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Review Article

Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer

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Abstract: Many normal biological processes, such as reproduction, fetal development and wound healing, are critically dependent on controlled degradation of extracellular matrix (ECM) macromolecules. However, excessive degradation of matrix components occurs in pathologic tissue destruction, e.g. in atherosclerosis, rheumatoid arthritis, and cancer. Matrix metalloproteinases (MMPs) are degradative enzymes that play an important role in all aspects of tumor progression by enhancing tumor-induced angiogenesis and destroying local tissue architecture and basement membranes to allow tumor invasion and metastasis. Efficient breakdown of the ECM surrounding invasive cancer islands involves interplay between tumor cells, stromal cells, and inflammatory cells, all of which express a distinct set of MMPs. Besides the classical role of MMPs in degradation of ECM, MMPs may also indirectly influence the tumor microenvironment through the release of growth factors, cryptic sites or angiogenic factors, or through the generation of matrix fragments that inhibit tumor cell proliferation, migration and angiogenesis. This makes the contribution of MMPs to tumorigenesis much more complex than initially thought. Currently, a number of clinical studies have focused on testing MMP inhibitors as potential antineoplastic agents. In this review we discuss the present role of MMPs in the development and progression of cancer, focusing on non-melanoma skin cancers basal (BCC) and squamous (SCC) cell carcinoma, and the possible influence of MMPs in their differences.

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Proteolytic remodeling of the extracellular matrix

Coordinated breakdown, synthesis and remodeling of the extracellular matrix (ECM) are crucial events in normal physiological situations, such as embryonic development, reproduction, wound healing, and angiogenesis. On the other hand, excessive matrix degradation occurs in pathological conditions, such as chronic wounds, dermal photoageing, bullous skin diseases, atherosclerosis, rheumatoid arthritis, and cancer invasion and metastasis (see 1–3). Remodeling of the ECM requires cooperation of many different protease systems. Extracellular matrix-degrading proteolytic enzymes can be divided into four subgroups according to their amino acid residue or cofactor required for catalytic activity: (1) cysteine proteases, (2) aspartic proteases, (3) serine proteases, and (4) metalloproteinases, which contain a metal ion in the catalytic site. They can be further divided into several superfamilies. The members of one superfamily, the metzincins, bind zinc at the catalytic site and have a conserved 'Met-turn' motif, as well as conserved structural topology. Metzincins con-

Abbreviations: AP-1, activator protein-1; BCC, basal cell carcinoma; BM, basement membrane; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; EGF, epidermal growth factor; HNSCC, squamous cell carcinoma of the head and neck; IFN-, interferon-; IL-, interleukin-; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; SCC, squamous cell carcinoma; VEGF, vascular endothelial growth factor

sist of four groups: serralysins, matrixins (matrix metalloproteinases), astacins and adamalysins (see 4).

Matrix metalloproteinases

The matrix metalloproteinases (MMP) gene family presently consists of 21 human members. Matrix metalloproteinases are products of different genes dispersed to the genome, although there is an MMP gene cluster in choromosome 11. Matrix metalloproteinases differ structurally, in that each MMP has the ability to degrade a particular subset of matrix proteins. The protein products are, however, classified by shared functional and structural characteristics (Fig. 1). Matrix metalloproteinases can be divided into the following subgroups: (1) collagenases, (2) gelatinases, (3) stromelysins, and stromelysin-like MMPs, (4) matrilysins, membrane-type (MT) MMPs, and (6) other MMPs (Table 1, see 3,5). Together they can degrade all known protein components of the ECM. It is important to remember that most of the MMP substrates defined to date have been demonstrated *in vitro* but rarely *in vivo*.

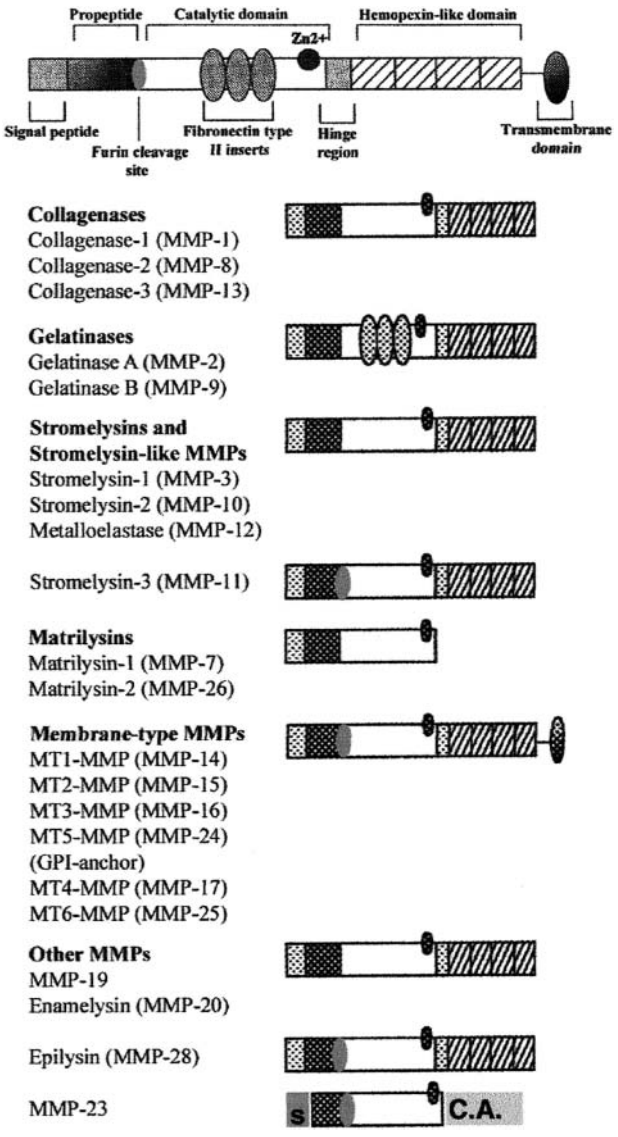

Figure 1. Structure of matrix metalloproteinases (modified from 145). s, signal anchor; CA, cysteine array.

The N-terminal signal peptide directs the protein for secretion into the endoplasmic reticulum and, eventually, out of the cell. All MMPs have a prodomain containing a highly conserved sequence, which has cysteine residue binding the catalytic zinc ion in a catalytic domain, thus keeping the pro-MMP in latent form. The catalytic domain contains the protein-degrading ability of the proteinase. Matrix metalloproteinases are classified on the basis of additional protein domains that contribute to their individual characteristics. A proline-rich hinge region connects the catalytic domain to the following hemopexin domain, which is assumed to mediate additional protein–protein interactions with substrates and with naturally occurring inhibitors. Fibronectin type II inserts in the catalytic domain of gelatinases modulate protein interactions important in substrate recognition. The transmembrane domain in the C-terminus localizes some cell-surface-bound MMPs to the cell membrane, and short sequence (furin site) between the prodomain and the catalytic domain provides an alternative cleavage site for MMP activation (see 3). These different combinations of protein motifs within the MMP family, together with individual gene sequences, generate the wide variety of characteristics that are within.

Collagenases

Collagenases-1, -2 and -3 are the major secreted proteinases capable of initiating degradation of native fibrillar collagens (type I, II, III, V and IX) and obviously play a crucial role in degradation of collagenous ECM in various situations. The fibrillar collagens are cleaved at a specific site to produce triple helical fragments, which denaturate spontaneously to gelatin at 37°C. These fragments can be further degraded by other MMPs (see 5). Matrix metalloproteinase-1, the first MMP determined by cDNA cloning (6), cleaves preferentially type III collagen while MMP-8 cleaves type I and II collagens (7,8). *In vitro* MMP-1 is expressed in various normal cells, such as keratinocytes, fibroblasts, endothelial cells, monocytes, macrophages, and chondrocytes (see 5). It can be detected *in vivo* in many physiological situations, including embryonic development and wound healing, but also in

Table 1. Matrix metalloproteinases

Enzyme	Substrates	Activated by
Collagenases		
Collagenase-1 (MMP-1)	Col I, II, III, VII, VIII, X, aggrecan, entactin/nidogen, MBP, serpins, α2M, perlecan, vitronectin, tenascin, fibrinogen, TNF precursor, IGFBP	MMP-3, -10, plasmin, kallikrein, chymase
Collagenase-2 (MMP-8)	Col I, II, III, aggrecan, serpins, α2M, fibrinogen	MMP-3, -10, plasmin
Collagenase-3 (MMP-13)	Col I, II, III, IV, IX, X, XIV, aggrecan, fibrillin, fibronectin, gelatin, LN-1, large tenascin C, osteonectin, serpins, PAI, fibrinogen	MMP-2, -3, -10, -14, -15, plasmin
Gelatinases		
Gelatinase A (MMP-2)	Col, I, IV, V, VII, X, gelatin, fibronectin, tenascin, fibrillin, osteonectin, entactin, aggrecan, vitronectin, decorin, MBP, decorin, plasminogen, α2M, LN-5, IGFBP, TNF precursor, pro-TGF-β, α1P1	MMP-1, -7, -13, -14, -15, -16, -24, 25, tryp-tase, thrombin, plasmin
Gelatinase B (MMP-9)	Col I, IV, V, VII, XI, XIV, gelatin, elastin, fibrillin, osteonectin, aggrecan, fibronectin, vitronectin, decorin, MBP, α2M, TNF precursor, IGFBP, plasminogen, pro-TGF-β, α1P1	MMP-2, -3, -13, plasmin
Stromelysins and tromelysin-like MMPs		
Stromelysin-1 (MMP-3)	Col III, IV, V, VII, IX, X, elastin, fibronectin, fibrillin, fibrinogen, gelatin, aggrecan, LN-1, nidogen, vitronectin, osteonectin, decorin, tenascin, α1P1, TNF precursor, MBP, E-cadherin, IGFBP, asminogen, osteopontin	Plasmin, kallikrein, chymase, tryptase
Stromelysin-2 (MMP-10)	Col III; IV, V, IX, X, elastin, fibronectin, gelatin, aggrecan, LN-1, nidogen	Elastase, cathepsin G, plasmin
Stromelysin-3 (MMP-11)	α1P1, IGFBP	Furin
Metalloelastase (MMP-12)	Elastin, col IV, fibronectin, LN-1, gelatin, vitronectin, entactin, proteoglycan, heparan and chondroitin sulfates, TNF precursor, plasminogen, fibrillin, fibrinogen, α1P1	Plasmin
Matrilysins		
Matrilysin-1 (MMP-7)	Col IV, elastin, fibronectin, LN-1, entactin, tenascin, osteonectin, aggrecan, vitronectin, MBP, decorin, versican, α1P1, osteopontin, E-cadherin, plasminogen, β4 integrin, α-prodefensin, Fas ligand, pro-TNF-α	MMP-3, plasmin
Matrilysin-2 MMP-26, endometase)	Col IV, gelatin, fibronectin, fibrin, α1PI, β-casein, TACE substrate	ND
Membrane-type MMPs		
MT1-MMP (MMP-14)	Col I, II, III, gelatin, fibronectin, LN-1, vitronectin, aggrecan, tenascin, nidogen, perlecan, ibrinogen/fibrin, fibrillin, α1PI, α2M, LN-5, CD-44, tTG	Plasmin, furin
MT2-MMP (MMP-15)	Fibronectin, LN-1, gelatin, aggrecan, tenascin, nidogen, perlecan, vitronectin, tTG	ND
MT3-MMP (MMP-16)	Col III, fibronectin, gelatin, laminin, aggrecan, casein, vitronectin, α2M, α1PI, tTG	Furin
MT4-MMP (MMP-17)	Gelatin, TNF-α precursor, fibrillin, fibronectin	ND
MT5-MMP (MMP-24)	ND	ND
MT6-MMP (MMP-25)	Col IV, gelatin, fibronectin, fibrin, LN-1	ND
Other MMPs		
MMP-19	Col IV, gelatin, LN-1, nidogen, tenascin, fibronectin, aggrecan, fibrinogen, COMP	Trypsin
Enamelysin (MMP-20)	Amelogenin, aggrecan, COMP	ND
MMP-23	ND	ND
Epilysin (MMP-28)	ND	ND

