Bredow et al., 1997).

Macrophage metalloelastase (MMP-12) is expressed in placenta, by alveolar macrophages in pulmonary emphysema and by fibroblasts in granulomatous diseases of the intestine and skin (Shapiro et al., 1993; Belaaouaj et al., 1995; Vaalamo et al., 1998, 1999). MMP-12 degrades elastin, type IV collagen, type I gelatin, fibronectin, laminin, vitronectin, proteoglycans, myelin basic protein, and α1-antitrypsin (Chandler et al., 1996).

Gelatinases

The gelatinase subgroup of MMPs consists of 72-kDa gelatinase (gelatinase-A, MMP-2) and 92-kDa gelatinase (gelatinase-B, MMP-9). MMP-2 is expressed by various cell types, including fibroblasts, keratino-

cytes, endothelial cells, chondrocytes, osteoblasts, monocytes and by different types of transformed cells (see Birkedal-Hansen et al., 1993). MMP-9 is produced by normal alveolar macrophages, polymorphonuclear leukocytes, osteoclasts, keratinocytes, invading trophoblasts, and by several types of transformed cells (see Birkedal-Hansen et al., 1993). Gelatinases degrade type IV, V, VII, X, XI, and XIV collagens, gelatin, elastin, proteoglycan core proteins, myelin basic protein, fibronectin, and precursors of TNF-α and IL-1ß (Birkedal-Hansen et al., 1993; Ito et al., 1996; Chandler et al., 1997). MMP-2 also degrades native type I collagen and activates latent MMP-9 and MMP-13 (Aimes and Quigley, 1995; Fridman et al., 1995; Knäuper et al., 1996b). MMP-9 also cleaves N-terminal telopeptide of type I collagen in acidic environment (Okada et al., 1995a). Thus, it is possible that MMP-2 and MMP-9 also play a role in the remodeling of collagenous ECM under certain conditions. Activation of MMP-2 by MT1-MMP occurs at the cell membrane by a mechanism involving interaction of the C-terminus of proMMP-2 with MT1-MMP/TIMP-2 complex (Sato et al., 1996a; Butler et al., 1998; Zucker et al., 1998).

Membrane-type MMPs

The MT-MMP subgroup contains four members: MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), and MT4-MMP (MMP-17) (Table 1). MT-MMPs contain a furin cleavage site between propeptide and the catalytic domain providing basis for furin-dependent activation of latent MT-MMPs prior to secretion. MT1-MMP activates latent MMP-2 and degrades type I, II, and III collagen, gelatin, fibronectin, laminin-1, vitronectin and cartilage proteoglycans (Ohuchi et al., 1997). MT1-MMP is expressed by dermal fibroblasts, malignant epithelial cells, and osteoclasts (Sato et al., 1994, 1997; Okada et al., 1995b). MT2-MMP is expressed in human placenta, brain, and heart. MT2-MMP also activates proMMP-2 and proMMP-13 and degrades laminin, fibronectin, and tenascin (Takino et al., 1995; d´Ortho et al., 1997). MT3-MMP expression has been detected in lung, placenta, kidney, ovary, intestine, prostate, spleen, heart, and skeletal muscle (Will and Hinzmann, 1995). MT3-MMP hydrolyzes gelatin, casein, type III collagen and fibronectin and it also activates proMMP-2 (Matsumoto et al., 1997; Shofuda et al., 1997). MT4-MMP is expressed in the brain, leukocytes, colon, ovary, testis, breast carcinomas, and breast cancer cell lines (Puente et al., 1996). The substrate specificity of MT4-MMP is not known.

Other MMPs

Two recently cloned human MMPs do not fit well into any subgroup mentioned above, based on structure and substrate specificity. MMP-19, initially named MMP-18 was cloned from human mammary gland and liver cDNA and it is expressed in a wide variety of human tissues (Cossins et al., 1996; Pendás et al., 1997b). The ability of MMP-19 to degrade native ECM components is not known. Enamelysin (MMP-20) was cloned from odontoblasts, has a restricted expression in dental tissues and degrades amelogenin (Llano et al., 1997).

