Department of Dermatology and Venereology
University of Helsinki
Finland

The expression and regulation of matrix metalloproteinases-12, -10 and -7 in epithelial cells and squamous cell cancer

ERJA KERKELÄ

Academic dissertation

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in the auditorium of the Department of Dermatology and Venereology, Meilahdentie 2, on November 30th, at 12 noon

Helsinki 2001

Supervised by:

Assistant Professor Ulpu Saarialho-Kere, M.D., Ph.D.
Department of Dermatology
Helsinki University Central Hospital
Helsinki, Finland

Reviewed by:

Docent Ilmo Leivo, M.D., Ph.D.
Department of Pathology
University of Helsinki
Helsinki, Finland

and

Professor Veli-Jukka Uitto, M.D., Ph.D.
Institute of Dentistry, Department of Oral Biology
University of Helsinki
Helsinki, Finland

Opponent:

Cornelia Mauch, M.D., Ph.D.
Department of Dermatology
University of Cologne
Cologne, Germany

ISBN 952-91-4086-X
ISBN 952-10-0202-6 PDF

Yliopistopaino
Helsinki 2001

To my family

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I-V). Some unpublished data is also included.

I Kerkelä E, Böhling T, Herva R, Uria JA, Saarialho-Kere U: Human macrophage metalloelastase (MMP-12) expression is induced in chondrocytes during fetal development and malignant transformation. Bone, in press.

II Saarialho-Kere U, Kerkelä E, Jeskanen L, Hasan T, Pierce R, Starcher B, Raudasoja R, Ranki A, Oikarinen A, Vaalamo M: Accumulation of matrilysin (MMP-7) and macrophage metalloelastase (MMP-12) in actinic damage. J Invest Dermatol 1999, 113:664-672.

III Kerkelä E, Ala-aho R, Jeskanen L, Rechardt O, Grénman R, Shapiro SD, Kähäri VM, Saarialho-Kere U: Expression of human macrophage metalloelastase (MMP-12) by tumor cells in skin cancer. J Invest Dermatol 2000, 114:1113-1119.

IV 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. Submitted.

V Kerkelä E, Ala-aho R, Jeskanen L, Lohi J, Grénman R, Kähäri VM, 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.

ABBREVIATIONS

BCC	basal cell carcinoma
BM	basement membrane
bFGF	basic fibroblast growth factor
cDNA	complementary DNA
DEJ	dermal epidermal junction
ECM	extracellular matrix
EGF	epidermal growth factor
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GM-CSF	granulocyte macrophage colony stimulating factor
HD	hemidesmosome
HGF	hepatocyte growth factor
HME	human macrophage metalloelastase
HNSCC	squamous cell carcinoma of the head and neck
IFN-γ	interferon-γ
IL-1β	interleukin-1β
KGF	keratinocyte growth factor
MME	mouse metalloelastase
MMP	matrix metalloproteinase
PDGF	platelet-derived growth factor
PG	proteoglycan
RT-PCR	reverse transcriptase polymerase chain reaction
TAM	tumor associated macrophages
TIMP	tissue inhibitor of metalloproteinases
TGF-α	transforming growth factor-α
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α
SCC	squamous cell carcinoma
UV	ultraviolet
VEGF	vascular endothelial growth factor

Erja Kerkelä

The Expression and Regulation of Matrix Metalloproteinases-12, -10 and -7 in Epithelial Cells and Squamous Cell Cancer

Department of Dermatology and Venereology
Helsinki University Central Hospital and University of Helsinki

ABSTRACT

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes which take part in the sophisticated coordination of extracellular matrix (ECM) synthesis and breakdown. Together they can cleave essentially all matrix proteins. Degradation of connective tissue is required both in normal physiologic processes and in pathologic tissue remodeling such as cancer. The aim of this study was to investigate the expression and regulation of metalloelastase (MMP-12), stromelysin-2 (MMP-10) and matrilysin (MMP-7) in epithelial cancers and to evaluate their role in the clinical behavior of tumors. Furthermore, we studied the role of MMP-12 during human development. Our results revealed a restricted expression of MMP-12 in hypertrophic chondrocytes in developing bone, beginning from the gestational age of 8 weeks, but not in other fetal tissues. Expression of MMP-12 mRNA could not be detected in normal adult cartilage or osteosarcomas but in chondrosarcomas in both macrophages and cancer cells. TNF-α induced MMP-12 transcription in chondrosarcoma-derived cells.

MMP-12 was detected in basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) of the skin and vulva both in epithelial cancer cells and macrophages *in vivo*, as well as in various cultured SCC cell lines and transformed HaCaT cells, whereas pre-malignant tumors and primary skin keratinocytes were negative. MMP-7 and -12 proteins were also upregulated in areas of elastotic material in solar damage, including actinic keratosis, a condition that can develop into SCC. In vulvar SCCs, MMP-12 mRNA was expressed in cancer cells only in the histologically more aggressive grade II/III samples, thereby correlating with the dedifferentiation of the tumors. In contrast, macrophage-derived MMP-12 mRNA was most abundant in well-differentiated grade I tumors. The amount of signal for MMP-12, regardless of its origin, did not significantly correlate with metastases, the amount of blood vessels or patient survival. MMP-12 production was induced by transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) in cells derived from cutaneous SCC and epithelial MCF-10f cells, while only by TGF-β1 in HaCaTs. In SCCs of the vulva, MMP-7 mRNA was detected in 50 % of tumors mainly in epithelial tumor cells, the expression generally concentrating in more aggressive tumors. In HaCaTs it was upregulated by TNF-α. MMP-10 mRNA was expressed in SCCs and BCCs only in epithelial laminin-5 positive cancer cells with a pattern totally different from its close homologue stromelysin-1 (MMP-3). The production of MMP-10 was upregulated in cutaneous SCC cells by TGF-α and KGF, and by IFN-γ in combination with TGF-β1 and TNF-α, both in SCC and HaCaT cells.

In conclusion, this study shows that MMP-12 can be expressed *in vivo* and *in vitro* by transformed epithelial cells. The level of cancer cell derived MMP-12 correlates with histological aggressiveness, while that of macrophages predicts less aggressive behavior of the tumors, suggesting a dual role for MMP-12 in cancer progression. According to the present results, there is a possibility that MMP-12 mRNA expression in m acrophages might serve as a new prognostic marker in patients with SCC. Furthermore, MMP-12 plays an important role in ECM remodeling during fetal bone development and is induced when chondrocytes undergo malignant transformation. In contrast, MMP-10 expression does not correlate with the invasive behavior of SCCs but may be induced by the wound healing and inflammatory matrix remodeling events associated with skin tumors.

1. INTRODUCTION

Controlled degradation of ECM macromolecules is critical for many normal biological processes, such as reproduction, wound healing and fetal development. On the other hand, excessive degradation of matrix components can lead to pathologic tissue destruction occurring, for example, in inflammatory and cardiovascular diseases, and in cancer. Matrix metalloproteinases (MMPs) are degradative enzymes that seem to 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 in tumorigenesis, 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.

To date, over 20 MMPs have been characterized. They share several structural and functional characteristics and together they are able to degrade practically all proteins of the ECM. According to their structural features and substrate specificity, they are divided into the subgroups of collagenases, gelatinases, stromelysins and stromelysin-like MMPs, matrilysins, membrane-type MMPs and other MMPs.

The aim of this study was to investigate the role and expression of metalloelastase (MMP-12), stromelysin-2 (MMP-10) and matrilysin (MMP-7) in premalignant lesions of the skin as well as in epithelial cancers both *in vivo* and *in vitro*, and to evaluate the role of MMP-12 in the clinical behavior of squamous cell carcinomas (SCC), in particular. Regulation of these MMPs was studied in various cultured epithelial cell lines, including different SCC cells and transformed HaCaT keratinocytes. Furthermore, we studied the role of MMP-12 during human development and in bone lesions. The principal methods used include *in situ* hybridization, immunohistochemistry, cell culture, Northern analysis, Western blot, and quantitative RT-PCR.

2. REVIEW OF THE LITERATURE

2.1. STRUCTURE OF THE SKIN

Human skin is the largest multifunctional organ of the body (Figure 1). It has multiple specified tasks, such as supporting the body with connective tissue, and protection from external injuries, micro-organisms and radiation. It also provides sensation, thermoregulation, and biochemical/metabolic and immune functions. The skin can be divided into an upper epithelial layer, *epidermis*, and an underlying connective tissue, *dermis*. These are separated by the basement membrane zone. Epithelium is not only found in skin, since epithelial cells also cover internal body cavities and tubes. In addition, they cover or line all the body organs, including organs of the digestive system. Epithelium also forms the secretory portion of the glands and their ducts. Different kinds of epithelia can be roughly divided into simple and stratified epithelium (Chan 1997; Archer 1998).

2.1.1. Epithelium

The stratified squamous epithelium of the skin consists mainly of *keratinocyte* layers (Figure 1). It contains also *melanocytes*, which produce pigment, *Langerhans cells*, which can process and introduce antigens, and *Merkel cells*. The epidermis has four layers: the basal, squamous and granular cell layer, and the stratum corneum. Basal cells form a single cell layer on an intact basement membrane. The epidermis regenerates constantly as basal cells divide and migrate towards the surface. Basal cells are rounded and flatten out as they differentiate. At the same time, nuclei disappear and cells get filled with keratin filaments and keratohyalin. Flattened cells provide a tight layer to protect the skin from external irritants. Finally, superficial flattened cells mainly contain keratinfilaments and are sloughed from the stratum corneum. (see Eckert 1989; Lever and Schaumburg-Lever, 1990a)

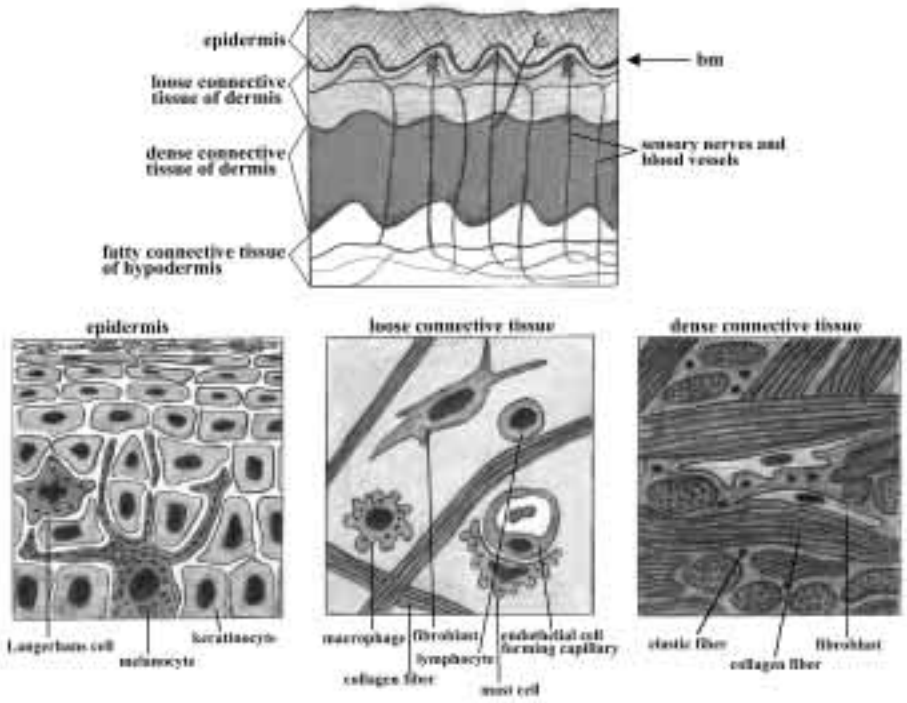


Figure 1. Structure of the skin (modified from Alberts et al, 1994b).

2.1.2. Extracellular matrix

The extracellular matrix (ECM) of interstitial connective tissue is formed by complex and intricate networks in which protein molecules are precisely organized. These networks determine the specific architecture of the tissues and provide cells with biological information and substratum for adhesion and migration. The variation in the relative amounts of macromolecular components and their organization in different tissues, together with the different cells present, create the diversity of the form of connective tissue ranging from bone to transparent matrix of the cornea. In interstitial connective tissue, the ECM is secreted by fibroblasts, and by some specialized cells such as chondrocytes in cartilage and osteoblasts in bone. The two main classes of macromolecules that making up the matrix are 1) fibrous proteins of two functional types: mainly structural (collagen and elastin) and mainly adhesive (fibronectin and laminin), and 2) proteoglycans, proteins with covalently linked polysaccharide chains called glycosaminoglycans (GAGs). Most of the structural ECM molecules, including collagens, noncollagenous glycoproteins and proteoglycans, are chimeric and share common domains. Besides fibroblasts, there are other cells present; *mast cells* are particularly abundant in skin, nasal mucosa and gut, but can be found in other tissues as well. *Macrophages* have many functions, e.g., phagocytosis, antigen presentation and secretion of growth factors. *Plasma cells* are B lymphocytes which produce antibodies. Furthermore, there are many blood-derived cells in the ECM, including *neutrophils*, *eosinophils*, *monocytes* and *lymphocytes* (Figure 1) (see Alberts et al, 1994a; Aumailley and Gayraud, 1998).