Modified from (46,115,145) with additional data from (180–183) and from articles referred to in the text.
Col, collagen; COMP, cartilage oligomeric matrix protein; IGFBP, insulin-like growth factor binding protein; LN, laminin; MBP, myelin basic protein; α2M, α2-macroglobulin; PAI, plasminogen activator inhibitor; α1PI, α1-proteinase inhibitor; tTG, tissue transglutaminase; ND, not detected.

chronic cutaneous ulcers and many cancers (2,9). Matrix metalloproteinase-8 is synthesized by maturing leukocytes in bone marrow, stored intracellularly in granules and released in response to extracellular stimuli (10). In addition, it has been detected, for example, in human chondrocytes, and synovial fibroblasts (11,12). Matrix metalloproteinase-13 was originally cloned from a breast tumor cDNA library (13) and degrades an exceptionally wide spectrum of substrates. Matrix metalloproteinase-13 cleaves preferentially type II collagen and also gelatin more effectively than other collagenases (14). It is expressed during bone development and gingival wound repair (15,16) as well as in pathological situations, such as in squamous cell carcinomas (SCCs) of different organs, chondrosarcomas and melanoma (see 17).

Gelatinases

Gelatinase A (MMP-2, 72 kDa gelatinase) is expressed in a variety of normal and transformed cells, including fibroblasts, keratinocytes, endothelial cells and chondrocytes. Gelatinase B

(MMP-9, 92 kDa gelatinase) is produced by keratinocytes, monocytes, macrophages, and many malignant cells. Gelatinases are able to degrade type IV, V, VII, X, XI, and XIV collagens, gelatin, elastin, proteoglycan and fibronectin among others (5) and they are believed to have a particularly important role in cancer invasion. Gelatinases demonstrate specific cell–MMP interactions: MMP-2 binds to integrin αvβ3 and MMP-9 to CD44 (18). Furthermore, MMP-2 knock-out mice exhibit reduced angiogenesis, impaired tumor growth and decreased metastasis (19). In MMP-9-deficient mice, endochondral ossification is impaired, resulting in abnormal skeletal growth (20).

Stromelysins and stromelysin-like MMPs

Stromelysins-1 and -2 (MMPs-3, -10) are highly homologous in primary structure and substrate specificity. Matrix metalloproteinase-3 is expressed by a variety of cells, e.g. keratinocytes, fibroblasts and chondrocytes. It alters the cellular microenvironment and can act as a natural tumor promoter and influence mammary tumor initiation (21). It is able to activate TGF-β, HB-EGF, IGFBP-3, TNF-α and IL-1β (22). Matrix metalloproteinase-10 was initially identified in an adenocarcinoma cDNA library (23). It was later isolated also from a rheumatoid synovial cell cDNA library and shown to be differentially expressed from MMP-3 in human fibroblasts (24). Matrix metalloproteinase-10 has been detected in tumor cells at least in SCCs of the head, neck and lung (25,26). Furthermore, MMP-10 is expressed by migrating keratinocytes in skin wounds (27) and by migrating enterocytes in intestinal ulcers (28). Stromelysin-3 (MMP-11) was cloned from breast cancer tissue (29). It is expressed in mesenchymal cells located close to epithelial cells during physiological and pathological tissue remodeling. It has a furin site in its prodomain and thus is processed intracellularly before being released as a mature enzyme (30). It is expressed in most invasive human carcinomas and is associated with a poor clinical outcome, e.g. in breast cancer (see 31). Mice lacking MMP-11 show reduced tumorigenesis in response to chemical mutagenesis (32). Matrix metalloproteinase-11 does not seem to cleave matrix components but may increase tumorigenesis by decreasing cancer cell death (33). Human macrophage metalloelastase (MMP-12) was initially found in alveolar macrophages of cigarette smokers (34). It is the most effective MMP against elastin but has a broad selection of other substrates. The expression of MMP-12 *in vivo* has been demonstrated mainly in macrophages, such as intestinal ulcerations (28). Furthermore, it degrades elastic fibers in atherosclerosis (35), and aneurysms (36). Matrix metalloproteinase-12-deficient mice have impaired macrophage-mediated proteolysis and tissue invasion (37). Interestingly, granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates tumor-infiltrating macrophages to produce metalloelastase, which cleaves plasminogen into angiostatin (38,39). Thus, MMP-12 may prevent tumor growth by inhibiting angiogenesis.

Matrilysins

Matrilysin (MMP-7) and endometase (MMP-26) are the smallest MMPs, lacking the hinge region and hemopexin domain. Matrix metalloproteinase-7 was originally identified as the small putative uterine metalloproteinase (PUMP) (23). Unlike most other MMPs expressed or released only in response to injury or disease, MMP-7 is expressed by non-injured, non-inflamed exocrine and mucosal epithelium in many tissues (40). In addition, MMP-7 is markedly up-regulated in the epithelium of injured airways (41) and the intestine (42), suggesting a key role in the repair of epithelium other than that of skin. Repair of the injured trachea is, indeed, markedly impaired in MMP-7-deficient mice (41). Matrix metalloproteinase-7 has been suggested to play a role in early stages of tumorigenesis (see 43). It is up-regulated in many tumors, especially of epithelial origin, such as those of the breast (29), lung and upper respiratory tract (25) and skin (44). In addition, intestinal tumorigenesis is reduced in MMP-7-deficient mice (45). Heparan sulfate proteoglycans bind matrilysin (18) and it is able to release soluble Fas ligand from the cell surface (46). Interestingly, MMP-7 can also inhibit tumor angiogenesis by generating angiostatin (47), which has also been shown *in vivo* (48).

Matrilysin-26 was cloned from fetal (49), placenta (50), and human endometrial tumor (51) cDNAs. Matrix metalloproteinase-26 has an unusual cysteine-switch propeptide sequence and also a threonine residue adjacent to the Zn-binding site that has been defined as a specific feature of matrilysin. Matrix metalloproteinase-26 is detected in placenta and uterus and is also widely expressed in diverse tumor cell lines and in malignant tumors by RT-PCR (52).

Membrane-type MMPs

The first membrane-type MMP (MT1-MMP) was detected on the surface of invasive tumor cells in 1994 (53) and altogether six distinct MT-MMPs have been found so far (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24; MMP-25 54–58).

Their domain structure is similar to other MMPs, except for a transmembrane domain and a cytoplasmic tail at the C-terminus. They also contain a furin site between the propeptide and catalytic domain (Fig. 1), cleaving of which leads to their activation (59). Membrane-type 4- and MT6-MMP have a glycosylphosphatidylinositol (GPI)-anchor binding them to the cell surface (60,61). Localization of MT-MMPs at the cell surface implies that they might have a significant role in regulation of cell–matrix interactions and activation of other MMPs. Indeed, MT-MMPs activate pro-MMP-2 (62–65), and MT1- and MT2-MMP also MMP-13 (66). The expression of MT-MMPs, especially MT1-MMP, has been detected in tumor cells or adjacent stromal cells in a variety of human cancers (56,57,65,66,67). Membrane type-MMP deficient mice are one of the few MMP knock-outs that have a phenotype, as they have impaired bone development and angiogenesis (68,69).

Other MMPs

Matrix metalloproteinase-19 was cloned from mammary gland (70) and liver (71) and isolated also as an autoantigen from the inflamed rheumatoid synovium as RASI (72). It has a domain structure typical to MMPs, except that it also contains a sequence of 36 residues in C-terminus lacking homology to any other MMP. Matrix metalloproteinase-19 is widely expressed in tissues, including placenta, lung, pancreas, ovary, spleen and intestine (71). Matrix metalloproteinase-19 is capable of degrading many components of the ECM and BM, but cannot activate any pro-MMPs (73). The loss of MMP-19 may be a marker of the invasive phenotype, since a progressive loss of MMP-19 expression from *in situ* carcinoma to invasive breast cancer has been reported (74). Enamelysin (MMP-20) is exclusively expressed in ameloblasts and odontoblasts of developing teeth (75) and degrades amelogenin, and thus apparently plays a crucial role in tooth enamel formation. Matrix metalloproteinase-23, cloned from an ovary cDNA library (76), is expressed mainly in the ovary, testis and prostate and might have a specialized role in reproductive processes. It has a unique structure with a short prodomain and also shortened C-terminal domain showing no similarity to hemopexin. It lacks a classic cysteine switch, but it possesses two novel motifs: a cysteine array (CA) and a novel Ig-fold (Fig. 1). It might establish a third subclass of membrane-bound MMPs and, thus, a new subclass of the MMP superfamily (77). Epilysin (MMP-28) was cloned from testis and keratinocyte cDNA libraries (78). It has a furin activation site and its splicing pattern differs from that of other MMP genes. By Northern analysis, it is expressed at high levels particularly in testis, but also in other tissues. It is also detected by immunohistochemistry in injured epidermis. According to Marchenko et al. (51), lung is the main organ expressing MMP-28, and it is also expressed in a broad range of carcinomas by RT-PCR (79).