Regulation of MMP activity

In general, MMPs are not constitutively expressed by cells *in vivo*, but their expression is induced in response to exogenous signals, e.g. cytokines or growth factors, and altered cell-matrix and cell-cell interactions (see Birkedal-Hansen et al., 1993; Kähäri and Saarialho-Kere, 1997). As exceptions to this rule, collagenase-2 (MMP-8) and 92-kDa gelatinase (MMP-9) are stored in secretory granules of neutrophils and eosinophils (Ståhle-Bäckdahl and Parks, 1993) and matrilysin (MMP-7) in secretory epithelial cells in exocrine glands of e.g. skin, gastrointestinal tract, and airways (Saarialho-Kere et al., 1995; Dunsmore et al., 1998). Expression of MMPs is primarily regulated at the level of transcription, although modulation of MMP mRNA half-life by growth factors and cytokines has also been observed. The proteolytic activity of MMPs is regulated by zymogen activation and inhibition of proteolytic activity by specific inhibitors, i.e. TIMPs and by non-specific proteinase inhibitors, e.g. α1-proteinase inhibitor and α2-macroglobulin.

Transcriptional regulation

Expression of several MMPs (MMP-1, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12, and MMP-13) in unstimulated cells in culture is low, but it is induced at transcriptional level e.g. by growth factors and cytokines, oncogenes, hormones, and contact to ECM (for review, see Birkedal-Hansen et al., 1993). The promoters of these inducible MMPs contain a conserved AP-1 binding site between -65 and -79 with respect to transcription start site (see Benbow and Brinckerhoff, 1997; Westermarck and Kähäri, 1999). The extracellular stimuli result in activation of the nuclear AP-1 transcription factor complexes (dimers composed of members of Fos and Jun families) (Karin et al., 1997), which bind to the AP-1 *cis*-element in the promoter and stimulate transcription of the MMP genes. The expression of the components of the classical AP-1 dimer, c-Jun and c-Fos is induced as a result of activation of three distinct classes of mitogen-activated protein kinases (MAPKs), i.e. extracellular signal-regulated kinase (ERK), stress-activated protein kinase/Jun N-terminal kinases (SAPK/JNKs), and p38. In general, ERK1,2 cascade is activated by mitogenic signals, resulting in phosphorylation of various substrates, including Elk-1, and in subsequent activation of c-*fos* transcription. SAPK/JNKs and p38 are activated by cytokines (TNF, IL-1) and cellular stress, such as UV light, resulting in phosphorylation of c-Jun and ATF-2

by JNKs, and ATF-2 by p38, which then induce c-*jun* transcription (see Karin et al., 1997).

The promoter regions of the AP-1 responsive MMPs also contain one or multiple PEA3 (polyomavirus enhancer A-binding protein-3) elements, which serve as binding sites for transcription factors of ETS family and cooperate with AP-1 element for maximal activation of MMP-1, MMP-3, and MMP-9 promoter (Wasylyk et al., 1991; Westermarck et al., 1997). However, the PEA3 site in the human MMP-13 promoter may not have a significant role in the transcriptional regulation of human MMP-13 gene (Pendás et al., 1997b; Tardif et al., 1997). Expression of ETS-1 has been demonstrated in stromal fibroblasts adjacent to invading tumor cells and in endothelial cells during tumor vascularization (Wernert et al., 1992, 1994; Bolon et al., 1995).

MMP-2 promoter is relatively unresponsive to stimulation in cultured cells and it lacks not only the adjacent AP-1 and PEA3 elements, but also the classical TATA box, although it has been suggested that a sequence at -26 bp drives basal activity (Templeton and Stetler-Stevenson, 1991). MT1-MMP is also constitutively expressed by different types of cells in culture (Lohi et al., 1996). However, in vulvar SCC cells expression of MT1-MMP is susceptible to enhancement by growth factors (Johansson et al., 1999).