2.1.2.1. Connective tissue components

Collagen is the most abundant structural component of the ECM and also the most abundant protein in the human body. The collagen gene family has now over 30 members, which code 19 different proteins (Trojanowska et al, 1998). They are secreted by a variety of cells including the cells of the interstitial connective tissue. The distribution and amount of each type varies greatly in different tissues. A characteristic property of collagens is to form highly organized, stiff, triple-stranded polymers in which three polypeptides, α-chains, are wound around one another in a ropelike superhelix. They are rich in proline, hydroxyproline and glycine and have classical Gly-X-Y triplets in their triplehelical domains of the polypeptides. Most of the collagens are synthesised as procollagens and processed in the extracellular space (Prockop and Kivirikko, 1995). Collagens can be divided into subgroups according to their structure and distribution. The main collagen types found in connective tissue are *fibrillar collagens*, i.e. type I, II, III, V and XI collagens. Type I and III are the major collagens in skin, and type I and II in the bone and cartilage. The type I collagen fibers provide mechanical strength while type III collagen gives flexibility to the tissues (Mauch and Krieg, 1993). Nonfibrillar collagens form sheet-like structures or connect ECM components to collagen fibrils (Prockop and Kivirikko, 1995; Aumailley and Gayraud, 1998). They include *network-forming* collagens (IV, VIII, X), fibril-associated collagens with interrupted triple helices (FACIT) (IX, XII, XIV, XVI, XIX), membrane-associated collagens with interrupted triple helices (MACIT) (XIII, XVII), proteins with multiple triple-helical domains and interruptions (MULTIPLEXINs) (XV, XVIII) and orphans (VI, VII) (Pihlajaniemi and Rehn, 1995).

Elastin and elastic fibers: The elastic properties of many tissues such as the lung, dermis and the large blood vessels are due to the presence of elastic fibers in the extracellular space. The elastic fiber is a complex structure containing elastin, microfibrillar proteins, lysyl oxidase, and probably, proteoglycans. Elastin is the predominant protein of mature elastic fibers and endows the fiber with the characteristic property of elastic recoil. Elastin is widely distributed in vertebrate tissues and possesses an unusual chemical composition rich in glycine, proline, hydrophobic amino acids (elastic repeats), as well as alanine-rich, lysine-containing domains that form crosslinks, all consistent with its characteristic physical properties. It performs various functions, acting statically

in dermis to endure long-term forces and dynamically in arteries to store and release energy rapidly. It is secreted as a soluble precursor, tropoelastin, before forming the amorphous component of elastic fibres. Tropoelastin is secreted into the extracellular space where it becomes highly cross-linked into a rubber-like network through the activity of lysyl oxidase. In elastin gene, hydrophobic and cross-linking domains are encoded in separate exons and there is significant alternative splicing, resulting in multiple isoforms. The elastin gene promoter contains many potential binding sites for various modulating factors, suggesting a complex pattern of transcriptional regulation. The microfibrils contain several proteins, including fibrillin (Sakai et al, 1986), and probably act as an organizing scaffold in the formation of the elastin network. Elastin is produced e.g. in endothelial cells and skin fibroblasts. Of the proteolytic enzymes, only elastases can properly degrade elastin (Mechan et al, 1991; Rosenbloom et al, 1993; Debelle and Tamburro, 1999).

Proteoglycans (PGs) are found primarily in the ECM or associated with the cell surface, where they bind to other matrix- and cell-associated components. They include a group of complex macromolecules in which one or more polysaccharide chains are linked to a central protein core. The predominant polysaccharides are known as glycosaminoglycans and consist of repeated disaccharides. PG structures are diverse in type, size and composition of polysaccharide attached, as well as in primary sequence, domain arrangement, degree of substitution and distribution of the polysaccharide chains along the protein core. Although there are common features among the glycosaminoglycans, six distinct classes are recognized according to fine structural differences: e.g., *chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin*, as well as *keratan sulfate* and *hyaluronate.* There are more than 40 different full-length core proteins, e.g., the aggrecan gene family which consists of four distinct proteins: *aggrecan, versican, neurocan* and *brevican*. Several other families of PGs are known, e.g., the cell-associated proteoglycans comprising the integral membrane *syndecan* family and the BM proteoglycan, *perlecan*, as well as small interstitial PGs *decorin* and *biglycan*. PGs carry out a variety of biological functions, including growth modulation, ionic filtration, matrix organization, cell-adhesion and structural scaffolds. Spatial immobilization of growth factors and cytokines may be one of the most important functions of proteoglycans (Wight et al, 1991; Schwartz 2000).

Fibronectin, a high molecular weight glycoprotein synthesized by many types of cells, is involved in the attachment of cells to the surrounding ECM. Fibronectin has affinity to the other main components of ECM, e.g., collagen and glycosaminoglycans. It also interacts with cell surface integrins. The attachment of cells to fibronectin (or the lack of it) plays a significant role in morphogenetic events, since during development, embryonic cells migrate on or through fibronectin-rich matrices and get cues that direct the cells along their migratory pathways. Malignantly transformed cells tend to lack cell-associated fibronectin. If fibronectin is indeed the main mechanism that anchors cells to the ECM, disturbances of this mechanism could play an important role in malignancy and many other diseases (see Yamada 1991; Ruoslahti 1999; Armstrong et al, 2000).

Integrins comprise a large family of transmembrane glycoprotein receptors that mediate cell-matrix or cell-cell adhesion and can transduce signals that regulate gene expression and cell functions. These signals regulate the activities of cytoplasmic kinases, growth factor receptors, and ion channels and control the organization of the intracellular actin cytoskeleton. Many integrin signals concentrate on cell cycle regulation, directing cells to live or die, to proliferate, or to exit the cell cycle and differentiate (Giancotti and Ruoslahti, 1999). Integrins are composed of two subunits, α and β, and each $\alpha\beta$ combination (currently more than 20) has its own binding specificity and signaling properties. Most integrins recognize several ECM proteins. Conversely, individual matrix proteins, such as fibronectin, laminins, collagens and vitronectin, bind to several integrins (Petruzzelli et al, 1999). Integrins can signal through the cell membrane in either direction: the

extracellular binding activity of integrins is regulated from the inside of the cell (inside-out signaling), while the binding of the ECM elicits signals that are transmitted into the cell (outside-in signaling) (see Giancotti and Ruoslahti 1999).

Tenascins comprise a family of strikingly large ECM glycoproteins, presently including tenascins -C, -R, -W, -X, and -Y. Tenascin is a special cell interaction protein capable of mediating both adhesive and repulsive interactions as well as binding to certain proteoglycans and fibronectin. It has a characteristic hexameric quaternary structure with six arms linked to a central globular domain. Tenascins are tightly regulated and display highly restricted and dynamic patterns of expression. Their expression is closely associated with morphogenetic events, including embryonic migration and induction particularly during neural development, skeletogenesis, and vasculogenesis. These molecules are reexpressed in the adult during normal processes, such as wound repair, nerve regeneration, and tissue involution, as well as in pathological conditions like vascular disease and tumorigenesis (Yamada et al, 1996; Jones et al, 2000).

2.1.2.2. Dermis

The connective tissue of the skin, the dermis, is derived from the mesoderm. It consists of collagenous and elastic fibers embedded into ground substrate. All three components are formed by fibroblasts. The dermis is divided into subepithelial papillary dermis of the loose connective tissue and reticular layer, which lie on adipose tissue (Figure 1). The papillary dermis has numerous blood vessels that penetrate from the deeper layers. Collagen fibers form a finely woven meshwork. The reticular layer has thick collagen bundles and it comprises the largest portion of the dermis. A small number of fibroblasts are interspersed between the collagen bundles. Dermis consists mainly of fibrillar collagens type I and type III (75 % of dry weight). Thin elastic fibers (1 %) are found throughout the dermis between the collagen bundles. The amorphous ground substrate consists mainly of proteoglycans, including dermatan sulfates, hyaluronic acid, chondroitin and heparan sulfates (see Lever and Schaumburg-Lever, 1990a; Archer et al, 1998).

2.1.3. Basement membrane

Basement membranes (BM) are highly specialized structures that separate basal cells from underlying connective tissue. Their structure is relatively similar in different organs. The cutaneous BM zone is located between the epidermis and the dermis and is called *the dermal-epidermal junction (DEJ)* (Figure 2). Besides separating two distinct compartments, DEJ provides adhesion and dynamic interface between them, thus determining the overall structural integrity of the skin. The DEJ restricts the movement of molecules between two compartments on the basis of size and charge, but it allows the passage of migrating and invading cells under normal (i.e. melanocytes and Langerhans cells) or pathological (i.e. lymphocytes and cancer cells) conditions. It also supports the epidermis and influences the behavior of keratinocytes by regulating cell polarity, proliferation, migration and differentiation. In addition, its role is important during morphogenesis and development, wound healing and remodeling of the skin (see Burgeson and Christiano, 1997).

Ultrastructurally, the DEJ is divided into four zones. First, the cell membranes of the basal keratinocytes contain multiprotein junctional complexes called *hemidesmosomes*, into which the epithelial cytokeratins insert (Figure 2). *Lamina lucida* is an electron-lucent region, which anchoring filaments pass through. *Lamina densa*, an electron-dense area, is connected to hemidesmosomes with anchoring filaments. *Sub-lamina densa* contains anchoring fibrils (Chan 1997). Each of these zones also contains ubiquitous BM components, e.g. laminin and nidogen in upper regions, network-forming type IV collagen and heparan sulphate proteoglycan (perlecan) mainly in lamina densa, and type VII collagen, the major component of the anchoring fibrils in the sub-lamina densa. Epithelial

cells generally attach to the BM via actin, α3β1 integrin and laminins (laminins 10/11, 5 and 6/7), which, in turn, connect to type IV collagen via nidogen (Burgeson and Christiano, 1997).

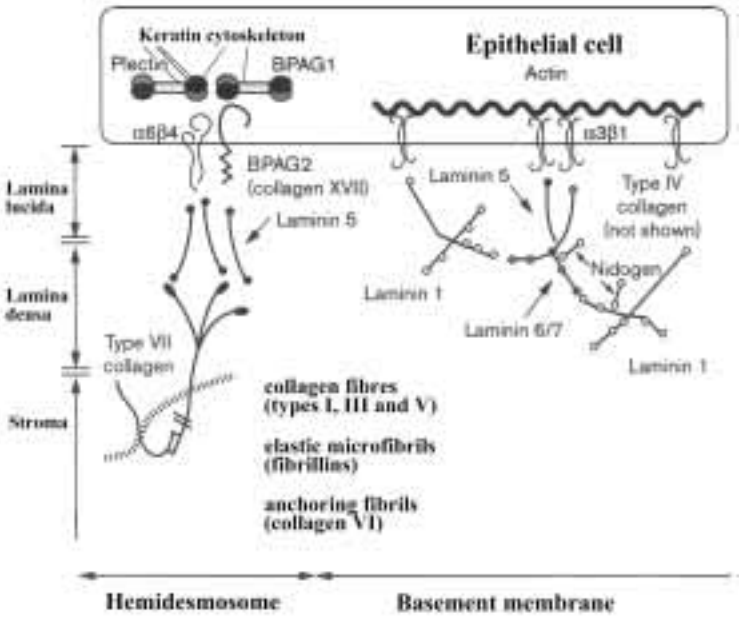


Figure 2. Proposed structure of the BM and hemidesmosome (modified from Burgeson and Christiano, 1997). This model depicts the relative locations of the molecules situated within the DEJ and interactions assumed to occur between them.

In stratified epithelia, such as in skin, and other complex epithelia (e.g., cornea, parts of the gastrointestinal tract), hemidesmosomes (HD) promote the adhesion of epithelial cells to the underlying BM. They consist of three classes of proteins: the cytoplasmic plaque proteins, *plectin* (McLean et al, 1996), *bullous pemphigoid antigen (BPAG) 1* (Stanley et al, 1988) together with *intermediate filament associated protein* and HD 1, act as linkers of the cytoskeleton; the transmembrane proteins, *α6β4 integrin* (Stepp et al, 1990; Sonnenberg et al, 1991) and *BPAG 2* (also known as collagen XVII) (Giudice et al, 1992), connect the cell interior to the ECM; finally, the BM associated proteins of the ECM, such as *laminin-5* (kalinin) (Rousselle et al, 1991), which is linked to type VII collagen (Rousselle et al, 1997). Laminin-5 is a cruciform-shaped molecule consisting of three nonidentical chains within a supramolecular network. Laminin-5 supports cell binding and spreading and is a major ligand for the α6β4 integrin. Interaction between these two molecules seems to be crucial for the formation of HD and the maintenance of stable adhesion (Borradori and Sonnenberg, 1999). α6β4 integrin is able to transduce signals from the ECM to the interior of the cell, which critically modulate the organization of the cytoskeleton, proliferation, apoptosis, and differentiation, all processes of crucial importance in development, wound healing and tumor invasion. It has been suggested that cancer cells use the interaction between α6β4 integrin and laminin-1 and -5 as their mechanism of adhesion during the invasion through surrounding tissues (Tani et al, 1996). Besides interaction with α6β4, laminin-5 can also bind to the α6β1 and α3β1 integrins. In quiescent tissues, it may predominantly be a static adhesive substrate, while during regenerative or wound healing responses it may have a major role in epithelial cell motility through action involving matrix metalloproteinase cleavage (Giannelli et al, 1997; Koshikawa et al, 2000). Laminin-5 is actually the first ECM component to be expressed and deposited by migrating keratinocytes during wound healing *in vivo* (Zhang and Kramer, 1996).