Regulation of MMPs

Matrix metalloproteinases are highly regulated with regard to both secretion and activity. In normal tissue, MMPs are expressed at very low levels, if at all, but their production and activation is rapidly induced when active tissue remodeling is needed (see 3). Matrix metalloproteinase proteins are secreted by the constitutive secretory pathway, except in the case of macrophages and neutrophils, in which MMP-8 and -9 can be stored and released. In addition, MMP-7 is constitutively expressed in the ductal epithelium of exocrine glands (40). Regulation occurs at multiple levels, including transcription, modulation of mRNA half-life, secretion, compartmentalization, zymogen activation and inhibition of proteolytic activity. Matrix metalloproteinases have natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), and certain non-specific proteinase inhibitors, such as α2-macroglobulin. In addition, serine protease inhibitors, e.g. α1-antitrypsin and plasminogen activator inhibitors-1 and -2, control general proteolytic activity, including MMPs.

Transcriptional regulation

At the transcriptional level, a variety of cytokines and growth factors (e.g. TNF-α, EGF, bFGF, IL-1, PDGF, IL-6 and TGF-β) induce production of MMPs, depending on the situation and cell type. Also oncogenes, hormones and various chemical agents (e.g. phorbol esters) as well as cell–cell and cell–matrix interactions can induce or repress the expression of MMPs (see 3). Regulatory elements of MMP promoters are remarkably conserved (see 80). Extracellular stimuli regulate MMP activity through the activator protein (AP)-1 binding site, which is situated in the proximal promoter near the transcription initiation site in inducible MMP genes (MMPs-1, -3, -7, -9, -10, -12, -13 and -19). Activator protein-1 site binds dimeric transcription factors of the Jun and Fos families. The expression of the transcription factors are activated by extracellular signals through three distinct mitogen-activated protein kinase (MAPK) pathways, i.e. extracellular signal-regulated kinase (ERK1, 2), stress-activated protein kinase/Jun N-terminal kinases (SAPK/JNK), and p38. In general, ERK1,

2 cascade is activated by mitogenic signals, while SAPK/JNKs and p38 are activated by cytokines and cellular stress, such as UV light (81). Matrix metalloproteinase-2, -11, -28 and MT1-MMP genes do not have an AP-1 site in their promoters, but contain putative regulatory elements (82,83).

Transcriptional activity usually depends on the AP-1 site interacting with several other *cis*-acting sequences located throughout the promoter. Multiple polyomavirus enhancer A-binding protein-3 (PEA-3) sites are present in all promoters except MMP-2 and bind members of the ETS family of transcription factors (84). Individual AP-1 and PEA3 sites often play distinct roles in basal and induced transcription. ETS and AP-1 factors can synergistically activate MMP transcription, and other transcription factors often contribute, particularly in response to growth factors and cytokines. Besides MMP expression, collaboration between ETS and AP-1 has been shown to regulate the promoters of many genes involved in tumor progression (85).

Proenzyme activation

In most cases the activity of MMPs is controlled extracellularly: they are secreted in an inactive, latent form (pro-MMP). MT-MMPs, MMP-11, -23 and -28 are activated intracellularly by Golgi-associated furin-like proteases. Matrix metalloproteinase-23 is both activated and released from the cell surface by a single cleavage (77). The latency of MMPs is dependent on cysteine switch. The highly conserved cysteine residue in the prodomain binds covalently to the zinc-ion in the catalytic domain. Disruption of the Cys-zinc bond and cleavage of the prodomain leads to a conformational change and activation of pro-MMPs (3,86). Various compounds, e.g. organomercurials (APMA), SH-reactive agents, reactive oxygen and detergents can react with this cystein and convert it to a non-binding form (3). Matrix metalloproteinases can also activate other MMPs (see Table 1). Secreted pro-MMPs can be activated *in vitro* also with plasmin, trypsin, kallikrein, chymase, and mast cell tryptase. The uPa/plasmin system, as well as other proteinases, activate pro-MMPs, including MMP-2, -9, and -12, in many physiological situations (87). These activation cascades between MMPs and other proteinases form complex networks in regulating tissue proteolysis.

Tissue inhibitors of MMPs

Matrix metalloproteinases activity is further modulated through interactions with their natural inhibitors: TIMPs-1, -2, -3, and -4 (88,89). In general, TIMPs can inhibit the activity of all MMPs *in vitro*, except MT1- and MT3-MMPs, which are not inhibited by TIMP-1 (62,90). However, various TIMPs have preferential inhibitory capabilities against different pro-MMPs. For instance, TIMP-1 preferentially forms a complex with pro-MMP-9, while TIMP-2 and -4 reduces pro-MMP-2 activation by MT1-MMP (see 89). The tertiary structure of TIMPs is very similar, as the N-terminus is necessary for MMP inhibition (91,92) while the C-terminal end affects their specificity (93). The mechanism involves forming non-covalent stoichiometric complexes with the zinc-binding site of the active form of MMPs (94).

Except for TIMP-3, which is bound to ECM (95), TIMPs are present in a soluble form in most tissues and body fluids but show differences in tissue distribution (89). The expression of TIMP-1 and -3 is up-regulated by various growth factors, cytokines, retinoids, and glucocorticoids, while the expression of TIMP-2 is mainly constitutive. Tissue inhibitors of metalloproteinases play an important role in many biological processes, including fetal development, arthritis, angiogenesis and cancer. An imbalance between TIMP and MMP activities is considered to result in excessive degradation of matrix components in tumor invasion, but the balance between various TIMPs may also be a critical factor in determining the degradative potential of cells in normal and pathological conditions. Besides antitumor activity, TIMPs also have growth promoting activity in an MMP-independent fashion (see 96). Other functions of TIMPs include matrix binding, inhibition of angiogenesis and induction of apoptosis (97).

Proteolysis in cancer

Tumorigenesis is a complex, multistage process, where a normal cell undergoes genetic changes resulting in the ability to invade, and spread to, distant sites of the body. Interactions between the tumor and its microenvironment result in the production of proteolytic enzymes crucial for this process. In order for metastasis to occur, a cell must be able to detach from the primary tumor, and invade through BM and interstitial ECM. It must survive the circulation to arrest at the distant capillary bed. In order for the tumor to establish itself in a distant place, the tumor cell must leave the circulation and invade through the matrix again. Furthermore, it has to respond to growth factors, proliferate as a secondary colony, induce angiogenesis and evade host defences (see 22,98).

In the adult organism, *angiogenesis*, i.e. forming of new blood vessels from existing ones, is normally limited to events of tissue repair/remodeling.

In order to maintain continuous growth, malignant tumors depend critically on neovascularization, which enables nutrient and oxygen supply. The ability of the tumor to promote angiogenesis is thought to be one of the early events in the transition of a tumor from the preneoplastic stage to neoplastic phenotype. Studies of various human cancers have also shown a correlation between an increased number of tumor blood vessels and poor prognosis (see 99). The switch into angiogenic phenotype depends on a net balance between positive stimulators (e.g. angiogenin, VEGF or bFGF) and negative regulators (e.g. thrombospondin-1, angiostatin, endostatin) of blood vessel growth. Obviously the understanding of the cellular events and molecular regulation of angiogenesis has huge clinical implications (see 100).

In a developing tumor, there is constant communication between the tumor cells and the surrounding stroma, including matrix components, fibroblasts, endothelial and inflammatory cells (see 101). Fibroblasts participate in the degradation of ECM by secreting proteases and their activators. Alterations in the tumor stroma involve also the synthesis ECM, as well as changes in cell behavior, adhesion and migration. Some reactions in tumor stroma can be viewed as a non-specific host response in order to slow down tumor progression. Under normal physiological conditions, inflammation is a protective reaction elicited by the host, which serves to eliminate pathogens and initiate repair. Many malignancies may also arise from the areas of inflammation as part of the normal host response, and as such 'tumors may be viewed as wounds that do not heal' (102). The inflammatory component of a developing neoplasm includes macrophages, neutrophils and mast cells, which are variably loaded with different cytokines, growth factors, cytotoxic mediators, potent angiogenic factors or inhibitors including proteinases, TNF-α, ILs and IFNs, which all can influence the developing tumor (see 103–105).

MMPs in tumor progression

The initial observation of the importance of MMPs in cancer biology was that the ability of tumor cells to invade the surrounding tissue correlated with increased MMP levels (106). Subsequently, many MMP family members have been isolated from tumor cell lines or found to be overexpressed in various tumor tissues. The early hypothesis was that tumors expressed MMPs to make holes in the ECM, thereby initiating the development of metastasis. Although MMPs are typically present at the invasive front, MMP levels can, however, be elevated already in the early stages of tumor progression. Recent work extends the role of MMPs to multiple stages of tumor progression, including growth, cell migration and angiogenesis (see 22).

Matrix metalloproteinases enable tumor angiogenesis as they allow endothelial cells to invade through BMs to form new blood vessels. They also regulate endothelial cell attachment, proliferation, migration and growth, either directly or by the release of growth factors (see 107). The importance of MMPs in angiogenesis is substantiated by the fact that synthetic and endogenous MMP inhibitors also reduce angiogenic responses (see 108). Furthermore, MMP-deficient mice (MMP-2, -9 and -14) demonstrate defective angiogenesis (19,20). Endothelial cells can produce at least MMP-1, -2, -9, -19 and MT1-MMP, but then again, inflammatory cells and fibroblasts express many MMPs and also contribute to angiogenic phenotype. For instance, infiltration by mast cells and activation of MMP-9 coincides with the 'angiogenic switch' in premalignant lesions during squamous epithelial carcinogenesis (109). Matrix metalloproteinase-9 promotes tumor angiogenesis in other mouse tumor models as well (110,111).

Additional evidence for the critical role of MMPs in tumorigenesis has been provided with transgenic and knock-out mice. For instance, mice lacking MMP-7 and MMP-2 show reduced tumor progression (19,45). In addition, chemical mutagenesis results in decreased tumorigenesis in MMP-11-deficient mice (32). Mice lacking MMP-9 show reduced keratinocyte hyperproliferation and a decreased incidence of invasive tumors during skin carcinogenesis (112). Furthermore, overexpression of MMP-3 and MMP-7 in transgenic mice led to enhanced tumorigenesis in a breast cancer model (21,113). Similarly, overexpression of MMP-1 enhanced tumor formation in a chemically induced skin carcinogenesis model (114).

Besides the classic role of MMPs in the degradation of ECM in tumorigenesis, new roles are emerging that make the contribution of MMPs much more complex than initially thought. For instance, MMPs cleave receptors involved in cell adhesion, unmask cryptic sites of interaction, activate growth factors, and act on ECM components or other proteins to uncover hidden biologic activities that can affect cell proliferation, migration and angiogenesis. Matrix metalloproteinases are thereby involved in creating an environment suitable for tumor progression (see 4,9,115). Furthermore, MMP-mediated degradation of ECM components may generate inhibitors of angiogenesis and cell growth. Matrix metalloproteinases are now thought to be the most important proteases responsible for generating the potent angiogenesis

inhibitor angiostatin, which is a NH_2-terminal fragment of plasminogen and effectively inhibits endothelial cell proliferation. Matrix metalloproteinases capable of cleaving angiostatin are MMP-3, -7, -9 and -12, of which MMP-12 is the most potent (39,47).