Zymogen activation

Most MMPs are secreted as latent precursors or zymogens, which are proteolytically activated in extracellular space (for review, see Nagase, 1997). The latency of MMPs is dependent on "cystein switch" formed by covalent interaction of the conserved cystein in the propeptide with the catalytic zinc (van Wart and Birkedal-Hansen, 1990). Various compounds, e.g. organomercurials (APMA) can react with cystein, converting it to nonbinding form. Alternatively, chaotropic agents (e.g. KI, NaSCN) can cause the propeptide to fold back, disrupting the cystein-zinc bond. In either case, the catalytic site is exposed and the enzyme then cleaves the propeptide autolytically. The propeptide of most MMPs can be cleaved by a number of other extracellular proteinases, e.g. plasmin and other MMPs (Table 1). For example, MMP-1 is activated by plasmin, trypsin and neutrophil elastase and superactivation of MMP-1 is achieved by further cleavage of propeptide by MMP-3 or MMP-10 (He et al., 1989). All four MT-MMPs and MMP-11 contain a potential cleavage site for the prohormone convertases (e.g. furin) which occur in the Golgi complex and pericellular space (Murphy and Knäuper, 1997), suggesting that they are processed by these proteinases (Pei and Weiss, 1995; Sato et al., 1996b). Activation of latent MMP-2 and MMP-13 at cell membrane by MT1-MMP provides a potent way of directing their activity to pericellular environment.

Inhibition of MMP activity

The proteolytic activity of MMPs is strictly controlled in the pericellular space by non-specific inhibitors, e.g. α2-macroglobulin and by specific inhibitors, TIMPs. α2-macroglobulin inactivates susceptible proteinases by entrapment following cleavage of the bait region and it is a 150-fold better substrate for MMP-1 than triple helical type I collagen (see Birkedal-Hansen et al., 1993).

TIMPs

At present, four members of the TIMP gene family are known: TIMP-1, -2, -3, and -4. All four TIMPs share structural features, especially 12 conserved cystein residues, which form six disulfide bonds (for review see Douglas et al., 1997; Gomez et al., 1997). TIMPs bind to the zinc-binding catalytic site of the MMPs with 1:1 molar ratio. In addition, TIMP-2 and TIMP-1 can bind to the hemopexin domain of latent MMP-2 and MMP-9, respectively. TIMP-1 potently inhibits the activity of most MMPs, with the exception of MT1-MMP and MMP-2. TIMP-2 also inhibits activity of most MMPs, except MMP-9. TIMP-3 has been shown to inhibit the activity of MMP-1, -2, -3, -9, and -13 (Apte et al., 1995; Knäuper et al., 1996a). Human TIMP-4 effectively inhibits activity of MMP-2, -9, and -7 (Greene et al., 1996; Douglas et al., 1997).

TIMPs are expressed by a variety of cell types and they apparently play an important role e.g. in tissue development, angiogenesis, cancer cell invasion and metastasis by regulating MMP activity and stimulating cell growth (Douglas et al., 1997; Gomez et al., 1997). TIMP-1, TIMP-2, and TIMP-4 are secreted in soluble form, whereas TIMP-3 is associated with the ECM (Leco et al., 1994; Kishnani et al., 1995). Expression of TIMP-1 in cultured cells is up-regulated e.g. by growth factors, cytokines, and phorbol ester, whereas the expression of TIMP-2 is mainly constitutive (Gomez et al., 1997). Expression of TIMP-3 is induced in response to mitogenic stimulation and during normal cell cycle progression (see Gomez et al., 1997) and it is inhibited by TNF-α in fibroblasts (Mattila et al., 1998). Expression of TIMP-4 is mainly restricted to the human heart (Greene et al., 1996).

Proteolysis of ECM during tumor invasion

Interaction of tumor cells with stromal ECM components and cells is important for the growth and invasion of a malignant tumor. Tumor growth involves alterations in the stromal ECM (Iozzo, 1995) and malignant tumors often induce a fibroproliferative response in the adjacent stroma, characterized by increased expression of type I and III procollagens (Kauppila et al., 1996). The formation of tumor stroma is often viewed as a non-specific host response in an attempt to wall off the tumor, and it is thought to have a negative influence on tumor progression. In this context it is interesting that MMP-3, MMP-7, MMP-9, and MMP-12 have recently been shown to generate angiostatin from plasminogen, indicating that their

expression in peritumoral area may in fact serve to limit tumor-induced angiogenesis (Dong et al., 1997; Patterson and Sang, 1997; Lijnen et al., 1998).