2.2. PROTEOLYTIC REMODELING OF THE ECM

The coordinated degradation of the ECM macromolecules is critical for many biological processes. Proteolytic activity is required in normal physiologic processes, such as reproduction (ovulation, menstruation and mammary gland involution), wound healing, angiogenesis and fetal development. However, excessive degradation of matrix components occurs in pathologic tissue destruction, e.g., in chronic wounds, dermal photoageing, bullous skin diseases, atherosclerosis, rheumatoid arthritis, as well as tumor cell invasion and metastasis (see Birkedal-Hansen et al, 1993; Kähäri and Saarialho-Kere, 1997; Nagase and Woessner, 1999; Curran and Murray, 2000; Ravanti and Kähäri, 2000). Remodeling of the ECM requires cooperation of many different protease systems. ECM degrading proteolytic enzymes can be divided into four subgroups according to their amino acid residue or cofactor required for catalytic activity. 1) *Cysteine proteases* include two superfamilies, and for example most cathepsins belong to this group (Chapman et al, 1997). 2) *Aspartic proteases* include, e.g., cathepsins D and E, renin and pepsin (Hoegl et al, 1999). 3) *Serine proteases* include for instance, tissue-type and urokinase-type plasminogen activators (tPa, uPa), thrombin, furin and plasmin (Plow et al, 1995). 4) *Metalloproteinases* 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 they have conserved "Met-turn" motif, as well as conserved structural topology. Metzincins consist of four groups; *serralysins*, *matrixins* (matrix metalloproteinases), *astacins* and *adamalysins* (see Stöcker et al, 1995; Bergers and Coussens, 2000a).

2.2.1. Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases are a group of enzymes which together can degrade all the known protein components of the ECM. The first MMP member, collagenase, was found in the tadpole tail (Gross and Lapière, 1962). Presently, the MMP gene family consists of 21 members and the family is still growing. MMPs are products of different genes dispersed to the genome, although there is an MMP gene cluster in choromosome 11 (Massova et al, 1998). MMPs differ structurally and by the fact 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 (Figure 3). MMPs can be divided into the following subgroups: 1) collagenases, 2) gelatinases, 3) stromelysins and 4) stromelysin-like MMPs, 5) membrane type (MT) MMPs, and 6) other MMPs according to their structure and substrate specificity (Table I) (see Birkedal-Hansen et al, 1993; Nagase and Woessner, 1999). It is important to remember that most of the MMP substrates defined to date have been demonstrated *in vitro* and their importance in biological context is not known.

The N-terminal *signal peptide*, rich in hydrophobic amino acids, directs the protein for secretion into the endoplasmic reticulum and, eventually, out of the cell. All except MMP-17 (Puente et al, 1996) have a signal peptide. MMP-23 has a signal anchor, which binds it to the cell surface (Pei et al, 2000). All MMPs have a *pro-domain* acting as an internal inhibitor to keep the enzyme inactive until proteinase activity is required. It contains a highly conserved sequence, which has cysteine residue binding the catalytic zinc ion and thus keeping the proMMP in latent form. Activation occurs with disruption of the Cys-zinc bond and cleavage of the pro-domain leading to a conformational change to the catalytically active form (Van Wart and Birkedal-Hansen, 1990). The catalytic core-domain contains the active, protein-degrading ability of the proteinase. This domain includes a metal-binding site for Zn^{2+}, the binding of which is essential for proteolytic activity (and explains the word 'metallo' in its name). MMPs are classified on the basis of additional protein domains that contribute to their individual characteristics (Nagase and Woessner, 1999). A proline-rich *hinge region* connects the catalytic domain to the following *hemopexin* domain. The active form of matrilysins contains only the catalytic domain characteristic of all MMPs. Structurally unique MMP-23 also

lacks the hemopexin domain. All other MMPs have an additional C-terminal domain with homology to a serum protein called hemopexin. There is a cysteine residue at both ends, which fold the domain into a four-bladed propeller structure. The hemopexin domain is suspected to mediate additional protein–protein interactions with substrates and with naturally occurring inhibitors TIMPs (Sanchez-Lopez et al, 1993; Baragi et al, 1994).

In some of the MMP members there are additional functional domains present. *Fibronectin type II inserts* in the catalytic domain of gelatinases modulate protein interactions important in substrate (gelatin) recognition (Murphy et al, 1994). The *transmembrane* domain in the C-terminus localizes some cell-surface-bound MMPs to the cell membrane and short sequence between the prodomain and the catalytic domain, i.e. *furin* domain provides an alternative cleavage site for MMP activation (Nagase and Woessner, 1999). These different combinations of protein motifs within the MMP family, together with individual gene sequences, generate the wide variety of characteristics within it.

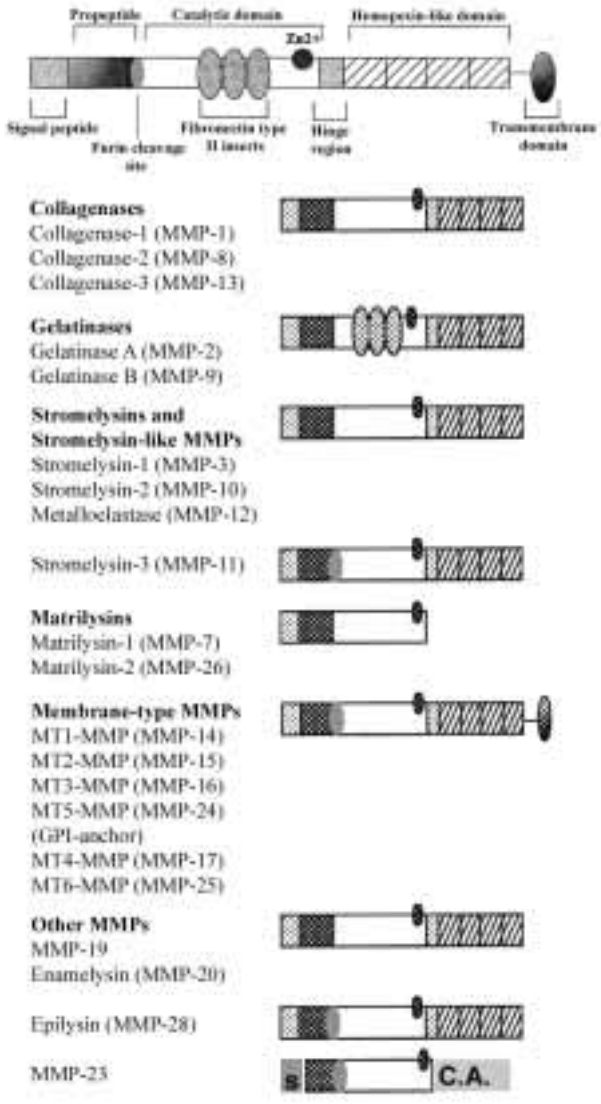


Figure 3. Structure of matrix metalloproteinases (modified from Johansson and Kähäri, 2000). s, signal anchor; C.A., cysteine array

Table I. Human Matrix Metalloproteinases

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

(Modified from Murphy and Knäuper, 1997; Johansson and Kähäri, 2000; McCawley and Matrisian, 2001) Col, collagen; COMP, cartilage oligomeric matrix protein; IGFBP, insulin-like growth factor binding protein; LN, laminin; MBP, myelin basic protein; PAI, plasminogen activator inhibitor; TACE, TNF-α converting enzyme; α2M, α2-macroglobulin; α1PI, α1-proteinase inhibitor; ND, not detected

2.2.1.1. 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 ¾ N-terminal and ¼ C-terminal triple helical fragments, which denaturate spontaneously to gelatin at 37°C. These fragments can be further degraded by other MMPs, such as gelatinases (see Birkedal-Hansen et al, 1993). MMP-1, the first MMP determined by cDNA cloning (Goldberg et al, 1986), cleaves preferentially type III collagen while MMP-8 cleaves type I and II collagens (Welgus et al, 1981; Hasty et al, 1987). MMP-13 cleaves preferentially type II collagen and also gelatin more effectively than other collagenases (Knäuper et al, 1996a). *In vitro* MMP-1 is expressed in various normal cells, such as keratinocytes, fibroblasts, endothelial cells, monocytes, macrophages and chondrocytes (see Birkedal-Hansen et al, 1993). It can be detected *in vivo* in many physiological situations, including embryonic development and wound healing, but also in chronic cutaneous ulcers and many cancers (McGowan et al, 1994; Saarialho-Kere 1998; Stetler-Stevenson et al, 2001). MMP-8 is synthesized by maturing leukocytes in bone marrow, stored intracellularly in granules and released in response to extracellular stimuli (Hasty et al, 1986; Hasty et al, 1990). In addition, it has been detected in human articular cartilage chondrocytes, mononuclear fibroblast-like cells in the rheumatoid synovial membrane, and more recently, in gingival fibroblasts and bronchial epithelial cell (Cole et al, 1996; Hanemaaijer et al, 1997; Abe et al, 2001; Prikk et al, 2001). MMP-13 was originally cloned from a breast tumor cDNA library (Freije et al, 1994) and has a potent degrading activity against an exceptionally wide spectrum of substrates. It is expressed during fetal bone development, bone remodeling, and gingival wound repair (Ståhle-Bäckdahl et al, 1997; Johansson et al, 1997b; Ravanti et al, 1999), as well as pathological situations, such as chronic cutaneous ulcers (Vaalamo et al, 1997), rheumatoid arthritis (Ståhle-Bäckdahl et al, 1997), periodontitis (Uitto et al, 1998) and various invasive tumors, e.g. squamous cell carcinomas of different organs, chondrosarcomas and melanoma (Johansson et al, 1997c; Uria et al, 1998; Airola et al, 1999; Pendas et al, 2000).

2.2.1.2. Gelatinases

Gelatinase A (MMP-2, also known as *72 kDa gelatinase*) is expressed in a variety of normal and transformed cells, including fibroblasts, keratinocytes, endothelial cells, chondrocytes, and osteoblasts. *Gelatinase B* (MMP-9, *92 kDa gelatinase*) is produced by keratinocytes, monocytes, macrophages, PMN (polymorphonuclear) leukocytes and a large variety of malignant cells. Gelatinases are able to degrade type IV, V, VII, X, XI, and XIV collagens, gelatin, elastin, proteoglycan and fibronectin among others (Birkedal-Hansen et al, 1993). They have three tandem fibronectin type repeats in the catalytic domain, which are important for elastolytic activity (Shipley et al, 1996a). MMP-9 also has a unique proline-rich domain with unknown function homologous to the α2-chain of type V collagen between catalytic and hemopexin-like domains (Wilhelm et al, 1989). Gelatinases are believed to have a particularly important role in cancers due to their capacity to cleave BM components (Birkedal-Hansen 1995). MMP-2 is regulated differently from other MMPs and it is believed to have a unique role in cell-matrix interactions, including cancer invasion (Yu et al, 1996). Furthermore, MMP-2 knock-out mice exhibit reduced angiogenesis, impared tumor growth and decreased metastasis (Itoh et al, 1998). In MMP-9-deficient mice, hypertrophic chondrocytes develop normally, but apoptosis, vascularization, and ossification are delayed, resulting in progressive lengthening of the growth plate (Vu et al, 1998). These mice are also resistant to blister formation in an experimental bullous pemphigoid model (Liu et al, 1998).

2.2.1.3. Stromelysins and stromelysin-like MMPs

Stromelysins-1 and *-2* (MMPs-3 and -10) are highly homologous in primary structure and substrate specificity. They have been shown to degrade *in vitro,* e.g., collagen III and IV, gelatin, casein, aggrecan, elastin, and proteoglycan core proteins (Murphy et al, 1991b; Chandler et al, 1997) and MMP-3 also osteopontin and serine proteinase inhibitors α1-proteinase inhibitor and α1-antitrypsin (Winyard et al, 1991; Zhang et al, 1994; Agnihotri et al, 2001). It is expressed by a variety of cells, e.g., keratinocytes, fibroblasts and chondrocytes. MMP-3 is expressed by stromal cells during normal mammary development and is strongly upregulated during post-lactational mammary involution (Witty et al, 1995; Lund et al, 1996). It alters the cellular microenvironment and can act as a natural tumor promoter and influence mammary tumor initiation (Sternlicht et al, 1999).

MMP-10 was initially identified in an adenocarcinoma cDNA library (Muller et al, 1988), and since then its expression has been associated with cancer invasion, mostly in tumours of epithelial origin (Muller et al, 1991; Polette et al, 1991). It was later isolated also from a rheumatoid synovial cell cDNA library and shown to be differentially expressed from MMP-3 in human foreskin and rheumatoid synovial fibroblasts (Sirum et al, 1989). By Northern analysis, MMP-10 has been detected in tumor cells at least in SCCs of the head and neck and lung (Muller et al, 1991), and also in hepatocellular carcinoma using immunoblotting (Lichtinghagen et al, 1995). Furthermore, MMP-10 has an important role in cutaneous and intestinal wound healing. It is expressed at the wound edge by migrating basal keratinocytes during both human and murine wound repair (Saarialho-Kere et al, 1994; Madlener et al, 1996) and by migrating enterocytes in inflammatory bowel disease (Vaalamo et al, 1998). MMP-10 expression is important for the normal repair process and is upregulated by cytokines rather than cell-matrix interactions (Rechardt et al, 2000). Furthermore, MMP-3 and -10 are differentially expressed in human bone (Bord et al, 1998) and they play a critical role in the activation of procollagenases-1 (MMP-1), -2 (MMP-8) and -3 (MMP-13) (Nicholson et al, 1989; Knäuper et al, 1996b; Nagase 1998).