MMP expression in tumors

Tumor, stromal and inflammatory cells can all overexpress various MMPs. The stromal expression was originally reported by Basset *et al*. (29), who noticed that fibroblasts were responsible for producing MMP-11 in breast cancer. Since then it has been shown by *in situ* hybridization that stromal expression of MMPs is, in fact, more common than tumor cell expression. For example, MMP-1, -2, -3, -11, and -14 mRNAs are mainly expressed by stromal fibroblasts in the vicinity of tumor cells in many human cancers, including breast, colorectal, lung, prostate and ovarian cancers (see 67). There are exceptions, such as MMP-7 and -10, which are mainly expressed in tumor cells. Also MMP-13 is usually expressed in tumor cells, and often at the invading front (116). Tumor cells can induce MMP expression either directly or by secreting soluble factors belonging to the immunoglobulin superfamily, called EMMPRINs, that induce MMP expression in stromal fibroblasts. To date only MMP-1, -2, and -3 have, however, been shown to be induced by EMMPRINs (117,118).

The expression patterns (mRNA and proteins) of both MMPs and TIMPs, as well as their combinations, have been studied as prognostic indicators of clinical outcome in many cancers (119). These studies suggest that MMPs and TIMPs may not only be good targets for cancer therapy but may also have clinical value in identifying subgroups of patients with an increased risk for recurrence. Generally, the expression of high levels of multiple MMP family members correlates positively with tumor aggressiveness, including increased invasive capacity, metastasis and poor patient survival. A direct correlation between MMP expression and the invasive phenotype of human tumors has been detected, for instance, in lung, prostate, stomach, colon, breast, ovary, and oral squamous cell cancers (see 9,67). However, there is no single MMP consistently overexpressed in every tumor type, or a consistent pattern of MMP expression across a variety of human cancers. This reflects the heterogeneity of tumors and differential expression of MMPs during tumor progression. At least MMP-1, -2, -7, -9, -11 and MT1-MMP are frequently overexpressed in many human tumors (see 9,67,107).

Therapeutic MMP inhibitors

The role of MMPs in many pathological conditions and their low basal expression in normal adult tissues have made them a prominent therapeutic target in cancer and other diseases. Thus, the most efficient approach for cancer therapy would seem to be the inhibition of all MMP activity, as with first generation broad-spectrum MMP inhibitors. Matrix metalloproteinase inhibitors may slow tumor growth by enhancing the development of a fibrotic capsule around the tumor, by inhibiting angiogenesis or by inducing apoptosis in tumor cells. More recently, inhibitors that demonstrate selectivity for a specific MMP subtype have been developed to limit unpleasant side-effects (see 9,22,119). Gene delivery of TIMPs could also result in a beneficial therapeutic effect (120), but the lack of effective methods has limited the clinical utility of this approach. Synthetic inhibitors interfere with the catalytic machinery in the active site of the MMPs. The hydroxyamate inhibitor batimastat and its analog marimastat were the first inhibitors studied in detail. Non-peptidic MMP inhibitors, such as BAY 12–9566 and AG3340, were synthesized in an attempt to improve the oral bioavailability, pharmaceutical properties and specificity. Also, shark cartilage extract (Neovastat, AE-941, Aeterna), retinoids, tetracycline derivates and bisphosphonates have been used to inhibit MMP activity (see 121). Phase III clinical trials using marimastat, batimastat and BAY 12–9566 alone or in combination with chemotherapy in patients with advanced cancers have been disappointing. This may result from the fact that MMPs are important already in the early stage of tumor progression, and inhibition may not be useful once the tumor has metastasized. Thus, future trials should concentrate on patients with early stage cancers, and the measurements of MMP levels would help identify patients more likely to respond to MMP therapy (122). Although the development of specific MMP inhibitors may result in more effective compounds, the MMP system is extremely complex, and it is not yet clear which subtypes should be targeted therapeutically to achieve antitumor effects (108). The dual role of MMPs in angiogenesis also emphasizes the necessity to identify appropriate targets for MMP inhibition.

MMPs in BCC and SCC of skin

Basal cell carcinoma (BCC) is the most common malignancy in humans (Caucasian populations) especially in the elderly population (see 123). The BCC incidence in Europe, USA and Australia increases by 3–6% each year (124). Basal cell carci-

noma is usually slow growing and rarely metastasizes but can cause significant local destruction and disfigurement if treated inadequately. It is considered to arise from multipotential cells within the basal layer of the epidermis or follicular structures and can develop without a premalignant lesion in both a hereditary and sporadic fashion. Disruption of the hedgehog-patched pathway (PTCH-gene) is a key event in the development of BCC in both sporadic and hereditary forms (125). P53 mutations are present in approximately 56% of all types of BCC and are also frequent in SCCs of the head and neck (125,126). The PATCHED and p53 genes are major targets of UV radiation and their inactivation leads to uncontrolled cellular proliferation. Basal cell carcinoma typically occurs in areas of chronic sun exposure, which is thought to be the most important and common cause of BCC (127). Also, ionizing radiation, chemical carcinogens (e.g. arsenic), and possibly infection with human papillomaviruses, have been associated with BCC development. There are several clinical and histologic subtypes of BCC that may exhibit different patterns of behavior and thus more aggressive therapy is often necessary for aggressive BCC variants, such as infiltrating and morpheaform BCCs (see 123).

Squamous cell carcinoma is a malignant tumor of keratinocytes of the spinous layer of the epidermis. It is the second most common form of skin cancer in Caucasian persons (128), accounting for 20% of all cutaneous malignancies, and frequently arises on the sun-exposed skin of the middle aged and elderly (129). Squamous cell carcinoma can occur in many other tissues too, including the mouth, airways, esophagus, uterine cervix and vulva. Evidently, the primary cause of cutaneous SCC is cumulative lifetime sun exposure (especially UVB). Furthermore, ionizing radiation, immune suppression, chronic inflammation, and human papillomavirus (HPV) infection may lead to the development of SCC. There are no generally used reliable prognostic markers for SCC in routine use (130). The prognostic risk factors include, e.g. diameter, depth of invasion, histologic differentiation, rapid growth, anatomic site, immune suppression, and etiology, so that tumors arising from scars and chronic ulcers tend to be aggressive (129).

Five to 20% of sun-induced precancerous lesions, known as actinic keratoses, will transform into SCC in 10–25 years (129). As a result of the fact that some actinic keratoses express MMP-1 (131), its up-regulation has been associated with the early events in SCC development. On the other hand, MMP-1 is up-regulated in migrating keratinocytes in association with wound repair and thus does not serve as an ideal marker for keratinocyte transformation (132). Overexpression of MMP-2, -3, -10 and -13 has not been observed in actinic keratoses (131,133,134). Bowen's disease represents cutaneous SCC *in situ*. Matrix metalloproteinase-9 expression has been reported recently in dyskeratotic foci of Bowen's disease and in the infiltrative edges of microinvasive carcinomas (135), while Bowen's disease lesions are negative for MMPs-10, -11, -12 and -13 (133–137).

Matrix metalloproteinase-1, unlike other collagenases (138), MMP-3 (139), MMP-11 (137), MMP-2 and -9 (140), are mainly expressed by stromal cells surrounding malignant epithelial cells in BCCs. Matrix metalloproteinase-10 mRNA is expressed in BCCs only in epithelial cancer cells with a pattern totally different from its close homologue MMP-3, which is more abundantly expressed in surrounding fibroblasts and only rarely in sclerosing cancer islands (133,134). Recently, Cribier et al. (141) reported that MMP-11 was more intensely expressed in aggressive forms of BCC. In aggressive morpheaform (infiltrative) BCCs and recurrent BCCs, MMP-7 was localized at the tumor–stromal interface (44), whereas it was not expressed in other BCC subtypes. Matrix metalloproteinase-13 mRNA was seen in focal areas of keratinized cells and was associated with terminal differentiation of epithelial cells (133). Macrophages in clinically more aggressive, fibrosing BCCs expressed MMP-12 more often than the keratotic or adenoid BCCs (136). MT1-MMP expression was found generally in stromal fibroblasts surrounding tumor islands in BCCs (134). Matrix metalloproteinase-19 or -28 have not been detected in the BCC tumor islands but they are associated with benign hyperproliferation of the epidermis such as that occurring during wound repair (132; Impola and Saarialho-Kere, unpublished observations).

Also, TIMPs have been found in BCCs. According to Varani et al. (138), MMP inhibitor activity mainly reflected the presence of TIMP-1. Significantly higher levels of TIMP-2 were detected in less infiltrative BCC when compared with SCCs or infiltrative BCCs (142). Tissue inhibitors of metalloproteinase-3 was present in tumor cells at the tumor edge of infiltrative BCCs (143), while TIMP-1 and -2 were found in stromal cells (142,144). In cutaneous and oral SCCs, expression of TIMP-1, -2 and -3 is detected in stromal cells in the vicinity of the tumor (130,142), suggesting that their expression represents a host response to limit invasion and angiogenesis.

Squamous cell carcinomas of different organs express several MMP members (see 145). In SCC of the oral cavity, MMP-3 protein correlated with

tumor size, invasion, and high incidence of lymph node metastases (146). The level of active MMP-2 may serve as a predictive marker of metastasis in oral SCCs (147). In addition, the high activity of MMP-2 and -9 correlated with the invasiveness of oral SCC (148) and shorter disease-free survival after treatment (149). According to Sutinen et al. (150) MMP-1 was detected in stromal fibroblasts and also in some neoplastic islands in oral SCCs. Matrix metalloproteinase-2 was expressed in fibroblasts surrounding the carcinoma cells and in the peripheral cell layer in tumor islands. Tissue inhibitor of MMPs-3 was expressed in stromal cells in all oral SCCs, TIMP-1 was detected in some stromal cells, whereas TIMP-2 was not expressed at all. Expression of MMPs and TIMPs was consistently low in oral epithelial dysplasias. In HNSCC, MMP-11 mRNA was detected in stromal cells next to invasive cancer cells and the level of its expression correlated with increased local invasiveness (151). Matrix metalloproteinase a-9 protein correlated with angiogenic markers and poor survival in HNSCC patients (152). In esophageal SCCs, MMP-13 protein was detected in tumor cells colocalizing with MT1-MMP, and their expression was related to cancer aggressiveness (153). Furthermore, elevated protein expression of MMP-7, -9 and MT1-MMP, as well as gelatinase activity, was detected in carcinoma cells correlating with the depth of tumor invasion (154). The expressions of MMP-1 and MMP-7 were associated with poor prognosis (155,156).