Migration and invasion of malignant cells through ECM involves their attachment to matrix components via integrin receptors (Heino, 1996). During the multi-step process of metastasis formation, cancer cells detach from the primary tumor, invade the stromal tissue, enter the circulation, arrest at the periferal vascular bed, extravasate, invade the target organ interstitium and parenchyma, and proliferate as a secondary colony (Stetler-Stevenson et al., 1993; Johnsen et al., 1998) (Fig. 2). At any stage, tumor cells must overcome the host immune response and therefore only a fraction of circulating tumor cells successfully initiate metastatic colonies (Liotta et al., 1991). Tumor induced angiogenesis is essential for expansion of the primary tumor and metastases, and new blood vessels penetrating the tumor are frequent sites for tumor cell entry into the circulation (Liotta et al., 1991). It is conceivable that proteolytic degradation of ECM plays a crucial role in all the above mentioned aspects of tumor development.

MMPs in tumor invasion

A considerable body of evidence has accumulated implicating MMPs in cancer spread. In fact, several MMPs have been first purified and cloned from tumor cell lines or tumor tissues. However, all MMPs known so far are also expressed by non-malignant cells suggesting that there are no cancer specific MMPs. A number of studies have demonstrated a positive correlation between MMP expression, and invasive and metastatic potential of malignant tumors including,

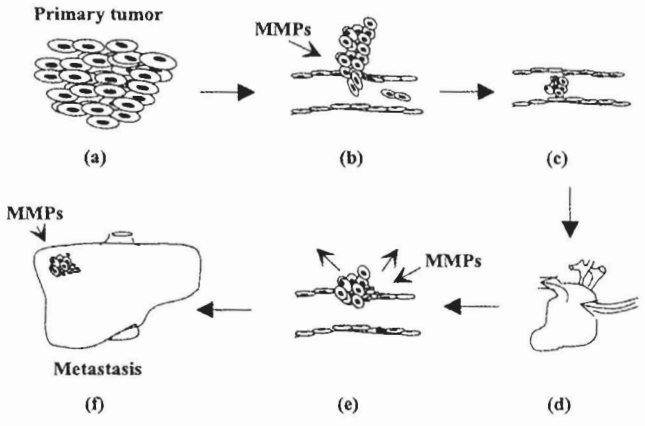

Fig. 2. Spread of malignant tumor. **a.** Primary tumor invades and spreads into adjacent normal tissue, usually coming in contact with blood vessels. **b, c.** Tumor cells penetrate the blood vessels and enter the circulation. **d.** Individual tumor cells are able to pass troughout the body with the help of junctions between lymphatics and blood vessels. At this step only few cells survive. **e.** Tumor cells that survive arrest in distant organs, extravasate, and invade the surrounding normal tissues. **f.** Extravasating tumor cells give rise to secondary tumors (in this example to the liver) and the process might be repeated again. (Modified from Franks and Teich, 1997).

colon, lung, head and neck, basal cell, breast, thyroid, prostate, ovarian, and gastric carcinomas (see Johnsen et al., 1998; Kähäri and Saarialho-Kere, 1999). In addition, studies with MMP inhibitors support the role of MMPs in tumor progression and metastasis (see Brown, 1998; Kähäri and Saarialho-Kere, 1999). Direct evidence for the role of distinct MMPs in tumor growth and invasion has recently been provided by mice with targeted disruption of a specific MMP gene. Interestingly, none of the MMP knock-outs reported have been embryonic lethal (Shapiro, 1998). Mice lacking MMP-7 show decreased intestinal tumorigenesis (Wilson et al., 1997), and MMP-11 deficient mice show impaired tumor formation in response to chemical mutagenesis (Masson et al., 1998). MMP-2 knock-out mice show reduced tumor growth and formation of metastases by Lewis lung carcinomas and B16-BL6 melanoma cells (Itoh et al., 1998).