Stromelysin-3 (MMP-11) has been cloned from invasive breast cancer tissue (Basset et al, 1990). It is expressed in mesenchymal cells located close to epithelial cells during physiological and pathological tissue remodeling processes. It has a furin cleavage site in its prodomain and thus is processed intracellularly before being released as a mature enzyme (Pei and Weiss, 1995). It is expressed in most invasive human carcinomas, and high MMP-11 levels are associated with a poor clinical outcome e.g. in breast cancer, suggesting a role during malignant processes (Rouyer et al, 1994). Mice lacking MMP-11 show reduced tumorigenesis in response to chemical mutagenesis (Masson et al, 1998). It does not seem to cleave matrix components but may increase tumorigenesis by decreasing cancer cell death (Boulay et al, 2001).

Human macrophage metalloelastase (HME, MMP-12) is often considered a member of the stromelysin subgroup. It was initially found in alveolar macrophages of cigarette smokers (Shapiro et al, 1993). Metalloelastase is the most effective MMP against elastin but has a broad selection of substrates including type IV collagen, fibronectin, laminin, gelatin, vitronectin, entactin, proteoglycan, heparan and chondroitin sulfates, α1-antitrypsin, myelin basic protein, and fibrinogen (Chandler et al, 1996; Gronski et al, 1997; Hiller et al, 2000). The expression of MMP-12 *in vivo* has been demonstrated mainly in macrophages, for example, in intestinal ulcerations (Vaalamo et al, 1998) and cutaneous diseases associated with granulomas or macrophage migration (Vaalamo et al, 1999). Furthermore, it degrades elastic fibers in atherosclerosis (Halpert et al, 1996), and aneurysms (Curci et al, 1999). MMP-12 deficient mice have impaired macrophage-mediated proteolysis and tissue invasion (Shipley et al, 1996b) and do not develop emphysema like wild-type mice (Hautamäki et al, 1997). Interestingly, granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates tumor-infiltrating macrophages to produce metalloelastase, which cleaves

plasminogen into angiostatin (Dong et al, 1997; Cornelius et al, 1998). Thus, MMP-12 may prevent tumor growth by inhibiting angiogenesis. MMP-12 is also able to reduce the number of functional urokinase-type plasminogen activator reseptors (uPAR) on endothelial and tumor cells. This leads to decrease in uPA binding, and since uPA is needed in the invasion and formation of new capillary structures, it inhibits angiogenesis and ultimately also tumor invasion and metastasis (Koolwijk et al, 2001).

2.2.1.4. Matrilysins

Matrilysin (MMP-7) and *endometase* (MMP-26) are the smallest MMPs, lacking the hinge region and hemopexin domain, which is considered to restrict substrate specificity (Park et al, 2000). MMP-7 was originally identified as the small putative uterine metalloproteinase (PUMP), when screening for stromelysin-related genes (Muller et al, 1988). It cleaves *in vitro* elastin, the core protein of proteoglycans, fibronectin, entactin, laminin-1, gelatin, and serpins among others (Murphy et al, 1991b; Sires et al, 1993; Halpert et al, 1996). Unlike most other MMPs expressed or released only in response to injury, disease, or inflammation, matrilysin is expressed by noninjured, noninflamed exocrine and mucosal epithelium in many adult tissues. Specifically, matrilysin is produced by all skin and salivary glands, airway ciliated cells, and by the ductal or glandular epithelium of the pancreas, liver, breast, intestine, and urogenital tissues (Rodgers et al, 1994; Saarialho-Kere et al, 1995; Wilson et al, 1995). In addition, matrilysin is markedly upregulated by migrating epithelium in the injured airway (Dunsmore et al, 1998) and intestine (Saarialho-Kere et al, 1996), suggesting that 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 (Dunsmore et al, 1998). MMP-7 has been suggested to play a role in early stages of tumorigenesis (see Fingleton et al, 1999). It is upregulated in many tumors, especially of epithelial origin, such as breast (Basset et al, 1990), lung and upper respiratory tract (Muller et al, 1991), skin (Karelina et al, 1994), stomach and colon (Newell et al, 1994; McDonnell et al, 1991). In addition, intestinal tumorigenesis is reduced in MMP-7-deficient mice (Wilson et al, 1997). Interestingly, MMP-7 can also inhibit tumor angiogenesis by generating angiostatin (Patterson and Sang 1997), which has also been shown *in vivo* (Pozzi et al, 2000).

MMP-26 was cloned from fetal (de Coignac et al, 2000), placenta (Uria and Lopez-Otin, 2000), and human endometrial tumor (Park et al, 2000) cDNAs. It efficiently cleaves fibrinogen and ECM proteins, including fibronectin, IV collagen, gelatin, vitronectin and α1-proteinase inhibitor (Park et al, 2000; Uria and Lopez-Otin, 2000; Marchenko et al, 2001a). MMP-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. MMP-26 is detected in placenta and uterus and is also widely expressed in diverse tumor cell lines as well as in malignant tumors from different sources, including carcinomas of lung, prostate and breast by RT-PCR (Marchenko et al, 2001a).

2.2.1.5. Membrane-type MMPs

The first membrane-type MMP (MT1-MMP) was detected on the surface of invasive tumor cells in 1994 (Sato et al) and altogether six distinct MT-MMPs have been found so far (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24; MMP-25) (Takino et al, 1995; Will and Hinzmann, 1995; Puente et al, 1996; Llano et al, 1999; Pei 1999b). Their domain structure is similar to other MMPs, except for a transmembrane domain and cytoplasmic tail at the C-terminus. They also contain a furin-sensitive motif between the propeptide and catalytic domain, cleaving of which in trans-Golgi network leads to activation of MT-MMPs (Sato et al, 1996). MT4-MMP and MT6-MMP have a glycosylphosphatidylinositol (GPI)-anchor binding them to the cell surface and thus form their own subgroup in the MT-MMP family (Itoh et al, 1999; Kojima et al, 2000). Localization of MT-MMPs

at the cell surface implies that they might have a significant role in the regulation of cell-matrix interactions and activation of other MMPs. Indeed, MT1-, MT2- MT3-, MT5-, and MT6-MMP activate proMMP-2 (Will et al, 1996; Pei 1999a; Miyamori et al, 2000; Velasco et al, 2000), and MT1- and MT2-MMP also MMP-13 (Knäuper et al, 1996c). Furthermore, it has recently been shown that MT1-, MT2-, and MT3-MMP can degrade cell surface tissue transglutaminase (tTG) at the leading edge of motile cancer cells thus suggesting a novel regulatory function of membrane-anchored MMPs in cancer cell adhesion and locomotion (Belkin et al, 2001).

MT1-MMP (MMP-14) digests e.g., types I, II and III collagens, fibronectin, vitronectin, tenascin, nidogen, laminin-5, perlecan, proteoglycan core proteins, fibrin, α1-proteinase inhibitor and α2-macroglobulin, as well as tumor TNF-α fusion protein (d'Ortho et al, 1997; Ohuchi et al, 1997; Hiraoka et al, 1998; Koshikawa et al, 2000). It seems to have an essential role in tumor cell invasion, mainly due to its ability to activate latent gelatinase A. The expression of MT1-MMP has been detected in tumor cells and adjacent stromal cells in a variety of human cancers, including head and neck, vulvar, lung, breast, colon, and liver carcinomas among others (Sato et al, 1994; Okada et al, 1995; Harada et al, 1998; Johansson et al, 1999).

MT2-MMP (MMP-15) is expressed in human brain, placenta and heart and in some cancers. It can degrade laminin, fibronectin and tenascin (Takino et al, 1995; d'Ortho et al, 1997). MT3-MMP is expressed in several human tissues, such as liver, placenta, colon, testis, intestine, pancreas, lung, kidney, and heart (Will et al, 1995). It degrades gelatin, casein, type II collagen, fibronectin, laminin-1, vitronectin, cartilage proteoglycan, α1-proteinase inhibitor, and α2-macroglobulin (Matsumoto et al, 1997; Shofuda et al, 1997; Shimada et al, 1999). The expression of MT3-MMP (MMP-16) is elevated after arterial injury, which also applies to MT1-MMP (Shofuda et al, 1998).

MT4-MMP (MMP-17) is expressed in brain, leukocytes, colon, ovary and testis. It has also been found in breast carcinomas (Puente et al, 1996) and some carcinoma cell lines (Grant et al, 1999; Kajita et al, 1999). It degrades gelatin, fibrinogen and fibrin and has TNF-α convertase activity (Wang et al, 1999; English et al, 2000). MT5-MMP (MMP-24) is expressed in brain, kidney, pancreas and lung, and in brain tumors (Llano et al, 1999). Its expression is also upregulated in kidneys of diabetic patients and suggests a novel role for MT5-MMP in the pathogenesis of renal tubular atrophy (Romanic et al, 2001). Murine MT5-MMP is shed from the cell surface as a soluble proteinase (Pei 1999a). MT6-MMP (MMP-25) is specifically expressed by peripheral blood leukocytes (Pei 1999b) and some brain tumors (Velasco et al, 2000). It is able to cleave type IV collagen, gelatin, fibronectin and fibrin (English et al, 2001).

2.2.1.6. Other MMPs

MMP-19 has been cloned from mammary gland (Cossins et al, 1996) and liver (Pendas et al, 1997) and isolated also as an autoantigen from the inflamed rheumatoid synovium as RASI (Sedlacek et al, 1998). It has a domain structure typical to MMPs, but it also contains a sequence of 36 residues in C-terminus lacking homology to any other MMP. MMP-19 is widely expressed in tissues, including placenta, lung, pancreas, ovary, spleen and intestine (Pendas et al, 1997) and also in acutely inflamed synovial tissue, especially in capillary endothelial cells, suggesting a role in angiogenesis (Kolb et al, 1999). MMP-19 is capable of degrading many components of the ECM and BM, e.g., type IV collagen, laminin, nidogen, tenascin-C, fibronectin, type I gelatin as well as aggrecan and cartilage oligomeric protein (COMP), but cannot activate any proMMPs (Stracke et al, 2000a; Stracke et al, 2000b).

Enamelysin (MMP-20) is exclusively expressed in ameloblasts and odontoblasts of developing teeth (Llano et al, 1997). Human enamelysin is able to degrade amelogenin, the major protein component

of the enamel matrix, as well as aggrecan and COMP (Stracke et al, 2000b). On the basis of its degrading activity on amelogenin and its highly restricted expression to dental tissues, human enamelysin apparently plays a crucial role in tooth enamel formation.

MMP-23, cloned from an ovary cDNA library (Velasco et al, 1999), is expressed mainly in ovary, testis and prostate and might have a specialized role in reproductive processes. It has a unique structure lacking structural features of diverse subclasses. It has 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. Little is known about its biochemical function. In mouse homologue, it was shown to contain an RRRR motif, which regulates its secretion and activation. It might establish a third subclass of membrane-bound MMPs and, thus, a new subclass of the MMP superfamily (Pei et al, 2000).

Epilysin (MMP-28) was cloned from testis and keratinocyte cDNA libraries (Lohi et al, 2001). 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*. (2001), lung is the main organ expressing MMP-28, and it is also expressed in a broad range of carcinomas as assessed by PCR (Marchenko and Strongin, 2001b).

2.2.2. Regulation of MMPs

MMPs are highly regulated, with both secretion and activity levels under tight control. In general, 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 Nagase and Woessner, 1999). MMP proteins are transcribed and secreted by the constitutive secretory pathway, except in the case of immune cells, such as macrophages and neutrophils, where MMPs can be stored in, and released from, secretory granules (MMP-8 and –9). In addition, MMP-7 is constitutively expressed in the ductal epithelium of exocrine glands (Saarialho-Kere et al, 1995). Regulation occurs at multiple levels, including transcription, modulation of mRNA half-life, secretion, localization, zymogen activation and inhibition of proteolytic activity. MMPs have natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), and certain non-specific proteinase inhibitors, such as α2-macroglobulin.

2.2.2.1. Transcriptional regulation

At 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 Nagase and Woessner, 1999).

Promoters of MMPs show remarkable conservation of regulatory elements (see Westermarck and Kähäri, 1999). Extracellular stimuli regulate MMP activity through AP-1 (activator protein-1) binding site, which is situated in the proximal promoter approximately at position -70 bp, with regard to the transcription initiation site in inducible MMP genes (MMPs -1, -3, -7, -9, -10, -12, -13, and -19) (Figure 4). Another AP-1 site is found in the promoters of MMP-1, MMP-3 and MMP-9. AP-1 site binds dimeric transcription factors of the Jun and Fos families, which stimulate the transcription of the MMP genes. 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 (TNF, IL-1) and cellular stress, such as UV light (Karin et al, 1997). MMP-2, MMP-11, MMP-28 and MT1-MMP genes do not have an AP-1 site in their promoters. The 5'-flanking sequence of the MT1-MMP gene contains putative regulatory elements, including one SP-1 site and four CCAAT-boxes, whereas there is no TATA-box (Lohi et al, 2000). The MMP-28 promoter has distinctive structural and functional features including GT-box, which dramatically reduces the transcriptional activity of the MMP-28 gene. Also SP-1 and SP-3 are potential regulators of it (Illman et al, 2001). MMP-11 is transcriptionally activated only by retinoic acid or by stromal cell interactions with tumor cells (Anglard et al, 1995; Ahmad et al, 1997).