Expression of MMP-7, -9, -13 and MT1-MMP have previously been detected in malignantly transformed keratinocytes in SCCs, but not in normal keratinocytes or in premalignant lesions of the skin (44,116,133; Fig. 2). Thus, their expression might serve as a marker for the transformation and invasion capacity of cutaneous SCC cells. More precisely, MMP-13 mRNA was expressed by tumor cells at the invading front, but occasionally also by stromal fibroblasts (116), while its potential activator, MMP-3, was expressed by stromal cells surrounding tumors (133). Matrix metalloproteinase-7 protein was expressed in SCC cells at the stromal interface surrounding tumor nests (44), which is also the case for MMP-9 (157). Matrix metalloproteinase-1 mRNA was detected in tumor and stromal cells in SCC of the skin (131), while MMP-11 was typically expressed exclusively in stromal cells (158). Also, the level of MMP-2 and -9 proteins was up-regulated in SCCs of the skin when compared with BCCs (159). Indeed MMP-9 expressed by inflammatory cells is functionally involved in distinct processes of epithelial carcinogenesis (112). Matrix metalloproteinase-28 was not detected in the invasive front of SCCs (132).

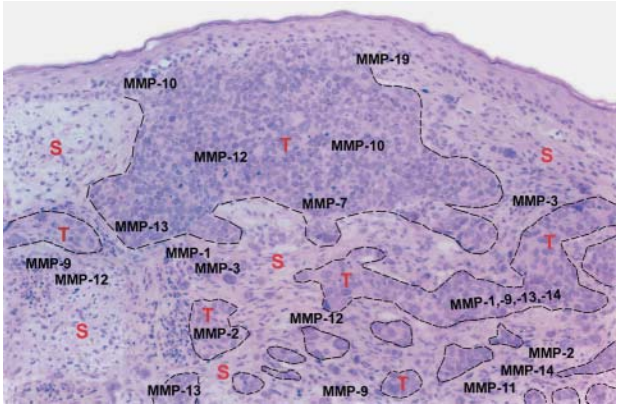

Figure 2. Expression of matrix metalloproteinases (MMPs) in a cutaneous squamous cell carcinoma. This schematic picture, made with a histological section of a poorly differentiated grade III SCC as a background, displays the most typical sites of expression for individual MMPs. Counterstaining was performed with hematoxylin and eosin; original magnification ×100. T, tumor; S, stroma.

The expression of MMP-12 has been shown mainly in macrophages, having a role in macrophage-related proteolysis of elastin in various pathological conditions for example. Interestingly, MMP-12 mRNA and protein are expressed not only by macrophages but also by transformed keratinocytes in cutaneous SCC (136; Fig. 2). Furthermore, the level of MMP-12 was higher in histologically more aggressive and poorly differentiated tumor types (grades II and III), suggesting a possible role in the invasion process (136). In vulvar SCCs, we found it was actually a cancer cell-derived MMP-12 that correlated with the aggressiveness and dedifferentiation of the tumors, while macrophage-derived MMP-12 was most abundant in grade I tumors, thus predicting less aggressive behaviour of the tumors (160). Larger cohorts of patients with HNSCC are needed to predict whether macrophage-derived MMP-12 would serve as a marker of good prognosis.

Matrix metalloproteinase-10 mRNA was expressed in SCCs only in epithelial laminin-5 positive cancer cells while its close homologue MMP-3 was expressed mostly in the stroma. Matrix metalloproteinase-10 expression does not correlate with the invasive behavior of SCCs, but might be induced by the inflammatory matrix remodeling events associated with skin tumors (134). Membrane type 1-MMP mRNA expression was found both in cancer cells and in stromal fibroblasts surrounding cutaneous SCCs. MT1-MMP-positive cancer cells were detected especially at the invasive edge of tumors (134). In HNSCC, MT1-MMP protein localized on the cell surface of tumor cells, especially at the invasive areas (161), while its

mRNA was detected in stromal cells of the same cancer (162). Interestingly, a similar discrepancy has been reported for MMP-2 expression in HNSCC and in skin cancer (140). Thus, it is possible that stromal cells produce MT1-MMP protein, which migrates onto tumor cell surfaces binding fibroblast secreted pro-MMP-2 and activating it as originally reported by Sato et al. (53). Membrane type 1-MMP can activate latent MMP-13 (66) and, based on previous data (133), may serve this function at the epithelial front. Interestingly, as MT1-MMP mRNA was produced only by fibroblasts in BCC, it might have a task other than that produced by cancer cells in invasive SCCs. The limited invasion potential of BCC supports this result (134). Also Ohtani et al. (163) suggested a dual role for MT1-MMP: its expression on the surface of cancer cells might relate to invasive growth, while in fibroblasts it may participate in tissue remodeling processes caused by invasive cancer cells.

Cell migration, keratinocyte hyperproliferation and angiogenesis are essential in both wound healing and tumor invasion. Spatially and temporally controlled expression of several MMPs is essential for normal wound closure. Matrix metalloproteinase-1, -3, -9, and -10 are up-regulated in migrating or proliferating keratinocytes in association with wound repair and thus do not serve as ideal markers for keratinocyte transformation in skin (see 132). During wound repair, MMP-7, -12, -13, and -14 are not detected in keratinocytes and thus their expression in keratinocyte-derived cells arises cancer suspicion. Interestingly, at least a couple of the newer MMPs, namely MMP-19 and MMP-28, are down-regulated rather than induced in cancers (74,132).

Basal cell carcinomas are generally only locally aggressive while SCCs have high metastatic potential. As cell–matrix interactions are important up- and down-regulators for MMPs, the changes in the compositions of different BM components, may influence MMP expression. Type VII and IV collagens were less expressed in SCC than BCC (159). Type IV collagen is the major component of BM and represents the first barrier in tumor cell invasion. Thus, reduced expression of collagen IV, combined with an increased expression of both MMP-2 and MMP-9, could account for the increased invasive potential of SCC vs. BCC (159).

Laminin-5, a protein expressed predominantly in the BM zone, promotes static adhesion in quiescent tissues. However, it also stimulates cell migration and/or invasion after having been cleaved by MMPs such as MMP-2 and MT1-MMP (164,165). Laminin-5 production by cancer cells can not be detected in types of BCCs other than the infiltrative areas of sclerosing BCCs (133,134). In fact, the simultaneous loss of expression of $\alpha 6\beta 4$ integrin and laminin-5 was detected in BCC cells but not in SCC cells (159,166,167). In HNSCC, laminin5-gamma2 is expressed intracellularly in the peripheral basaloid cells of tumor islands at the invasion front and is not seen in premalignant specimens (168).

There is growing evidence that integrins can bind or up-regulate MMPs and the integrin profile of BCCs might explain their non-metastasizing behaviour. $\beta 1$ and $\beta 3$ integrins were prominently expressed at the periphery of BCC tumor islands, while in SCCs they were absent or variably expressed (169). Recently, $\alpha v\beta 6$ integrin has been shown to promote invasion of SCC cells through up-regulation of MMP-9 (170). Furthermore, MMP-7 can cleave $\beta 4$ integrin (171).

Table 2. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in cutaneous basal cell and squamous cell carcinomas

MMP	BCC Epithelium	Stroma	SCC Epithelium	Stroma
MMP-1	–	+	+	+
MMP-2	–	+	+	+
MMP-3	(+)*	+	+	+
MMP-7	(+)*	–	+	–
MMP-9	–	+	+	+
MMP-10	+	–	+	–
MMP-11	–	+	–	+
MMP-12	(+)*	+	+	+
MMP-13	+	–	+	(+)
MMP-14	–	+	+	+
TIMP-1	–	+	–	+
TIMP-2	–	+	–	+
TIMP-3	+	+	–	+

Table summarizes the *in vivo* results of several articles referred in the text.
+: expressed; (+): expressed only occasionally; *only present in the aggressive sclerosing subtype; –: not expressed.
MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; BCC, basal cell carcinoma; SCC, squamous cell carcinoma.

Loss or down-regulation of E-cadherin is a key event in the process of tumor invasion and metastasis. Koseki *et al*. (172) suggested that BCCs do not have much metastatic potential because of the retention of high levels of E-cadherin expression. Matrix metalloproteinases capable of cleaving E-cadherin are MMP-3 and -7 (173), which points out how MMPs may mediate invasion, not only by directly degrading matrix, but also by cleaving transmembrane proteins and receptors. E-cadherin expression is reduced in particularly infiltrative BCCs (174) that are also known to produce MMP-7 (44).

Summary and conclusions

The expression of various MMPs and TIMPs in cutaneous SCC and BCC of the skin *in vivo* is summarized in Table 2. Different MMPs might form a network, in which each has a distinct role in the cleavage of a particular matrix component or activation of other MMPs. Stromal cells are the major source of MMPs, but tumor cells, fibroblasts and inflammatory cells all express a distinct set of MMPs capable of complementing the proteolysis needed in tumor progression (see 175). The expression of MMPs is clearly more abundant in the epithelial compartment of SCCs than BCCs (Table 2), which might reflect the difference in the invasion capacity of these tumors. Low expression of TIMPs does not necessarily correlate with enhanced invasiveness, as malignant and metastatic tumors often overexpress TIMPs *in vivo* (46,176). However, MMPs are usually expressed more abundantly, as overexpression of TIMPs can block tumor growth (120,177,178). Interestingly, at least MMP-9, -12, and MT1-MMP are all expressed both in tumor and stromal cells in SCCs, so that epithelial expression is more frequent in poorly differentiated tumors when compared with less aggressive SCCs (or BCCs) (134,136,159,160). In contrast to SCC, TIMP-3 is expressed in cancer cells in BCC and may thus limit MMP activity. On the whole, individual MMPs may have different roles depending on the cell type or state of transformation. For example, MMP-7 and -9 may both induce tumor angiogenesis or inhibit it by generation of angiostatin (48,109,179). Thus, increased expression of MMPs may both confer invasiveness to the tumor cells and, paradoxically, lead to the production of molecules limiting their growth. Similarly, if a particular MMP is expressed by a tumor cell, it may contribute to invasion, while, when expressed by a stromal or inflammatory cell it may have another role, such as regulatory or even protective, in host defense. Expanding knowledge on the role of MMPs in cancer suggests that their functions are much more complex than initially thought. In skin cancer they may not only be good targets for antineoplastic therapy but may turn out to have clinical utility in identifying subgroups of SCC patients at increased risk for recurrence.