In malignant tumors many MMPs are not produced by neoplastic cells, but by non-malignant stromal cells (Basset et al., 1997). For example, in SCCs invading tumor cells and stromal cells express distinct MMPs, which may complement each other's substrate specificity and form networks of crosstalking MMP cascades, in which a single MMP cleaves a particular native or partially degraded ECM component and activates other latent MMPs (Fig. 3). Tumor cells also secrete factors, such as extracellular MMP inducer (EMMPRIN), which enhances the expression of MMP-1, MMP-2, and MMP-3 by fibroblasts (Guo et al., 1997). In addition, many growth factors and cytokines secreted by tumor infiltrating inflammatory cells as well as by tumor or stromal cells are capable of modulating MMP expression.

Squamous cell carcinoma

Squamous cell carcinoma (SCC) can develop in squamous epithelia of many organs including the skin, oral cavity, larynx, pharynx, airways, oesophagus, uterine cervix, and vulva. SCC is the most frequent malignant tumor in the oral and maxillofacial region, and its metastatic and invasive ability results in poor

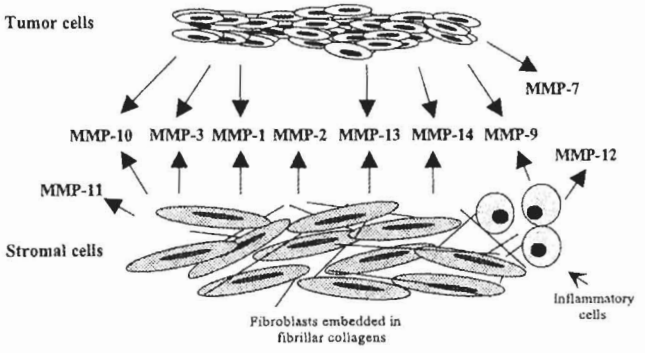

Fig. 3. Expression of MMPs by tumor cells, stromal fibroblasts, and inflammatory cells in SCC.

prognosis. Cutaneous SCCs that arise secondary to inflammatory and ulcerative processes have a much higher rate of metastasis than those developed in sun-damaged skin. SCCs of vulva also have a high invasion and metastasis capacity (Krag, 1991).

SCC of the skin clinically presents as a shallow ulceration surrounded by an elevated, indurated border. The tumor consists of irregular masses of epidermal cells that invade the dermis, composed of normal and atypical squamous cells characterized by variations in the cell size and shape, hyperplasia and hyperchromasia of the nuclei, absence of intracellular bridges, and keratinization of individual cells. SCC cells show a reduction in the number of desmosomes at the cell surface, as compared to normal epidermal squamous cells and microvilli extending into widened intercellular spaces. The more malignant the tumor, the greater is the number of atypical squamous cells (Lever and Schaumburg-Lever, 1990; Krag, 1991). Keratinization of well-differentiated SCCs leads to formation of horn pearls composed of concentric layers of squamous cells, the center of the horn pearl usually showing incomplete keratinization.

MMPs in squamous cell carcinomas of the head and neck

SCCs of skin and oral cavity are characterized by high ability to invade. Accordingly, increased collagenolytic activity has been detected in explants derived from the head and neck SCCs (Burman and Carter, 1985). MMP-1 expression is low in normal, hyperplastic and dysplastic oral mucosa, whereas abundant levels of MMP-1 mRNA have been detected both in tumor cells and stromal fibroblasts of invasive SCCs of the head and neck region (Polette et al., 1991; Gray et al., 1992; Johansson et al., 1997c; Airola et al., 1997; Sawatsubashi et al., 1998; Sutinen et al., 1998). A recent study has shown that expression of MMP-1 is associated with poor prognosis in oesophageal cancer (Murray et al., 1998).

Immunoreactivity for MMP-3 has been detected in small cancer cell nests in the invasive front of SCCs of the oral cavity and skin, but not in normal oral epithelium (Kusukawa et al., 1995; Airola et al., 1997). MMP-3 expression correlates with tumor size, depth of tumor invasion, diffuse invasive mode, and high incidence of lymph node metastasis (Kusukawa et al., 1995). Abundant expression of MMP-10 has also been detected in tumors showing high local invasion (Muller et al., 1991; Polette et al., 1991).