Although the importance of AP-1 site is undeniable, transcriptional activity usually depends on its interaction with several other cis-acting sequences located throughout the promoter (Figure 4). Multiple polyomavirus enhancer A-binding protein-3 (PEA-3) sites are present in all the promoters except MMP-2. They are located adjacent to at least one AP-1 element, except in MMP-12 (Figure 4) and bind members of the ETS family of transcription factors (Wasylyk et al, 1991). Individual AP-1 and PEA3 sites often play distinct roles in basal and induced transcription, probably due to the function of the specific transcription factors binding to these sites. 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 (Denhardt 1996). The expression of ETS-1 colocalizes with several MMPs at the invasive edge of many tumors; ETS-1 is also expressed during tumor angiogenesis (Wernert et al, 1992). AP-1 site plays a major role in the repression of MMPs by TGF-β, retinoids and glucocorticoids (Benbow et al, 1997). A TGF-β-inhibitory element (TIE) has been described in some promoters, and, at least in MMP-3 promoter, it mediates TGF-β repression (see Vincenti 2001).

Induction of MMP-12 in macrophages by cytokines and growth factors (IL-1, TNF-α, M-CSF, VEGF, PDGF-BB), as well as its inhibition by TGF-β1, depended on signaling through an AP-1 site at -81 base pairs. The inhibitory effect of TGF-β1 on MMP-12 was dependent on Smad3 transcription factor (Feinberg et al, 2000) (Figure 4). Furthermore, AP-1 site is critical for the induction of MMP-12 promoter activity by GM-CSF. AP-1 binding activity was increased by GM-CSF treatment and the AP-1 complex induced by GM-CSF consisted of multiple Jun and Fos isoforms (Wu et al, 2000). MMP-12 gene activity can, interestingly, be regulated in an allele-specific manner. A common functional polymorphism within the MMP-12 promoter influences the binding affinity of AP-1 and, thus, coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease (Jormsjo et al, 2000).

Both AP-1 and PEA3 sequences are required for the induction of MMP-7 by tumor promoter 12-O-tetradecanoylphordol-13-acetate (TPA) and EGF (Figure 4). In addition, induction of MMP-7 expression and activity by Ki-*ras* oncogene is mediated through AP-1. Interestingly, *ras* mutations are associated with colon tumor progression, as is MMP-7. MMP-7 can be both upregulated and downregulated by TGF-β and has several TIE elements in its promoter (Figure 4) (Wilson and Matrisian, 1998). MMP-7 is also a target gene for β-catenin-Tcf transcription complex, whose activity is thought to play a crucial role in the initiation of intestinal tumorigenesis, and it functions in co-operation with PEA3 and AP-1 factors (Crawford et al, 2001). MMP-10 is responsive for various growth factors, like TGF-α, EGF, KGF, TNF-α and TGF-β1, as well as phorbol ester in keratinocytes (Windsor et al, 1993; Madlener et al, 1996), but functional studies at the transcriptional level have not been performed.

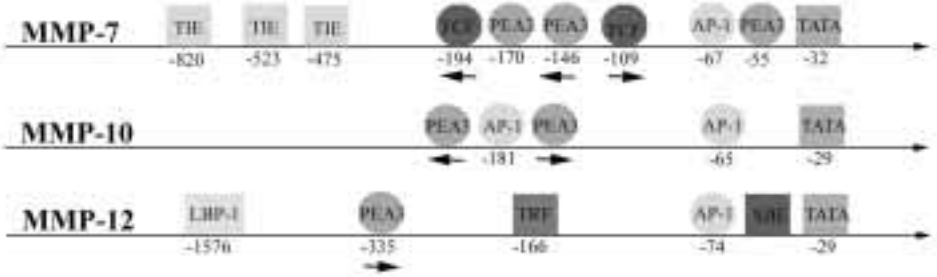


Figure 4. Regulatory elements of promoter regions of MMP-7, -10, and –12 (modified from Westermarck and Kähäri, 1999). LBP, leader binding protein; SBE, STAT binding element; TCF, Tcf binding site; TRF, octamer binding protein.

2.2.2.2. Proenzyme activation

In most cases the activity of MMPs is controlled extracellularly: they are secreted in an inactive, latent form that requires the removal of the prodomain to attain the catalytically active state. MMP-11, MMP-28 and MT-MMPs are activated intracellularly by Golgi-associated furin-like proteases. MMP-23 is both activated and released from the cell surface by a single cleavage (Pei et al, 2000). The latency of MMPs is dependent on cysteine switch (Springman et al, 1990). The highly conserved cysteine residue in the prodomain binds covalently to the Zinc-ion in the catalytic domain, and this interaction has to be disrupted in order to activate proMMPs (Nagase and Woessner, 1999). Various compounds, e.g. organomercurials (APMA), SH-reactive agents, reactive oxygen and detergents can react with this cystein and convert it to a nonbinding form (Springman et al, 1990; Nagase and Woessner, 1999).

Secreted proMMPs can be activated *in vitro* with plasmin, trypsin, kallikrein, chymase, and mast cell tryptase. MMPs can also activate other MMPs (see Table I). A classical example is the activation of proMMP-2 with MT1-MMP, which starts with TIMP-2 and MT1-MMP forming a receptor complex to the cell surface. TIMP-2 has a free C-tail, which binds proMMP-2, and then TIMP-2-free MT1-MMP can activate proMMP-2 (Zucker et al, 1998). At low levels TIMP-2, thus, enhances the activation process, whereas at high levels it inhibits MMP-2 activation (see Seiki 1999). Also TIMP-4 can form complex with proMMP-2, while TIMP-1 forms complex with proMMP-9, and TIMP-3 with both proMMPs-2 and -9 (Goldberg et al, 1989; Bigg et al, 1997; Butler et al, 1999). The uPa/plasmin system, as well as other proteinases, activate proMMPs, including MMPs-2, -9, and -12, in many physiological situations (Carmeliet et al, 1997; Mazzieri et al, 1997). These activation cascades between MMPs and other proteinases form complex networks in regulating tissue proteolysis.

2.2.2.3. Inhibitors of MMPs

MMP activity is further modulated through interactions with their natural inhibitors, the specific tissue inhibitors of matrix-metalloproteinases (TIMPs) (Edwards et al, 1996; Gomez et al, 1997). In addition, serine protease inhibitors (serpins), which include α1-antitrypsin and plasminogen activator inhibitors (PAI)-1 and –2, control general proteolytic activity, including MMPs, in tissues. Also non-specific serum proteinase inhibitors (e.g. α-macroglobulin) can inhibit the proteolytic activity of MMPs. α-macroglobulin is abundant in many human tissues and is important in inhibiting MMP activity in serum and body fluids. It can also inhibit serine-, cysteine-, and aspartate proteinases (Birkedal-Hansen et al, 1993). Due to the role of MMPs in many pathological conditions, various chemical inhibitors have also been synthesized in order to use them as therapeutic agents (Brown et al, 1999) (see chapter 2.3.4.3. at page 35).

Four TIMP family members have been described to date (TIMPs-1, -2, -3, and -4), most of which are capable of binding and inhibiting the activity of all the members of the MMP family. (Edwards et al, 1996; Gomez et al, 1997). In general, TIMPs can inhibit the activity of all MMPs *in vitro*, except MT1-MMP and MT3-MMP, which are not inhibited by TIMP-1 (Will et al, 1996; Shimada et al, 1999). The tertiary structure of TIMPs is very similar. However, TIMPs demonstrate differences in tissue distribution, as well as in their ability to form complexes with the inactive form of MMP and, thus, the ability to control MMP activation. The N-terminus is necessary for MMP inhibition (Murphy et al, 1991a; Langton et al, 1998) while the C-terminal end affects their specificity (Goldberg et al, 1992; Ogata et al, 1995; Bigg et al, 1997). The mechanism involves forming non-covalent stoichiometric complexes with the zinc-binding site of the active form of MMPs (Gomis-Rüth et al, 1997). Furthermore, TIMPs can inhibit the activation of many proMMPs and various TIMPs have preferential inhibitory capabilities against different proMMPs. For instance, TIMP-1 preferentially forms complex with proMMP-9, while TIMP-2 and TIMP-4 reduced proMMP-2 activation by MT1-MMP. TIMP-4 also binds to MT1-MMP inhibiting its autocatalytic processing (Gomez et al, 1997; Hernandez-Barrantes et al, 2001).

Except for TIMP-3, which is bound to ECM (Leco et al, 1994), TIMPs are present in a soluble form in most tissues and body fluids (Gomez et al, 1997) and expressed by a variety of cell types. The latest member of the TIMP family, TIMP-4, is mainly expressed in heart (Greene et al, 1996), suggesting a tissue-specific function. The expression of TIMP-1 and TIMP-3 is upegulated by various growth factors and cytokines, including TGF-β, EGF, TNF-α, ILs, retinoids, and glucocorticoids, while the expression of TIMP-2 is mainly constitutive (Birkedal-Hansen 1995).

TIMPs 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 ECM components in tumor invasion and metastasis, and their reciprocal expression levels have been assessed for prognostic significance (Birkedal-Hansen 1995). Besides antitumor activity, TIMPs may also have growth promoting activity in an MMP-independent fashion (Henriet et al, 1999). Other functions of TIMPs include matrix binding, inhibition of angiogenesis and the induction of apoptosis (Brew et al, 2000). The balance between various TIMPs may also be a critical factor in determining the degradative potential of cells in normal and pathological conditions.

2.2.3. MMPs in development

Embryonic development requires an accurate coordination of programs for growth, differentiation, ECM formation and angiogenesis. These processes are modulated by a diverse group of macromolecules, including ECM glycoproteins, their receptors, i.e. integrins, and ECM-degrading enzymes as well as their inhibitors. Studies suggest that, besides degrading ECM components, MMPs appear to regulate many fundamental cellular processes of development, including morphogenesis, apoptosis, cell migration, proliferation and invasion. They are involved e.g., in ovulation, embryonic growth, trophoblast invasion, and the development of the skeleton and organs (see Birkedal-Hansen et al, 1993; Werb and Chin, 1998; Werb et al, 1999). Tissue morphogenesis depends on specific movements of the cells which sometimes have to migrate over long distances. Studies have implicated MMPs in the migration of a variety of epithelial, mesenchymal, and neuronal cells either through the ECM or on a specific substrate. Organ cultures show that MMPs are also involved in altering ECM, permitting invasion, selection of branch points, and regulation of epithelial-to-mesenchymal transition during branching morphogenesis. Interaction between ECM components and cell surface molecules regulate cell behavior, and MMPs affect cell survival and

proliferation both positively and negatively by regulating survival signals generated by specific adhesive events (Vu and Werb, 2000).

MMPs in human development: The role of MMPs in human development has mainly been elucidated by studying their expression in specific tissues. MMP-1 has been detected in developing fetal skin from 6 to 20 weeks. It is associated with the development of interfollicular epidermis, the collagenous dermis, and the development of nerves and hair follicles (McGowan et al, 1994). It also participates in blood vessel development in fetal skin (Karelina et al, 1995). MMP-2 is involved in appendageal development and contributes to the remodeling of the BM zone during fetal skin morphogenesis. The absence of type VII collagen, which is the major component of anchoring fibrils, correlated directly with the presence of MMP-2 (Karelina et al, 2000). Expression of MMPs has also been demonstrated during intrahepatic bile duct development, where MMP-2, -3, and -9 were expressed only in the ductal plate, while MMP-1 was also present in migrating biliary cells and immature bile ducts (Terada et al, 1995). Furthermore, MMP-1, -2, -7 and -13 proteins were localized in the early human liver anlage already at the 6^{th} gestational week in different cell types at different time points. MMP-13 was found exclusively in hematopoietic cells, as was MMP-7 from the 7^{th} week onward (Quondamatteo et al, 1999). By RT-PCR, MT1-MMP was detected in fetal membranes (Fortunato et al, 1998). MMP-13 mRNA has been detected in hypertrophic chondrocytes in developing human bone beginning at week 10 and osteoblasts and fibroblasts in calvarial bone at week 16 (Johansson et al, 1997b; Ståhle-Bäckdahl et al, 1997). Also MMP-10 protein has been localized to chondrocytes of the growth plate in the fetal rib, while MMP-3 was in osteocytes in the surrounding fibrous tissue. They were both also expressed by osteoblasts at sites of bone formation, and MMP-10 in osteoclasts as well (Bord et al, 1998). MMP-1, MMP-2, and MMP-9 were expressed in developing human fetal testis and ovary (Robinson et al, 2001). Obviously, it is crucial that MMPs and their inhibitors, TIMPs, function coordinately during various stages of development. Expression of TIMP-3 mRNA has been detected in developing bone, kidney, and in mesenchymal cells, whereas TIMP-1 was only detected in osteoblasts of developing bone (Airola et al, 1998). All four TIMPs have been detected in ovary and testis (Robinson et al, 2001).