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References

1. Kähäri V, Saarialho-Kere U. Matrix metalloproteinases in skin. Exp Dermatol 1997: 6: 199–213.
2. Saarialho-Kere U. Patterns of matrix metalloproteinase and TIMP expression in chronic ulcers. Arch Dermatol Res 1998: 294: S47–S54.
3. Nagase H, Woessner J F. Matrix metalloproteinases. J Biol Chem 1999: 274: 21491–21494.
4. Bergers G, Coussens L M. Extrinsic regulators of epithelial tumor progression: metalloproteinases. Curr Opin Genet Dev 2000: 10: 120–127.
5. Birkedal-Hansen H, Moore W G, Bodden M K et al. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 1993: 4: 197–250.
6. Goldberg G, Wilhelm S M, Kronberger A, Bauer E A, Grant G A, Eisen A Z. Human fibroblast collagenase. Complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem 1986: 261: 6600–6605.
7. Welgus H, Jeffrey J J, Eisen A Z. The collagen substrate specificity of human skin fibroblast collagenase. J Biol Chem 1981: 256: 9511–9515.
8. Hasty K, Jeffrey J J, Hibbs M S, Welgus H G. The collagen substrate specificity of human neutrophil collagenase. J Biol Chem 1987: 262: 10048–10052.
9. Stetler-Stevenson W, YuA E. Proteases in invasion: matrix metalloproteinases. Semin Cancer Biol 2001: 11: 143–153.
10. Hasty K, Pourmotabbed T F, Goldberg G I. Human neutrophil collagenase. A distinct gene product with homology to other matrix metalloproteinases. J Biol Chem 1990: 265: 11421–11424.
11. Cole A, Chubinskaya S, Schumacher B et al. Chondrocyte matrix metalloproteinase-8. Human articular chondrocytes express neutrophil collagenase. J Biol Chem 1996: 271: 11023–11026.
12. Hanemaaijer R, Sorsa T, Konttinen Y T et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. J Biol Chem 1997: 272: 31504–31509.
13. Freije J, Diez-Itza I, Balbin M et al. Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J Biol Chem 1994: 269: 16766–16773.
14. Knäuper V, Lopez-Otin C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996a: 271: 1544–1550.

15. Johansson N, Saarialho-Kere U, Airola K et al. Collagenase-3 (MMP-13) is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts during human fetal bone development. Dev Dyn 1997a: 208: 387–397.
16. Ravanti L, Häkkinen L, Larjava H et al. Transforming growth factor-beta induces collagenase-3 expression by human gingival fibroblasts via p38 mitogen-activated protein kinase. J Biol Chem 1999: 274: 37292–37300.
17. Pendas A, Uria J A, Jimenez M G, Balbin M, Freije J P, Lopez-Otin C. An overview of collagenase-3 expression in malignant tumors and analysis of its potential value as a target in antitumor therapies. Clin Chim Acta 2000: 291: 137–155.
18. Parks W C, Shapiro S D. Matrix metalloproteinases in lung biology. Respir Res 2001: 2: 10–19.
19. Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 1998: 58: 1048–1051.
20. Vu T, Shipley J M, Bergers G et al. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 1998: 93: 411–422.
21. Sternlicht M, Lochter A, Sympson C J et al. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 1999: 98: 137–146.
22. McCawley L, Matrisian L M. Matrix metalloproteinases: multifunctional contributors to tumor progression. Mol Med Today 2000: 6: 149–156.
23. Muller D, Quantin B, Gesnel MC, Millon-Collard R, Abecassis J, Breathnach R. The collagenase gene family in humans consists of at least four members. Biochem J 1988: 253: 187–192.
24. Sirum K, Brinckerhoff C E. Cloning of the genes for human stromelysin and stromelysin 2: differential expression in rheumatoid synovial fibroblasts. Biochemistry 1989: 28: 8691–8698.
25. Muller D, Breathnach R, Engelmann A et al. Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. Int J Cancer 1991: 48: 550–556.
26. Polette M, Claver C, Muller D, Abecassis J, Binninger I, Birembaut P. Detection of mRNAs encoding collagenase-1 and stromelysin-2 in carcinomas of the head and neck by in situ hybridization. Invasion Metast 1991: 11: 76–83.
27. Rechardt O, Elomaa O, Vaalamo M et al. Stromelysin-2 is upregulated during normal wound repair and is induced by cytokines. J Invest Dermatol 2000: 115: 778–787.
28. Vaalamo M, Karjalainen-Lindsberg M L, Puolakkainen P, Kere J, Saarialho-Kere U. Distinct expression profiles of stromelysin-2 (MMP-10), collagenase-3 (MMP-13), macrophage metalloelastase (MMP-12), and tissue inhibitor of metalloproteinases-3 (TIMP-3) in intestinal ulcerations. Am J Pathol 1998: 152: 1005–1114.
29. Basset P, Bellocq J P, Wolf C et al. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 1990: 348: 699–704.
30. Pei D, Weiss S J. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature 1995: 375: 244–247.
31. Rouyer N, Wolf C, Chenard M P et al. Stromelysin-3 gene expression in human cancer: an overview. Invasion Metastasis 1994: 14: 269–275.
32. Masson R, Lefebvre O, Noel A et al. In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. J Cell Biol 1998: 140: 1535–1541.
33. Boulay A, Masson R, Chenard M P et al. High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase. Cancer Res 2001: 61: 2189–2193.
34. Shapiro S, Kobayashi D, Ley T J. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. J Biol Chem 1993: 268: 23824–23829.
35. Halpert I, Sires U I, Roby J D et al. Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc Natl Acad Sci U S A 1996: 93: 9748–9753.
36. Curci J, Liao S, Huffman M D, Shapiro S D, Thompson R W. Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. J Clin Invest 1999: 102: 1900–1910.
37. Shipley J, Wesselschmidt R L, Kobayashi D K, Ley T J, Shapiro S D. Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. Proc Natl Acad Sci U S A 1996: 93: 3942–3946.
38. Dong Z, Kumar R, Yang X, Fidler I J. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell 1997: 88: 801–810.
39. Cornelius L, Nehring L C, Harding E et al. Matrix metalloproteinases generate angiostatin: effects on neovascularization. J Immunol 1998: 161: 6845–6852.
40. Saarialho-Kere U, Crouch E C, Parks W C. Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. J Invest Dermatol 1995: 105: 190–196.
41. Dunsmore S, Saarialho-Kere U K, Roby J D et al. Matrilysin expression and function in airway epithelium. J Clin Invest 1998: 102: 1321–1331.
42. Saarialho-Kere U, Vaalamo M, Puolakkainen P, Airola K, Parks W C, Karjalainen-Lindsberg M L. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. Am J Pathol 1996: 148: 519–526.
43. Fingleton B, Heppner Goss K J, Crawford H C, Matrisian L M. Matrilysin in early stage intestinal tumorigenesis. APMIS 1999: 107: 102–110.
44. Karelina T, Goldberg G I, Eisen A Z. Matrilysin (PUMP) correlates with dermal invasion during appendageal development and cutaneous neoplasia. J Invest Dermatol 1994: 103: 482–487.
45. Wilson C, Heppner K J, Labosky P A, Hogan B L, Matrisian L M. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A 1997: 94: 1402–1407.
46. Sternlicht M D, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001: 17: 463–516.
47. Patterson B, Sang Q A. Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J Biol Chem 1997: 272: 28823–28825.
48. Pozzi A, Moberg P E, Miles L A, Wagner S, Soloway P, Gardner H A. Elevated matrix metalloprotease and angiostatin levels in integrin alpha 1 knockout mice cause reduced tumor vascularization. Proc Natl Acad Sci U S A 2000: 97: 2202–2207.
49. de Coignac A, Elson G, Delneste Y et al. Cloning of MMP-26. A novel matrilysin-like proteinase. Eur J Biochem 2000: 267: 3323–3329.
50. Uria J, Lopez-Otin C. Matrilysin-2, a new matrix metalloproteinase expressed in human tumors and showing the