The levels of active MMP-2 in tumor cell nests of metastatic SCCs are significantly higher than in non-metastatic cancer suggesting that active MMP-2 could serve as a predictive marker of metastasis in oral SCCs (Kawamata et al., 1998). Interestingly, MMP-2 and MT1-MMP exhibit similar expression patterns in SCCs of the head and neck, consistent with the role of MT1-MMP in proMMP-2 activation (Okada et al., 1995a; Yoshizaki et al., 1997). Expression of MT1-MMP in SCCs appears to be associated with high a degree of differentiation (Yoshizaki et al., 1997). In SCCs of the skin and oral cavity, interactions between malignant keratinocytes and adjacent stromal fibroblasts are critical in directing expression of MMP-9 to the tumor-stroma interface (Lengyel et al., 1995; Borchers et al., 1997). In addition, MMP-9 is actively expressed by eosinophils and stored by neutrophils in cutaneous SCCs (Ståhle-Bäckdahl and Parks, 1993). MMP-11 transcripts have been observed in stromal fibroblasts surrounding tumor cell islands of invasive head and neck SCCs, and the level of MMP-11 expression correlates with increased local invasion in these tumors (Muller et al., 1993).

Our recent observations show that in SCCs of the skin, oral cavity, pharynx, and larynx MMP-13 is primarily expressed by tumor cells at the invading front of the tumor, but in some cases also by stromal fibroblasts (Airola et al., 1997; Johansson et al., 1997c). Interestingly, no expression of MMP-13 has been detected in premalignant tumors of skin, in intact or re-epithelializing epidermis, healthy oral mucosa, or in normal keratinocytes in culture (Airola et al., 1997; Johansson et al., 1997b,c; Vaalamo et al., 1997; Uitto et al., 1998). Therefore, expression of MMP-13 appears to serve as a marker for squamous epithelial cell transformation. In SCCs of the head and neck abundant expression of MMP-13 correlates with the invasion and metastasis capacity of the tumor, indicating that MMP-13 expression is also an indicator for invasive capacity of SCCs (Johansson et al., 1997c).

The expression of TIMPs in SCCs *in vivo* has been less extensively studied than that of MMPs. In cutaneous and oral SCCs expression of TIMP-1, TIMP-2, and TIMP-3 is detected in stromal cells adjacent to the tumor (Wagner et al., 1996; Airola et al., 1998, Sawatsubashi et al., 1998; Sutinen et al., 1998), suggesting that their expression represents a host attempt to limit tumor invasion and tumor-induced angiogenesis. This notion is supported by observations of Polette et al. (1991) indicating that the presence of TIMP-1 and TIMP-2 in SCCs correlates with less aggressive growth.

MMPs in SCCs of the female genital tract

SCC of the vulva accounts for 3% of all genital cancers in women and is the most common primary malignant tumor of the vulva. It usually occurs in elderly women, and may show extensive local invasion and metastases in inguinal lymph nodes. Well-differentiated SCCs of the vulva usually have a good prognosis if they are confined to vulva and inguinal nodes, but invasion to other pelvic organs, metastasis to iliac lymph nodes, or evidence of blood-borne metastasis results in less favorable prognosis (Krag, 1991). In younger women vulvar SCC is associated with human papillomavirus (HPV) infection, whereas vulvar carcinoma in older women is seldom associated with HPV infection, but involves mutation of p53 tumor suppressor gene

(Hording et al., 1994; Kagie et al., 1997). We have shown that MMP-13 is specifically expressed by tumor cells in invasive vulvar SCCs. MMP-13 expression is especially abundant in vulvar carcinomas showing metastasis to lymph nodes and is associated with expression of MT1-MMP by tumor cells and MMP-2 by stromal cells (Johansson et al., 1999). In our material of vulvar SCC cell lines, the presence of mutated p53 did not correlate with MMP-13 expression, indicating that p53 inactivation alone does not render squamous epithelial cells capable of expressing MMP-13 (Johansson et al., 1999). Nevertheless, expression of MMP-13, MT1-MMP, and MMP-7 by vulvar SCC cells *in vivo*, but not by normal vulvar epithelial cells provides evidence that the expression of these MMPs can be used as an indicator for malignant transformation of keratinocytes (Johansson et al., 1999).