The skeleton develops either by intramembranous or endochondral ossification (flat bones of the skull or long bones and spine, respectively). Endochondral ossification is a process where avascular tissue (cartilage) is turned into highly vascularized bone tissue. Chondrocytes proliferate and mature to form columns of hypertrophic cartilage which is then resorbed and replaced by bone matrix. Ossification begins with the invasion of calcified hypertrophic cartilage by capillaries. Terminal hypertrophic chondrocytes undergo apoptosis, cartilage matrix is degraded, and osteoblasts form the trabecular bones, which are subsequently remodelled by osteoclasts. Cartilage is normally highly resistant to vascularization, except hypertrophic cartilage during this process (Werb et al, 1999). Thus, angiogenesis requires the invasion of endothelial cells and localized proteolytic degradation of ECM. Transgenic mice have proven useful when determining the contribution of individual MMPs to biological processes, including development (Shapiro et al, 1998). Two MMPs have been implicated to be particularly important in bone development: MMP-9 and MT1-MMP. In MMP-9-deficient mice, vascular invasion was impeded, which was coupled with a delay in apoptosis of hypertrophic chondrocytes and ossification of hypertrophic cartilage. As a result, long bones were shorter than in control animals (Vu et al, 1998). It has been suggested that MMP-9 would regulate the release of an angiogenic factor from hypertrophic cartilage. Thus, the lack of MMP-9 would lead to decrease in vascular invasion. MMP-9 may have also other functions, such as regulation of apoptosis or resorption of cartilage (Vu and Werb, 2000). MT1-MMP knock-out mice also have severe defects in skeletal development and angiogenesis which are different from that in MMP-9-null mice (Holmbeck et al, 1999; Zhou et al, 2000). The mice have craniofacial dysmorphism, arthritis, osteopenia, dwarfism, and fibrosis of soft tissues due to inadequate collagenolytic activity that is essential for modeling of skeletal and extraskeletal connective tissues. The shortening of

bones is a consequence of impaired endochondral ossification. The defect in angiogenesis is not probably restricted to cartilage alone.

Developmental defects resulting from the inactivation of other MMP genes have not been apparent. Many processes that are affected *in vitro* by MMPs do not seem to be affected by the lack of relevant MMP *in vivo*. For example, kidney development requires MMP-9 and MT1-MMP *in vitro*, but in MMP-9 or MT1-MMP knock-out mice kidney development is not affected (Vu and Werb, 2000). There may be compensatory mechanisms *in vivo* allowing only defects where individual MMP plays a crucial function to be seen. MMPs have overlapping substrate specificities and are simultaneously expressed in various developmental stages. Furthermore, the mechanisms of relevant MMPs are difficult to understand by looking at only the end result of its deficiency, since these events usually include a very complex interplay between many cellular processes (Shapiro et al, 1998; Vu and Werb, 2000).

2.3. PROTEOLYSIS IN CANCER

2.3.1. Tumor invasion and metastasis

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. When a tumor cell acquires this ability it is called “malignant“. Invasive growth is associated with excessive degradation of various matrix structures. Interactions between the tumor and its microenvironment result in the production of proteolytic enzymes crucial for this process. The ability to form tumors at distant sites (metastasis, secondary lesion) is characteristic of highly malignant tumors with poor clinical outcome. The loss of an intact BM as the first barrier is, therefore, the initial step of tumor invasion (Figure 5). In order for invasion and metastasis to occur, a cell or group of cells must be able to detach from the primary tumor, invade through BM and interstitial ECM. The tumor cell enters the circulation either through lymphatic vessels or through blood vessels in a process called intravasation. It must survive the circulation to arrest at the distant capillary bed. In order for the tumor to establish itself in a distant place, it must repeat the steps in reverse. This process of extravasation requires the tumor cell to leave the circulation, adhere to subendothelial BM, attain access into the organ parenchyma and invade through matrix again. Furthermore, it has to respond to growth factors, proliferate as a secondary colony, induce angiogenesis and evade host defences (see Ellis and Fidler, 1996; McCawley and Matrisian, 2000).

2.3.2. Tumor angiogenesis

In the adult organism, angiogenesis, i.e. forming of new blood vessels from existing ones, is normally limited to events of tissue repair/remodeling such as that occurring during menstruation, mammary gland involution, wound healing and inflammation. Angiogenesis is a complicated process that requires: 1) degradation of the BM and ECM surrounding blood vessels; 2) chemotaxis of endothelial cells toward an angiogenic stimulus; 3) proliferation of endothelial cells; and 4) remodeling of the BM and synthesis of new matrix components as the new blood vessel forms (Ellis and Fidler, 1996). It is now well established that in order to maintain continuous growth, invasion and formation of metastases, malignant tumors depend critically on neovascularization, which enables nutrient and oxygen supply (Folkman 1995). The ability of the tumor to promote angiogenesis is thought to be one of the early events in the transition of a tumor from pre-neoplastic stage to neoplastic phenotype. Pathological studies of various human cancers have also shown a correlation between an increased number of tumor blood vessels and poor prognosis (see Weidner 1995). New blood vessels penetrating the tumor are frequent sites of tumor cell entry to the circulation. Metastatic cells themselves must again undergo angiogenesis to grow a clinically

detectable size. The switch into angiogenic phenotype depends on a net balance between positive and negative regulators of blood vessel growth. Therefore, the increased production of angiogenic stimulators, such as angiogenin, VEGF or bFGF, is not sufficient for angiogenic phenotype, but inhibitors (e.g. thrombospondin-1, angiostatin, endostatin) must be decreased as well. It is obvious that the understanding of the cellular events and molecular regulation of angiogenesis has tremendous clinical implications (see Folkman 1995; Hanahan and Folkman, 1996).

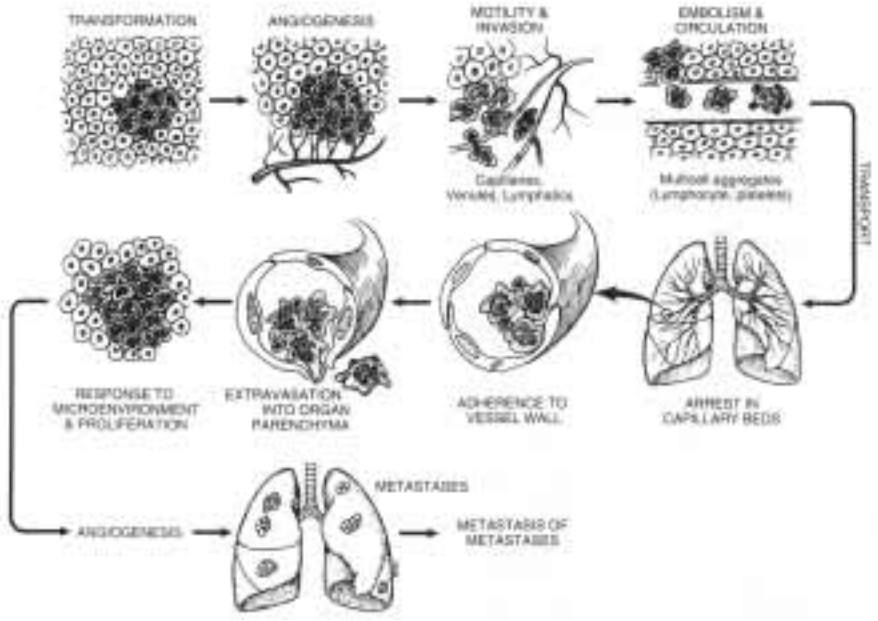


Figure 5. Multiple stages of tumor progression (modified from Ellis and Fidler, 1996).

2.3.3. Stromal contribution to tumorigenesis

Interactions of tumor cells with stromal components are important for tumorigenesis (see Iozzo 1995). As tumor progresses from a hyperplastic, premalignant stage through angiogenic switch to an invasive metastatic cancer, there is constant communication between tumor cells and the surrounding stromal components, including fibroblasts, endothelial cells, inflammatory cells and structural matrix components (see McCawley and Matrisian, 2001). Alterations in the stroma of malignant tumors involve the synthesis and breakdown of ECM components, as well as changes in cell behavior, adhesion and migration. The reactions in tumor stroma are often viewed as a non-specific host response in order to slow down tumor progression. Stromal fibroblasts also participate in the degradation of ECM by secreting matrix degrading proteases as well as their activators (Wernert 1997).

Inflammation is a protective reaction elicited by the host in response to infection, injury or tissue damage. Under normal physiological conditions, this response serves to eliminate pathogens and initiate repair. Many malignancies may also arise from the areas of infection and inflammation as part of the normal host response, and as such “tumors are often viewed as wounds that do not heal“ (Wernert 1997). The inflammatory component of a developing neoplasm includes a diverse leukocyte population, e.g., macrophages, neutrophils, eosinophils, and mast cells, all of which are variably loaded with different cytokines and cytotoxic mediators including serine-, cysteine-, and metalloproteinases, as well as soluble mediators of cell killing, such as TNF-α, ILs and IFNs (see Coussens et al, 2001). After activation, mast cells can release stored and newly synthesized inflammatory mediators, including factors known to enhance angiogenic phenotype such as heparin,

metallo- and serine proteinases, and various growth factors (bFGF, VEGF) (Coussens et al, 1999). Macrophages also have the potential to mediate tumor cytotoxicity and to stimulate antitumor lymphocytes. Tumor cells can benefit from the activities of *tumor-associated macrophages (TAMs)*, which can produce factors directly promoting tumor growth (like IL-1 and PDGF). Furthermore, proteases secreted by these macrophages degrade the surrounding tissue and facilitate tumor cell expansion and invasion. TAMs can also promote angiogenesis by secreting potent angiogenic proteases, cytokines, and growth factors, like bFGF, VEGF and TNF-α, or inhibit angiogenesis by producing inhibitors (like angiostatin or GM-CSF). Macrophages are, in fact, expected to influence every stage of angiogenesis (Wahl and Kleinman, 1998; Joseph and Isaacs1998; Ono et al, 1999).

2.3.4. MMPs in tumor progression

The initial observation of the importance of MMPs in cancer biology was that tumor cells with the ability to penetrate the surrounding tissue correlated with increased MMP levels (Liotta et al, 1980). Subsequently, many MMP family members have been isolated from tumor cell lines or found to be overexpressed in various tumor tissues, especially the malignant tumor front. They are also regulated both by oncogenes and growth factors involved in cancer progression (Crawford et al, 1996). The early hypothesis was that tumors expressed MMPs to make holes in the ECM in order to allow the movement of tumor cells across the BM, 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 (McCawley and Matrisian, 2000).

MMPs 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. The importance of MMPs in angiogenesis is substantiated by the fact that synthetic and endogenous MMP inhibitors also reduce angiogenic responses (see Stetler-Stevenson 1999). For example, a peptide called PEX prevents MMP-2 binding to the integrin $\alpha_v\beta_3$, which reduces angiogenesis and tumor growth in a model system (Brooks et al, 1998). Furthermore, MMP-deficient mice (MMP-2, -9 and -14) all demonstrate defective angiogenesis (Itoh et al, 1998; Vu et al, 1998; Zhou et al, 2000). 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 (Coussens et al, 1999). MMP-9 promotes tumor angiogenesis in other mouse tumor models as well (Bergers et al, 2000b; Yu and Stamenkovich, 2000). The crucial role of MMP-2 and MT1-MMP in angiogenesis has perhaps been studied the most (see Foda and Zucker, 2001)**.**

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 show reduced intestinal tumorigenesis (Wilson et al, 1997), and besides defective angiogenesis, MMP-2 knock-out mice exhibit reduced tumor progression (Itoh et al, 1998). In addition, chemical mutagenesis results in decreased tumorigenesis in MMP-11-deficient mice (Masson et al, 1998). Mice lacking MMP-9 show reduced keratinocyte hyperproliferation at all neoplastic stages and a decreased incidence of invasive tumors during skin carcinogenesis (Coussens et al, 2000). Furthermore, overexpression of MMP-3 and MMP-7 in transgenic mice led to enhanced tumorigenesis in breast cancer model (Rudolph-Owen et al, 1998; Sternlicht et al, 1999). Similarly, overexpression of MMP-1 enhanced tumor formation in a chemically induced model on skin carcinogenesis (D'Armiento et al, 1995).

Besides the classic role of MMPs in the degradation of ECM in tumorigenesis, new roles are emerging which make the contribution of MMPs much more complex than initially thought. As ECM is not static, cells receive clues from their environment through attachments to the matrix and thus respond to changes in their environment. MMP activity, either directly or indirectly, is vital for this process. 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 which can affect cell proliferation, migration and angiogenesis (Figure 6). MMPs are thereby involved in creating an environment suitable for tumor progression (see Bergers and Coussens, 2000a; McCawley and Matrisian, 2001; Stetler-Stevenson et al, 2001). Furthermore, MMP-mediated degradation of ECM components may generate also inhibitors of angiogenesis and cell growth. MMPs are now thought to be the most important proteases responsible for generating the potent angiogenesis inhibitor angiostatin, which is an NH_2-terminal fragment of plasminogen and effectively inhibits endothelial cell proliferation. MMPs capable of cleaving angiostatin are MMP-3, -7, -9 and -12, of which MMP-12 is the most potent (Patterson and Sang, 1997; Cornelius et al, 1998).