minimal domain organization required for secretion, latency, and activity. Cancer Res 2000: 60: 4745–4751.
51. Park H, Ni J, Gerkema F E, Liu D, Belozerov V E, Sang Q X. Identification and characterization of human endometase (Matrix metalloproteinase-26) from endometrial tumor. J Biol Chem 2000: 275: 20540–20544.
52. Marchenko G, Ratnikov B I, Rozanov D V, Godzik A, Deryugina E I, Strongin A Y. Characterization of matrix metalloproteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. Biochem J 2001a: 356: 705–718.
53. Sato H, Takino T, Okada Y et al. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994: 370: 61–65.
54. Takino T, Sato H, Shinagawa A, Seiki M. Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library. MT-MMPs form a unique membrane-type subclass in the MMP family. J Biol Chem 1995: 270: 23013–23020.
55. Will H, Hinzmann B. cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment. Eur J Biochem 1995: 231: 602–608.
56. Puente X, Pendas A M, Llano E, Velasco G, Lopez-Otin C. Molecular cloning of a novel membrane-type matrix metalloproteinase from a human breast carcinoma. Cancer Res 1996: 56: 944–949.
57. Llano E, Pendas A M, Freije J P et al. Identification and characterization of human MT5-MMP, a new membrane-bound activator of progelatinase a overexpressed in brain tumors. Cancer Res 1999: 59: 2570–2576.
58. Pei D. Leukolysin/MMP25/MT6-MMP. a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. Cell Res 1999a: 9: 291–303.
59. Sato H, Kinoshita T, Takino T, Nakayama K, Seiki M. Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. FEBS Lett 1996: 393: 101–104.
60. Itoh Y, Kajita M, Kinoh H, Mori H, Okada A, Seiki M. Membrane type 4 matrix metalloproteinase (MT4-MMP, MMP-17) is a glycosylphosphatidylinositol-anchored proteinase. J Biol Chem 1999: 274: 34260–34266.
61. Kojima S, Itoh Y, Matsumoto S, Masuho Y, Seiki M. Membrane-type 6 matrix metalloproteinase (MT6-MMP, MMP-25) is the second glycosyl-phosphatidyl inositol (GPI)-anchored MMP. FEBS Lett 2000: 480: 142–146.
62. Will H, Atkinson S J, Butler G S, Smith B, Murphy G. The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. Regulation by TIMP-2 and TIMP-3. J Biol Chem 1996: 271: 17119–17123.
63. Pei D. Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. J Biol Chem 1999b: 274: 8925–8932.
64. Miyamori H, Takino T, Seiki M, Sato H. Human membrane type-2 matrix metalloproteinase is defective in cell-associated activation of progelatinase A. Biochem Biophys Res Commun 2000: 267: 796–800.
65. Velasco G, Cal S, Merlos-Suarez A et al. Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. Cancer Res 2000: 60: 877–882.
66. Knäuper V, Will H, Lopez-Otin C et al. Cellular mechanisms for human procollagenase-3 (MMP-13) activation. Evidence that MT1-MMP (MMP-14) and gelatinase a (MMP-2) are able to generate active enzyme. J Biol Chem 1996: 271: 17124–17131.
67. Nelson A, Fingleton B, Rothenberg M L, Matrisian L M. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000: 18: 1135–1149.
68. Zhou Z, Apte S S, Soininen R et al. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci 2000: 97: 4052–4057.
69. Holmbeck K, Bianco P, Caterina J et al. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 1999: 99: 81–92.
70. Cossins J, Dudgeon T J, Catlin G, Gearing A J, Clements J M. Identification of MMP-18, a putative novel human matrix metalloproteinase. Biochem Biophys Res Commun 1996: 228: 494–498.
71. Pendas A, Knäuper V, Puente X S et al. Identification and characterization of a novel human matrix metalloproteinase with unique structural characteristics, chromosomal location, and tissue distribution. J Biol Chem 1997: 272: 4281–4286.
72. Sedlacek R, Mauch S, Kolb B et al. Matrix metalloproteinase MMP-19 (RASI-1) is expressed on the surface of activated peripheral blood mononuclear cells and is detected as an autoantigen in rheumatoid arthritis. Immunobiology 1998: 198: 408–423.
73. Stracke J O, Hutton M, Stewart M et al. Biochemical characterization of the catalytic domain of human matrix metalloproteinase 19. Evidence for a role as a potent basement membrane degrading enzyme. J Biol Chem 2000: 275: 14809–14816.
74. Djonov V, Hogger K, Sedlacek R, Laissue J, Draeger A. MMP-19: cellular localization of a novel metalloproteinase within normal breast tissue and mammary gland tumours. J Pathol 2001: 195: 147–155.
75. Llano E, Pendas A M, Knäuper V et al. Identification and structural and functional characterization of human enamelysin (MMP-20). Biochemistry 1997: 36: 15101–15108.
76. Velasco G, Pendas A M, Fueyo A, Knäuper V, Murphy G, Lopez-Otin C. Cloning and characterization of human MMP-23, a new matrix metalloproteinase predominantly expressed in reproductive tissues and lacking conserved domains in other family members. J Biol Chem 1999: 274: 4570–4576.
77. Pei D, Kang T, Qi H. Cysteine array matrix metalloproteinase (CA-MMP) /MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation. J Biol Chem 2000: 275: 33988–33997.
78. Lohi J, Wilson C L, Roby J D, Parks W C. Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. J Biol Chem 2001: 276: 10134–10144.
79. Marchenko G, Strongin A Y. MMP-28, a new human matrix metalloproteinase with an unusual cysteine-switch sequence is widely expressed in tumors. Gene 2001b: 265: 87–89.
80. Westermarck J, Kähäri V M. Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J 1999: 13: 781–792.
81. Karin M, Liu Z G, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol 1997: 9: 240–246.
82. Illman S A, Keski-Oja J, Lohi J. Promoter characterization of the human and mouse epilysin (MMP-28) genes. Gene 2001: 275: 185–194.
83. Lohi J, Lehti K, Valtanen H, Parks W C, Keski-Oja J.

Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. Gene 2000: 242: 75–86.
84. Wasylyk C, Gutman A, Nicholson R, Wasylyk B. The c-Ets oncoprotein activates the stromelysin promoter through the same elements as several non-nuclear oncoproteins. EMBO J 1991: 10: 1127–1134.
85. Denhardt D. Oncogene-initiated aberrant signaling engenders the metastatic phenotype: synergistic transcription factor interactions are targets for cancer therapy. Crit Rev Oncog 1996: 7: 261–291.
86. Van Wart H, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci U S A 1990: 87: 5578–5582.
87. Carmeliet P, Moons L, Lijnen R et al. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet 1997: 17: 439–444.
88. Edwards D, Beaudry P P, Laing T D et al. The roles of tissue inhibitors of metalloproteinases in tissue remodelling and cell growth. Int J Obes Relat Metab Disord (Suppl) 1996: 3: S9–S1.
89. Gomez D, Alonso D F, Yoshiji H, Thorgeirsson U P. Tissue inhibitors of metalloproteinases: structure, regulation and biological function. Eur J Cell Biol 1997: 74: 111–122.
90. Shimada T, Nakamura H, Ohuchi E et al. Characterization of a truncated recombinant form of human membrane type 3 matrix metalloproteinase. Eur J Biochem 1999: 262: 907–914.
91. Murphy G, Houbrechts A, Cockett M I, Williamson R A, O'Shea M, Docherty J A. The N-terminal domain of tissue inhibitor of metalloproteinases retains metalloproteinase inhibitory activity. Biochemistry 1991: 30: 8097–8102.
92. Langton K, Barker M D, McKie N. Localization of the functional domains of human tissue inhibitor of metalloproteinases-3 and the effects of a Sorsby's fundus dystrophy mutation. J Biol Chem 1998: 273: 16778–16781.
93. Goldberg G, Strongin A, Collier I E, Genrich L T, Marmer B L. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. J Biol Chem 1992: 267: 4583–4591.
94. Gomis-Rüth F, Maskos K, Betz M et al. Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. Nature 1997: 389: 77–81.
95. Leco K, Khokha R, Pavloff N, Hawkes S P, Edwards D R. Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. J Biol Chem 1994: 269: 9352–9360.
96. Henriet P, Blavier L, DeClerck Y A. Tissue inhibitors of metalloproteinases (TIMP) in invasion and proliferation. APMIS 1999: 107: 111–119.
97. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biopys Acta 2000: 1477: 267–283.
98. Ellis L, Fidler I J. Angiogenesis and metastasis. Eur J Cancer 1996: 32A: 2451–2460.
99. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 1995: 147: 9–19.
100. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996: 86: 353–364.
101. Iozzo R. Tumor stroma as a regulator of neoplastic behavior. Agonistic and antagonistic elements embedded in the same connective tissue. Lab Invest 1995: 73: 157–160.
102. Wernert N. The multiple roles of tumour stroma. Virchows Arch 1997: 430: 433–443.
103. Joseph I, Isaacs J T. Macrophage role in the anti-prostate cancer response to one class of antiangiogenic agents. J Natl Cancer Inst 1998: 90: 1648–1653.
104. Ono M, Torisu H, Fukushi J, Nishie A, Kuwano M. Biological implications of macrophage infiltration in human tumor angiogenesis. Cancer Chemother Pharmacol 1999: 43: S69–S71.
105. Coussens L, Werb Z. Inflammatory cells and cancer: think different!. J Exp Med 2001: 193: F23–F26.
106. Liotta L, Tryggvason K, Garbisa S, Hart I, Foltz C M, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 1980: 284: 67–68.
107. Foda H, Zucker S. Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. Drug Discov Today 2001: 6: 478–482.
108. Stetler-Stevenson W. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest 1999: 103: 1237–1241.
109. Coussens L, Raymond W W, Bergers G et al. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 1999: 13: 1382–1397.
110. Bergers G, Brekken R, McMahon G et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000: 2: 737–744.
111. YuQ, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev 2000: 14: 163–176.
112. Coussens L, Tinkle C L, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000: 103: 481–490.
113. Rudolph-Owen L, Chan R, Muller W J, Matrisian L M. The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. Cancer Res 1998: 58: 5500–5506.
114. D'Armiento J, DiColandrea T, Dalal S S et al. Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis. Mol Cell Biol 1995: 15: 5732–5739.
115. McCawley L, Matrisian L M. Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol 2001: 13: 534–540.
116. Johansson N, Airola K, Grénman R, Kariniemi A L, Saarialho-Kere U, Kähäri V M. Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. Am J Pathol 1997b: 15: 499–508.
117. Kataoka H, DeCastro R, Zucker S, Biswas C. Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res 1993: 53: 3154–3158.
118. Biswas C, Zhang Y, DeCastro R G, Nakamura T, Kataoka H, Nabeshima K. The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res 1995: 55: 434–439.
119. Vihinen P, Kähäri V M. Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer 2002.
120. Ahonen M, Baker Ah, Kähäri V M. Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. Cancer Res 1998: 58: 2310–2315.
121. Hidalgo M, Eckhardt S G. Matrix metalloproteinase inhibitors: how can we optimize their development? Ann Oncol 2001: 12: 285–228.