The expression of MT1-MMP and MMP-2, two known activators of MMP-13, co-localized with the expression of MMP-13 in vulvar SCCs creating an optimal environment for pericellular activation of tumor cell-derived proMMP-13 (Johansson et al., 1999). Furthermore, in analogy with cutaneous SCCs (Pyke et al., 1992; Ståhle-Bäckdahl and Parks, 1993) expression of MMP-9 by invading tumor cells and tumor infiltrating inflammatory cells (Johansson et al., 1999) adds a further link to this MMP cascade, as MMP-9 can also activate proMMP-13. Since the substrate specificity of the above mentioned MMPs present in the peritumoral environment of SCCs is different, it can be proposed that each of these MMPs plays a distinct role in SCC invasion. It is therefore possible that specifically inhibiting the expression or activity of one MMP could disturb the balance in this complex network of proteases and inhibit invasion of SCC tumor cells (Fig. 3)

Cervical carcinoma is thought to arise in pre-existing areas of intraepithelial neoplasia (dysplasia) over a period of 10 to 20 years. A subset of mucosal HPVs, including HPV16, 18, 31, and 33, are frequently associated with various grades of squamous intraepithelial neoplasia (Aho et al., 1991; Storey et al., 1998). SCC is the most common carcinoma of the cervix accounting for 80% of cervical malignancies, whereas adenocarcinomas are less common (5 to 20%) (Krag, 1991). High levels of MMP-2, MMP-9 and MT1-MMP have been observed in cervical carcinoma cell lines (Tamakoshi et al., 1995; Gilles et al., 1996; Nuovo, 1997). *In vivo* studies have demonstrated high expression of MT1-MMP in both tumor and stromal cells of invasive cervical carcinomas and lymph node metastases (Gilles et al., 1996). We have also detected expression of MMP-13 in cell lines derived from cervical SCCs, although no expression of MMP-13 was detected in cervical SCCs *in vivo* in our material (Johansson et al., 1999). Interestingly, expression of MMP-13 in cervical SCC cell lines appeared to correlate with the presence of oncogenic HPV, suggesting that HPV plays a role in induction of MMP-13 expression (Johansson et al., 1999). No expression of MMP-13 was detected in adenocarcinomas of uterus and ovary, indicating that MMP-13 expression is specific for transformed squamous epithelial cells (Johansson et al., 1999).

Conclusion

A number of studies have provided evidence for the role of MMPs in tumor cell invasion and metastasis (for reviews, see Stetler-Stevenson et al., 1993; Basset et al., 1997; Johnsen et al., 1998; Kähäri and Saarialho-Kere, 1999). It is evident, that invasion of SCC cells *in vivo* involves interplay between tumor cells, stromal cells and inflammatory cells, all of which are capable of expressing distinct pattern of MMPs and thereby contributing to degradation of stromal ECM components (Fig. 3). These observations show that instead of determining the expression of a single MMP it may be more informative to compare the invasion capacity of malignant tumors with the expression of several MMPs and their activators. Furthermore, the expression of MMP-13, MT1-MMP, and MMP-7 can be used as indicator for transformation of keratinocytes, as these MMPs are not expressed by normal keratinocytes (Johansson et al., 1997b, 1999).

At present, several synthetic MMP inhibitors are in clinical trials to inhibit growth and invasion of malignant tumors *in vivo* (see Brown, 1998; Kähäri and Saarialho-Kere, 1999). Gene delivery of TIMP-1, -2, and -3 into malignant cells may also be a potent way of inhibiting tumor invasion and survival (Ahonen et al., 1998). Furthermore, an effective way of inhibiting the expression of MMPs may be blocking signaling pathways mediating activation of MMP transcription. Our recent findings together with observations by others indicate that inhibition of the activity of distinct mitogen-activated protein kinases (MAPKs) may serve as a potent way of inhibiting MMP expression (Gum et al., 1997; Reunanen et al., 1998; Simon et al., 1998; Westermarck et al., 1998; Ravanti et al., 1999). The ongoing and future clinical trials are expected to show whether the concept of MMP inhibition has a place in the therapeutic arsenal aimed at inhibiting growth, invasion, and metastasis of malignant tumors, including SCCs.

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