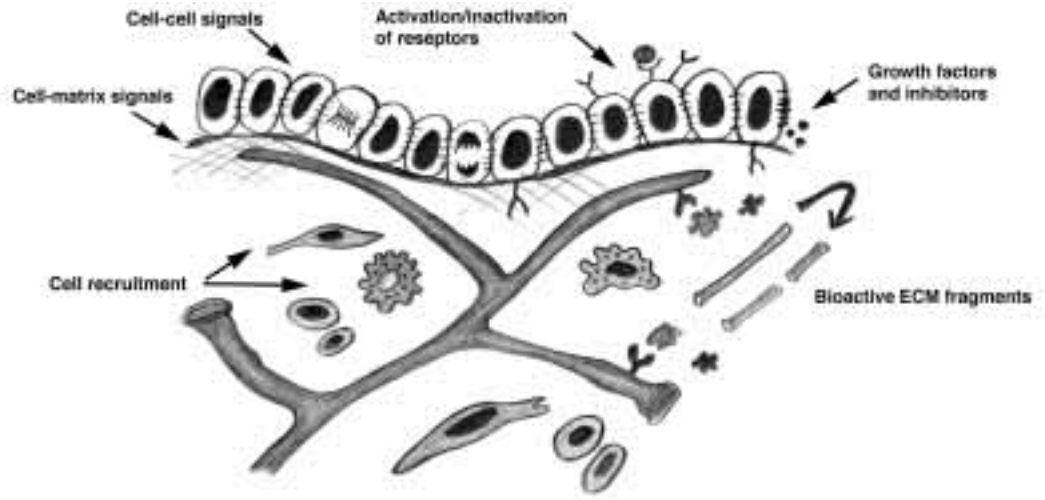


Figure 6. Expanded view of MMP-mediated proteolysis (modified from Bergers and Coussens, 2000). MMPs can cleave: reseptors (or proteins) involved in cell-matrix or cell-cell adhesion, latent cell surface ligands, matrix proteins generating new bioactive fragments and sequestered growth factors and inhibitors. Through these actions MMPs regulate, for example, angiogenesis, cell proliferation, migration and apoptosis.

2.3.4.1. MMP expression in tumors

MMP expression patterns show that, in addition to tumor cells, also surrounding stromal cells and inflammatory cells involved in cancer progression can overexpress various MMPs and complement the proteolytic capacities of one another. The stromal expression was originally reported by Basset *et al*. (1990), who noticed that stromal 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, -9, -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 Nelson et al, 2000). There are exceptions, such as MMP-7, which is mainly expressed in tumor cells. Also MMP-13 is usually expressed in tumor cells, and often at the invading front (Johansson et al, 1997c; Johansson et al, 1999). In breast cancer, most MMPs, namely MMP-1, MMP-2, MMP-3, MMP-11 and MT1-MMP were expressed by fibroblasts in the tumor stroma, while only MMP-7 and MMP-13 were expressed by tumor cells. MMP-9 localized to endothelial cells and MMP-12 to macrophages

(Heppner et al, 1996). Interestingly, in a mouse model of skin carcinogenesis, MMP-9 supplied by reactive stromal cells (macrophages, neutrophils and mast cells) contributes to skin carcinogenesis, and inflammatory cells thus act as “coconspirators” in cancer and may actually represent a useful target for anti-cancer therapy (Coussens et al, 2000). 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 MMPs-1, -2, and -3 have, however, been shown to be induced by EMMPRINs (Kataoka et al, 1993; Biswas et al, 1995).

MMP-9 mRNA has been demonstrated in inflammatory cells, but the protein can still be detected in tumor cells (Monsky et al, 1993). The same phenomenon has been observed with MMP-2 mRNA, as it is mainly expressed by stromal cells at the invading tumor front, but the protein localizes to tumor cells, e.g., in breast, colon and skin cancers, as well as hepatocellular and pancreatic adenocarcinomas (Pyke et al, 1992; Pyke et al, 1993; Heppner et al, 1996; Määttä et al, 2000). This discrepancy may be due to the fact that the half-life of MMP mRNA and the efficiency of translation differ between stromal and neoplastic cells. Alternatively, tumor cells can bind stromal-secreted MMPs (Brooks et al, 1998; Yu and Stamenkovich, 2000). Similar discrepancy in MT1-MMP mRNA and protein localization has been shown in head and neck carcinoma, for example. Thus, it is possible that MT1-MMP protein produced by stromal cells migrates onto tumor cell surfaces, as MMP-2 does (Yoshizaki et al, 1997). ProMMP-2 secreted by stromal fibroblasts could then be activated after binding to MT1-MMP at the tumor cell surface as originally reported by Sato *et al*. (1994). Results on MT1-MMP are somewhat conflicting, since expression of MT1-MMP has been reported in stromal cells and in neoplastic epithelium, or both, e.g. in colon, lung, breast, head and neck, gastric, pancreatic, and hepatocellular carcinoma (Nomura et al, 1995; Okada et al, 1995; Polette et al, 1996; Mori et al, 1997; Harada et al, 1998; Imamura et al, 1998; Shimada et al, 2000).

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. 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, where many MMPs are expressed at high levels (see Stetler-Stevenson et al, 1996). 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, which reflects the heterogeneity of tumors and differential expression of MMPs during tumor progression. At least MMP-1, MMP-2, MMP-7, MMP-9, MMP-11 and MT1-MMP are frequently overexpressed in many human tumors (see Nelson et al, 2000; Foda and Zucker, 2001; Stetler-Stevenson and Yu, 2001).

2.3.4.2. MMPs in skin cancer and other epithelial tumors

Solar elastosis and solar keratosis: Prolonged exposure of skin to sun light leads to dermal photoageing characterized by alterations in the composition of the dermal matrix, such as an increase in the amounts of elastin, fibrillin and versican (Bernstein et al, 1994; Bernstein et al, 1995b) and reduced amounts of type I collagen and decorin (Bernstein et al, 1995a; Bernstein et al, 1996b). The term *elastosis* indicates an increased amount of connective tissue fiber in the upper reticular dermis. The elastoses are a reaction to prolonged exposure to the sun and/or heat over many years. Those affected are generally elderly people who have little protective pigment or people who have been overexposed to the sun during their lives. In patients with clinically evident solar elastosis, staining reveals degeneration of collagen in the upper dermis. The elastotic material

usually consists of aggregates of thick bands, and in areas of severe solar degeneration it may extend into lower portions of the dermis (see Lever and Schaumburg-Lever 1990b).

Solar keratoses (actinic keratoses) represent *in situ* carcinoma with varying degrees of epidermal atypia or dysplasias and are very common in elderly people. Excessive exposure to sunlight over many years and inadequate protection against it are essential predisposing factors. Histologically, actinic keratoses are usually well-separated islands of abnormal keratinocytes with overlying parakeratosis. The upper dermis usually shows chronic inflammatory infiltrate. Unlike BCCs, the majority of SCCs in light-exposed areas may arise from pre-existing solar keratoses but they rarely metastasize (Marks et al, 1988; Lever and Schaumburg-Lever, 1990c).

Several members of the MMP family have been implicated in photoageing. UVA induced the expression of MMP-1 by dermal fibroblasts *in vivo* and the expression of MMP-1, -2, and -3 in culture (Scharffetter et al, 1991). UVB induced the expression of MMP-1, -3, and -9 in normal human epidermis *in vivo* (Koivukangas et al, 1994; Fisher et al, 1996). These MMPs may take part in the degradation of type I collagen seen in chronically photoaged skin (Bernstein et al, 1996a; Fisher and Voorhees, 1998). Of MMPs-1, -2, -3 and -13 only MMP-1 was expressed in actinic keratoses by *in situ* hybridization (Airola et al, 1997; Tsukifuji et al, 1999).

Basal cell carcinoma (BCC) is the most common malignancy in humans (Caucasian populations) especially in the elderly population (see Ramsey 2001). It is usually slow growing and rarely metastasizes but can cause significant local destruction and disfigurement if treated inadequately. With proper therapy, the prognosis is excellent and death extremely rare. BCC is considered to arise from multipotential cells within the basal layer of the epidermis or follicular structures. These cells are formed continuously during life and are capable of forming hair, sebaceous glands and apocrine glands. BCC typically occurs in areas of chronic sun exposure (UV radiation), which is thought to be the most important and common cause of BCC (Kricker et al, 1994). Also ionizing radiation, chemical carcinogens (eg. chronic exposure to 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. Histologically, BCCs showing no differentiation are called *solid* BCC, with the subgroups of *infiltrative* and *circumscribed*. Those that differentiate toward hair structures are called *keratotic*, toward tubular glands, *adenoid*, and toward sebaceous glands, *sebaceous* BCCs. Clinically, five types occur: 1) *Morpheaform* (sclerosing, fibrosing) BCC arises as thin strands of tumor cells that are embedded in a dense fibrous stroma. The strands of infiltrating BCC tend to be somewhat thicker, and they have a spiky irregular appearance. 2) *Nodular* BCC is the most common variety of BCCs. Tumor cells of nodular BCC typically have large, hyperchromatic, oval nuclei and little cytoplasm. The cells appear rather uniform and tumor aggregates may be of varying sizes. Nodular BCCs usually have limited potential for growth, but they can occasionally be infiltrative and aggressive, and in the face, even destroy the eyes and nose. 3) *Pigmented* BCC differs from the previous subtype only by the brown pigmentation. 4) *Superficial* BCC presents as scaly patches or papules, which mainly occur on the trunk and has little tendency to become invasive. 5) *Fibroepithelioma* usually consists of only one raised, moderately firm nodule, mainly in the back. It is important to recognize the various types, since more aggressive therapy is often necessary for aggressive BCC variants, such as infiltrating and morpheaform BCCs (see Lever and Schaumburg-Lever, 1990c; Ramsey 2001).

MMP-1, unlike other collagenases (Varani et al, 2000), MMP-3 (Majmudar et al, 1994), MMP-11 (Thewes et al, 1999), and both gelatinases, MMP-2 and MMP-9, (Pyke et al, 1992) are mainly expressed by stromal cells surrounding malignant epithelial cells in BCCs. In the more aggressive

morpheaform (infiltrative) BCCs and recurrent BCCs, MMP-7 localized at the tumor-stromal interface (Karelina et al, 1994), whereas it was not expressed in other BCC subtypes. MMP-13 mRNA was seen in focal areas of keratinized cells and associated with terminal differentiation of epithelial cells as assessed with immunostaining for involucrin and apoptosis (Airola et al, 1997). Also TIMPs have been found in BCCs. According to Varani *et al*. (2000), MMP inhibitor activity mainly reflected the presence of TIMP-1. Significantly higher levels of TIMP-2 have been detected in BCC when compared to SCCs (Wagner et al, 1996). TIMP-3 was present in tumor cells at the tumor edge of infiltrative BCCs (Airola et al, 1998).

Squamous cell carcinoma (SCC) can occur in many tissues and organs, including the skin, mouth, airways, esophagus, uterine cervix and vulva. Cutaneous squamous cell carcinoma (SCC) is the second most common form of skin cancer and frequently arises on the sun-exposed skin of the middle aged and elderly. Evidently, the primary cause of SCC is cumulative lifetime sun exposure (especially UVB). The mechanism of UV-induced photocarcinogenesis appears to involve the inactivation of the p53 tumor suppressor gene (Ziegler et al, 1994). Most SCCs arise from sun-induced precancerous lesions known as actinic keratoses. On a continuum with actinic keratosis is SCC in situ, a subset of which is referred to as Bowen's disease. Furthermore, ionizing radiation, immunosupressive agents, chronic inflammation, and human papillomavirus (HPV) infection may lead to the development of SCC. SCC is capable of local extension, regional lymph node and distant metastasis. Thus, high-risk SCC requires careful evaluation and treatment (Marks et al, 1988; Bernstein et al, 1996b; Goldman 2001).

SCC is a malignant tumor of keratinocytes. SCC *in situ* is characterized by severe atypia of the basal layer, intraepidermal nests of atypical cells or full-thickness intraepidermal proliferation of atypical keratinocytes. The atypical keratinocytes often extend deep down hair follicles. With invasive SCC, malignant keratinocytes extend through the BM and into the dermis. The neoplastic cells demonstrate varying degrees of squamous differentiation and atypia. SCC cells are characterized by variations in the cell size and shape, hyperplasia and hyperchromasia of the nuclei, absence of intracellular bridges and keratinization on cells. Most dermatopathologists report SCCs as well, moderately or poorly differentiated, which behave more aggressively clinically. Increased depth of invasion of SCC is strongly associated with local recurrence, metastasis and death (see Johnson et al, 1992; Goldman 2001).

Various SCCs express several MMP members (see Johansson and Kähäri, 2000). In cutaneous SCC, MMP-7 protein was expressed in tumor cells at the stromal interface surrounding tumor nests (Karelina et al, 1994). Together with MMP-9 (Karelina et al, 1993), they correlate with aggressive behavior of skin tumors. In SCCs of the skin and oral cavity, MMP-9 localizes to malignant keratinocytes at the tumor/stroma interface, which is due to interactions between tumor cells and adjacent fibroblasts (Lengyel et al, 1995; Borchers et al, 1997). Also the level of MMP-2 protein, together with MMP-9, is induced in SCCs of the skin when compared to BCCs (Dumas et al, 1999). Furthermore, MMP-13 mRNA expression was detected at the epithelial tumor front and less frequently in the surrounding stromal cells, while its potential activator, MMP-3, was expressed by stromal cells surrounding tumors (Airola et al, 1997). Also MMP-1 mRNA has been detected in tumor and/or stromal cells in SCC of the skin (Tsukifuji et al, 1999), while MMP-11 was expressed exclusively in stromal cells, as is typical of it (Asch et al, 1999).