122. Zucker S, Cao J, Chen W T. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene 2000: 19: 6642–6650.
123. Ramsey M. Basal Cell Carcinoma. In: Taylor R, Wells M J, Chan E F, Quirk C, Elston D M, eds. Emedicine Journal, Dermatology, Malignant Neoplasms. eMedicine.com Inc, 2001.
124. Armstrong B K, Kricker A. Skin cancer. Dermatol Clin 1995: 13: 583–594.
125. Ling G, Ahmadian A, Persson A et al. PATCHED and p53 gene alterations in sporadic and hereditary basal cell cancer. Oncogene 2001: 20: 7770–7778.
126. Lacour J P. Carcinogenesis of basal cell carcinomas: genetics and molecular mechanisms. Br J Dermatol 2002: 146 (Suppl. 6): 17–19.
127. Kricker A, Armstrong B K, English D R. Sun exposure and non-melanocytic skin cancer. Cancer Causes Control 1994: 5: 367–392.
128. Bernstein S, Lim K K, Brodland D G. The many faces of squamous cell carcinoma. Dermatol Surg 1996: 22: 243–254.
129. Johnson T, Rowe DE, Nelson BR. Squamous cell carcinoma of the skin (excluding lip and oral mucosa). J Am Acad Dermatol 1992: 26: 467–484.
130. Helliwell T R. Molecular markers of metastasis in squamous carcinomas. J Pathol 2001: 194: 289–293.
131. Tsukifuji R, Tagawa K, Hatamochi A, Shinkai H. Expression of matrix metalloproteinase-1-2 and – 3 in squamous cell carcinoma and actinic keratosis. Br J Cancer 1999: 80: 1087–1091.
132. Saarialho-Kere U, Kerkelä E, Suomela S, Jahkola T, Keski-Oja J, Lohi J. Epilysin (MMP-28) expression is associated with cell proliferation during epithelial repair. J Invest Dermatol 2002: 119: 14–21.
133. Airola K, Johansson N, Kariniemi A L, Kähäri V M, Saarialho-Kere UK. Human collagenase-3 is expressed in malignant squamous epithelium of the skin. J Invest Dermatol 1997: 109: 225–231.
134. Kerkelä E, Ala-aho R, Lohi J, Grénman R, Kähäri V M, Saarialho-Kere U. Differential patterns of stromelysin-2 (MMP-10) and MT1-MMP (MMP-14) expression in epithelial skin cancers. Br J Cancer 2001: 84: 659–669.
135. Verdolini R, Amerio P, Goteri G et al. Cutaneous carcinomas and preinvasive neoplastic lesions. Role of MMP-2 and MMP-9 metalloproteinases in neoplastic invasion and their relationship with proliferative activity and p53 expression. J Cutan Pathol 2001: 28: 120–126.
136. Kerkelä E, Ala-Aho R, Jeskanen L et al. Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. J Invest Dermatol 2000: 114: 1113–1119.
137. Thewes M, Worret W I, Engst R, Ring J. Stromelysin-3 (ST-3). Iimmunohistochemical characterization of the matrix metalloproteinase (MMP)-11 in benign and malignant skin tumours and other skin disorders. Clin Exp Dermatol 1999: 24: 122–126.
138. Varani J, Hattori Y, Chi Y et al. Collagenolytic and gelatinolytic matrix metalloproteinases and their inhibitors in basal cell carcinoma of skin: comparison with normal skin. Br J Cancer 2000: 82: 657–665.
139. Majmudar G, Nelson B R, Jensen T C, Johnson T M. Increased expression of matrix metalloproteinase-3 (stromelysin-1) in cultured fibroblasts and basal cell carcinomas of nevoid basal cell carcinoma syndrome. Mol Carcinog 1994: 11: 29–33.
140. Pyke C, Ralfkiaer E, Huhtala P, Hurskainen T, Dano K, Tryggvason K. Localization of messenger RNA for Mr 72,000 and 92,000 type IV collagenases in human skin cancers by in situ hybridization. Cancer Res 1992: 52: 1336–1341.
141. Cribier B, Noacco G, Peltre B, Grosshans E. Expression of stromelysin 3 in basal cell carcinomas. Eur J Dermatol 2001: 11: 530–533.
142. Wagner S, Ockenfels H M, Wagner C, Soyer H P, Goos M. Differential expression of tissue inhibitor of metalloproteinases-2 by cutaneous squamous and basal cell carcinomas. J Invest Dermatol 1996: 106: 321–326.
143. Airola K, Ahonen M, Johansson N et al. Human TIMP-3 is expressed during fetal development, hair growth cycle, and cancer progression. J Histochem Cytochem 1998: 46: 437–447.
144. Childers J W, Hernandez A D, Kim Jh, Stricklin G P. Immunolocalization of collagenase inhibitor in normal skin and basal cell carcinoma. J Am Acad Dermatol 1987: 17: 1025–1032.
145. Johansson N, Kähäri V M. Matrix metalloproteinases in squamous cell carcinoma. Histol Histopathol 2000: 15: 225–237.
146. Kusukawa J, Sasaguri Y, Morimatsu M, Kameyama T. Expression of matrix metalloproteinase-3 in stage I and II squamous cell carcinoma of the oral cavity. J Oral Maxillofac Surg 1995: 53: 530–534.
147. Kawamata H, Uchida D, Hamano H et al. Active-MMP2 in cancer cell nests of oral cancer patients: correlation with lymph node metastasis. Int J Oncol 1998: 13: 699–704.
148. Ikebe T, Shinohara M, Takeuchi H et al. Gelatinolytic activity of matrix metalloproteinase in tumor tissues correlates with the invasiveness of oral cancer. Clin Exp Metastasis 1999: 17: 315–323.
149. Yorioka C W, Coletta R D, Alves F, Nishimoto I N, Kowalski L P, Graner E. Matrix metalloproteinase-2 and – 9 activities correlate with the disease-free survival of oral squamous cell carcinoma patients. Int J Oncol 2002: 20: 189–194.
150. Sutinen M, Kainulainen T, Hurskainen T et al. Expression of matrix metalloproteinases (MMP-1 and – 2) and their inhibitors (TIMP-1-2 and – 3) in oral lichen planus, dysplasia, squamous cell carcinoma and lymph node metastasis. Br J Cancer 1998: 77: 2239–2245.
151. Muller D, Wolf C, Abecassis J et al. Increased stromelysin 3 gene expression is associated with increased local invasiveness in head and neck squamous cell carcinomas. Cancer Res 1993: 53: 165–169.
152. Riedel F, Gotte K, Schwalb J, Bergler W, Hormann K. Expression of 92-kDa type IV collagenase correlates with angiogenic markers and poor survival in head and neck squamous cell carcinoma. In T J Oncol 2000: 17: 1099–1105.
153. Etoh T, Inoue H, Yoshikawa Y, Barnard G F, Kitano S, Mori M. Increased expression of collagenase-3 (MMP-13) and MT1-MMP in oesophageal cancer is related to cancer aggressiveness. Gut 2000: 47: 50–56.
154. Murray G, Duncan M E, O'Neil P, McKay J A, Melvin W T, Fothergill J E. Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J Pathol 1998: 185: 256–261.
155. Ohashi K, Nemoto T, Nakamura K, Nemori R. Increased expression of matrix metalloproteinase 7 and 9 and membrane type 1-matrix metalloproteinase in esophageal squamous cell carcinomas. Cancer 2000: 88: 2201–2209.
156. Yamashita K, Mori M, Shiraishi T, Shibuta K, Sugimachi K. Clinical significance of matrix metalloproteinase-7 expression in esophageal carcinoma. Clin Cancer Res 2000: 6: 1169–1174.

157. Lengyel E, Gum R, Juarez J et al. Induction of M (r) 92,000 type IV collagenase expression in a squamous cell carcinoma cell line by fibroblasts. Cancer Res 1995: 55: 963–967.
158. Asch P, Basset P, Roos M, Grosshans E, Bellocq J P, Cribier B. Expression of stromelysin 3 in keratoacanthoma and squamous cell carcinoma. Am J Dermatopathol 1999: 21: 146–150.
159. Dumas V, Kanitakis J, Charvat S, Euvrard S, Faure M, Claudy A. Expression of basement membrane antigens and matrix metalloproteinases 2 and 9 in cutaneous basal and squamous cell carcinomas. Anticancer Res 1999: 19: 2929–2938.
160. Kerkelä E, Ala-aho R, Klemi P, Grénman S, Kähäri V M, Saarialho-Kere U. Metalloelastase (MMP-12) expression by tumor cells in squamous cell carcinoma of the vulva correlates with invasiveness, while that by macrophages predicts better outcome. J Pathol 2002: 198: 258–269.
161. Yoshizaki T, Sato H, Maruyama Y et al. Increased expression of membrane type 1-matrix metalloproteinase in head and neck carcinoma. Cancer 1997: 79: 139–144.
162. Okada A, Bellocq J P, Rouyer N et al. Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc Natl Acad Sci 1995: 92: 1135–1149.
163. Ohtani H, Motohashi H, Sato H, Seiki M, Nagura H. Dual over-expression pattern of membrane-type metalloproteinase-1 in cancer and stromal cells in human gastrointestinal carcinoma revealed by in situ hybridization and immunoelectron microscopy. Int J Cancer 1996: 68: 565–570.
164. Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson W G, Quaranta V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science 1997: 277: 225–228.
165. Koshikawa N, Giannelli G, Cirulli V, Miyazaki K, Quaranta V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J Cell Biol 2000: 148: 615–625.
166. Rossen K, Dahlstrom K K, Mercurio A M, Wewer U M. Expression of the alpha 6 beta 4 integrin by squamous cell carcinomas and basal cell carcinomas: possible relation to invasive potential? Acta Derm Venereol 1994: 74: 101–105.
167. Savoia P, Cremona O, Trusolino L, Pepino E, Marchisio P C. Integrins and basement membrane proteins in skin carcinomas. Pathol Res Pract 1994: 190: 950–954.
168. Patel V, Aldridge K, Ensley J F et al. Laminin-gamma2 overexpression in head-and-neck squamous cell carcinoma. Int J Cancer 2002: 99: 583–588.
169. Peltonen J, Larjava H, Jaakkola S et al. Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. Variable expression in basal and squamous cell carcinomas. J Clin Invest 1989: 84: 1916–1923.
170. Thomas G J, Lewis M P, Hart I R, Marshall J F, Speight P M. AlphaVbeta6 integrin promotes invasion of squamous carcinoma cells through up-regulation of matrix metalloproteinase-9. Int J Cancer 2001: 92: 641–650.
171. von Bredow D C, Nagle R B, Bowden G T, Cress A E. Cleavage of beta 4 integrin by matrilysin. Exp Cell Res 1997: 236: 341–345.
172. Koseki S, Ansai S, Aoki T, Hozumi Y, Mitsuhashi Y, Kondo S. E-cadherin expression in skin tumors using an AMeX immunohistostaining method. J Dermatol 2000: 27: 307–311.
173. Noë V, Fingleton B, Jacobs K et al. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. J Cell Sci 2001: 114: 111–118.
174. Pizarro A. E-cadherin expression is frequently reduced in infiltrative basal cell carcinoma. J Dermatol 2000: 27: 804–805.
175. Kähäri V, Saarialho-Kere U. Matrix metalloproteinases and their inhibitors in tumour growth and invasion. Ann Med 1999: 31: 34–45.
176. Salmela M T, Karjalainen-Lindsberg M L, Puolakkainen P, Saarialho-Kere U. Upregulation and differential expression of matrilysin (MMP-7) and metalloelastase (MMP-12) and their inhibitors TIMP-1 and TIMP-3 in Barrett's oesophageal adenocarcinoma. Br J Cancer 2001: 85: 383–392.
177. Khokha R. Suppression of the tumorigenic and metastatic abilities of murine B16–F10 melanoma cells in vivo by the overexpression of the tissue inhibitor of the metalloproteinases-1. J Natl Cancer Inst 1994: 86: 299–304.
178. Imren S, Kohn D B, Shimada H, Blavier L, DeClerck Y A. Overexpression of tissue inhibitor of metalloproteinases-2 retroviral-mediated gene transfer in vivo inhibits tumor growth and invasion. Cancer Res 1996: 56: 2891–2895.
179. Nishizuka I, Ichikawa Y, Ishikawa T et al. Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis in vivo. Cancer Lett 2001: 173: 175–182.
180. English W R, Puente X S, Freije J M et al. Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-alpha convertase activity but does not activate pro-MMP2. J Biol Chem 2000: 275: 14046–14055.
181. English W R, Velasco G, Stracke J O, Knäuper V, Murphy G. Catalytic activities of membrane-type 6 matrix metalloproteinase (MMP25). FEBS Lett 2001: 491: 137–142.
182. Agnihotri R, Crawford H C, Haro H, Matrisian L M, Havrda MC, Liaw L. Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). J Biol Chem 2001: 276: 28261–28267.
183. Kang T, Nagase H, Pei D. Activation of membrane-type matrix metalloproteinase 3 zymogen by the proprotein convertase furin in the trans-Golgi network. Cancer Res 2002: 62: 675–681.