In SCCs of the head and neck (HNSCC), stromelysin (MMP-3, and -10) transcripts were localized in tumor and stromal cells along disrupted BM (Polette et al, 1991). MMP-3 immunoreactivity correlated with tumor size, tumor invasion, and the high incidence of lymph node metastasis in SCC of the oral cavity (Kusukawa et al, 1995). MMP-11 mRNA was detected in stromal cells next to invasive cancer cells of the HNSCC and the level of its expression correlated with increased local

invasiveness (Muller et al, 1993). MMP-13 mRNA was expressed by tumor cells at the invading front, but occasionally also by stromal fibroblasts. Its expression correlated with the invasion and metastasis capacity of the HNSCCs (Johansson et al, 1997c). MT1-MMP protein was shown to localize on the cell surface of tumor cells, especially at the invasive areas (Yoshizaki et al, 1997). Its mRNA has, however, been detected in stromal cells of the same cancer (Okada et al, 1995). Interestingly, a similar discrepancy has been reported for MMP-2 expression in HNSCC and in skin cancer (Pyke et al, 1992) in accordance with the role of MT1-MMP in the activation of MMP-2 (Sato et al, 1994). Furthermore, the level of active MMP-2 may serve as a predictive marker of metastasis in oral SCCs (Kawamata et al, 1998). MMP-1 mRNA has been detected both in stromal fibroblasts surrounding invasive oral SCC, and in tumor cells (Polette et al, 1991; Gray et al, 1992; Sutinen et al, 1998). MMP-9 protein correlated with angiogenic markers and poor survival in HNSCC patients (Riedel et al, 2000).

In esophageal SCCs, high levels of MMP-1, -2, and -3 expression have been shown (Shima et al, 1992). MMP-1 is also associated with poor prognosis (Murray et al, 1998). MMP-13 protein was detected in tumor cells colocalizing with MT1-MMP, and their expression was related to cancer aggressiveness (Etoh et al, 2000). Furthermore, elevated protein expression of MMP-7, MMP-9 and MT1-MMP, as well as gelatinase activity, was detected in carcinoma cells correlating with the depth of tumor invasion (Ohashi et al, 2000). MMP-7 may also serve as a prognostic factor for patients with esophageal SCC (Yamashita et al, 2000).

Cancer of the vulva may arise from the skin, subcutaneous tissues, glandular elements of the vulva, or the mucosa of the lower third of the vagina. Vulvar cancer is relatively rare, being responsible for approximately 5% of gynecologic cancers. Approximately 85–90% of these tumors are epidermoid cancers (SCC). It is primarily a disease of postmenopausal women. Intraepithelial cancer of the vulva in women in their 20s and 30s has increased remarkably in recent years coincidentally with an increase in the incidence of diagnosis of dysplasia and carcinoma in situ of the cervix. SCCs of the vulva usually show extensive local invasion to other pelvic organs and metastases in iliac lymph nodes. Epidermoid cancers may be graded histologically from I to III (Kurman et al, 1994). Grade I tumors are well differentiated, often forming keratin pearls; grade II tumors are moderately well differentiated; and grade III tumors are composed of poorly differentiated cells. The extent of underlying inflammatory cell infiltration into the stroma surrounding the invasive tumor is variable. Well-differentiated tumors usually have a good prognosis. However, the gross size of the tumor is the most significant factor in prognosis. The primary determinant of nodal metastases is tumor size. A variant of epidermoid cancer, verrucous carcinoma, is a locally invasive tumor that seldom metastasizes to regional lymph nodes (Smith et al, 1994).

To date, there is only one report concerning MMPs in vulvar SCCs. MMP-13 was shown to be expressed especially in vulvar carcinomas showing metastasis to lymph nodes, and its expression was associated with the expression of MT1-MMP in tumor cells and MMP-2 in stromal cells. Also MMP-1 expression was detected in stromal as well as some tumor cells. In some samples, MMP-9 protein colocalized with MMP-13 (Johansson et al, 1999).

2.3.4.3. Therapeutic MMP inhibitors

Currently, a number of clinical studies focus on testing MMP inhibitors as potential antineoplastic agents. The low basal expression of MMPs in normal adult tissues have made them a prominent therapeutic target in cancer and other diseases, as it has been assumed that their effects on normal cell functions would be minimal. Thus, the most efficient approach for cancer therapy would seem to be the inhibition of all MMP activity by either TIMPs or the administration of synthetic MMP inhibitors. Indeed, the first generation broad-spectrum MMP inhibitors did just that, but unspecific

action caused some side effects of arthralgia, probably due to their broad substrate specificity and because MMP activity is required for maintaining healthy joints in adult tissue. More recently, inhibitors that demonstrate some selectivity for specific MMP family members have been developed in an attempt to limit unpleasant side effects (see McCawley and Matrisian, 2000; Stetler-Stevenson and Yu, 2001).

Theoretically, the ability of TIMPs to specifically inhibit the activity of several MMPs could result in a beneficial therapeutic effect. For instance, adenovirus-mediated delivery of TIMP-3 gene inhibits invasion and induces apoptosis in various normal and malignant cells (Bian et al, 1996; Ahonen et al, 1998; Baker et al, 1998). However, the lack of effective methods of systemic gene delivery has limited the clinical utility of this approach. Synthetic inhibitors designed to inhibit MMP activity work in a similar way to TIMPs by interfering with the catalytic machinery in the active site of the enzyme. The *hydroxyamate inhibitor batimastat* and its orally available analogue *marimastat* were the first inhibitors studied in detail. These compounds were designed to fit tightly within the active site of the MMP in a stereospecific manner (Brown et al, 1999; Hidalgo and Eckhardt 2001). The *nonpeptidic MMP inhibitors*, such as BAY 12-9566 and AG3340, were synthesized in an attempt to improve the oral bioavailability, pharmaceutical properties and specificity of the peptidic inhibitors. Specific peptide inhibitors may represent a useful alternative for selective targeting of therapeutic MMP inhibition in cancer. Gelatinase-specific inhibitors isolated from phage display peptide libraries suppressed migration of both tumor cells and endothelial cells *in vitro*, targeted to tumors and prevented tumor invasion and growth in animal models (Koivunen et al, 1999).

Retinoids may be effective in cancer therapy by blocking MMP synthesis and decreasing tumor invasiveness (Schoenermark et al, 1999). *Tetracycline derivates* interfere with both the activity and the production of MMPs and inhibit tumor growth and metastases in preclinical models. Also *bisphosphonates* have been used to inhibit MMP activity (Hidalgo and Eckhardt, 2001). Besides strategies targeted to inhibit MMP activity, the degradation of MMP mRNA by *antisense RNA* or ribozyme techniques may also provide an efficient alternative to prevent tumor cell invasion (Hasegawa et al, 1998; Stetler-Stevenson and Yu, 2001). The advantage of antisense strategies is their selectivity for a specific MMP subtype, thereby potentially resulting in fewer toxic effects. The inhibition of *MAP kinases* involved in signal transduction can also inhibit MMPs and the invasive potential of cancer cells (see Hidalgo and Eckhardt, 2001). Similarly, MMP promoter activity can be inhibited, for instance, by preventing activation of AP-1 site by oligomers.

Several synthetic inhibitors are in phase I/II/III trials for various forms of cancer. Phase III clinical trials using marimastat, batimastat and BAY 12-9566 alone or in combination with chemotherapy in patients with advanced lung, prostate, pancreas, brain, or gastrointestinal tract cancers showed no clinical efficacy. This may result from the fact that MMPs seem to be important already in the early stages of tumor progression, and inhibition may no longer be useful once metastases have been established. Thus, future trials should concentrate on patients with early stage cancers, and the measurements of MMPs levels from tissue or blood would help identifying patients more likely to respond to MMP therapy (Zucker et al, 2000). Although the development of specific MMP inhibitors may result in more effective compounds, the MMP system is, however, extremely complex, and it is not yet clear which subtypes should be targeted therapeutically to achieve antitumor effects (Stetler-Stevenson 1999). The dual role of MMPs in angiogenesis also emphasises the necessity to identify appropriate targets for MMP inhibition.

3. AIMS OF THE STUDY

The principal aim of this study was to examine the role of MMPs- 12, -10 and -7 in epithelial cancers. It has been thought that the inhibition of MMPs collectively would be the most effective way to prevent cancer growth and metastasis. However, multiple roles for MMPs are becoming evident in cancer biology; for example MMP-7 and -12, may also inhibit cancer growth. Thus, further knowledge is needed on the actual role and contribution of individual MMPs to the multiple stages of tumorigenesis in different cancer types. The expression of certain MMPs might also be used as prognostic markers when planning the aggressiveness of tumor treatment.

The specific aims of the study were as follows:

1) To investigate the expression and role of metalloelastase (MMP-12), stromelysin-2 (MMP-10) and matrilysin (MMP-7) in UV-damaged and premalignant lesions of the skin, in basal cell carcinoma, and squamous cell carcinomas of the skin and vulva.
2) To study the regulation of these MMPs in cultured epithelial cells, including primary keratinocytes, transformed HaCaT cells and various SCC cell lines.
3) To study how angiostatin-generating MMPs (MMPs -7, -9 and -12) affect tumor progression.
4) To evaluate whether there is correlation between the expression patterns of these MMPs and clinical behavior of tumors (e.g. aggressiveness, angiogenesis, metastasis and patient outcome).
5) To investigate MMP-12 expression during development.

4. MATERIALS AND METHODS

4.1. Tissue samples

This study was approved by the Ethics Committee of the Department of Dermatology, University of Helsinki, University Central Hospital.

Fetal tissues and bone samples (I): Formalin fixed, paraffin-embedded specimens were obtained from the Department of Pathology, Helsinki University Hospital (adult bone and bone lesions) and from Oulu University Hospital (fetal tissues). Fetal age was estimated by menstrual age and macroscopic and histological examination. All fetal material used for *in situ* hybridization originated from medical abortions at 7, 9, 10, 11, 12, 16, 17, 20, 21 and 23 weeks of gestation (n = 28). The following adult histological specimens were examined: normal bone and cartilage (n = 4), osteoid osteoma, chondroma, osteochondroma, osteoblastoma (n = 8), osteosarcoma (n = 4), and chondrosarcoma (n = 19). The diagnoses were confirmed by two experienced pathologists.

Skin samples (II): Formalin fixed, paraffin-embedded archival specimens of solar elastosis (n = 18, mean age 74 y, head and arm), solar keratosis (n = 8, mean age 67 y, head and ankle), anetoderma (n = 4, mean age 33 y, back and thigh), and mid-dermal elastolysis (n = 3, mean age 31 y, abdomen, arm) were obtained from the Department of Dermatopathology, University of Helsinki. Samples of normal skin from two groups of adults (n = 7, mean age 23 y, back, buttock and neck; n = 7, mean age 72 y, abdomen, back and face) and children under 5 y of age (n = 3; neck, back and buttock) were also examined.

Eight healthy individuals, aged 23-44, volunteered to photoprovocation and skin biopsies. The photoprovocation was done during winter as previously described (Hasan et al, 1997) with UVASUN (emission spectrum 340-400 nm) and with Philips (Eindhoven, The Netherlands) TL 20W/12 (emission spectrum 280-370 nm) light sources. Briefly, a 5-8 cm area of intact skin was irradiated on three successive days with 100 J UVA or with a 1,5 minimal erythema dose of UVB. Skin biopsies were obtained from six volunteers 3-5 days after the last provocation, fixed in formalin and embedded in paraffin. Two subjects were biopsied 24 h after one single provocation and samples were snap-frozen immediately. The study was approved by The Ethical Committees of Oulu and Tampere University Hospitals.

SKH-1 hairless mice were exposed to the sun spectrum of 90 % UVA and 10 % UVB radiation as previously described (Starcher et al, 1999). Briefly, groups of five mice were exposed for 30 s three times a week with the lamp (Dermalight 2001 mercury halide 400 w high pressure lamp with UVB filter) positioned 8 cm above the mice to provide 0,09 J/cm^2 of total irradiation, which is equivalent to approximately 1 minimal erythema dose, for up to 25 weeks. Non-irradiated age-matched mice served as controls.

Skin cancers (III, V): Formalin fixed, paraffin-embedded archival specimens of SCC and BCC were obtained from the Department of Dermatopathology, University of Helsinki. The diagnoses were confirmed by two experienced dermatopathologists. The following histologic specimens were examined: squamous cell carcinomas (SCC): grades I (n = 5/n = 9, respectively), II (n = 7/n = 9), III (n = 5), basal cell carcinomas (BCC) (n = 19) (histological subtypes: fibrosing (n = 11), keratotic (n = 3), adenoid (n = 5), actinic keratoses (n = 6, IV), melanomas (n = 12, II), and Bowen's disease (n = 5).

Vulvar SCCs (IV): The most representative samples from the formalin fixed, paraffin-embedded vulvar SCC specimens were selected from the files of the Department of Pathology, University of Turku, Finland. Samples were reclassified, sectioned, and processed for the study. The tumors were regraded (grade I, n = 11; grade II, n = 13; grade III, n = 9) according to Kurman *et al* (1994).

4.2. RNA probes

The generation and specificity of MMP-3, MMP-7, MMP-10, MMP-13 and mouse metalloelastase probes have been described (McDonnell et al, 1991; Shapiro et al, 1992; Saarialho-Kere et al, 1994; Johansson et al, 1997a). A 650-bp fragment of MMP-12 cDNA (corresponding to nucleotides 600-1250) was generated by PCR as previously described (Vaalamo et al, 1998), resulting in a product with SP6 (3´end) and T7 (5´end) RNA polymerase recognition elements. A 1240-bp fragment of MT1-MMP cDNA (Lohi et al, 1996) was subcloned to a pGEM vector containing an SP6 RNA polymerase recognition element. When linearized with BgI II, an antisense RNA probe could be transcribed *in vitro* containing 405 bp from 3´UTR of the MT1-MMP cDNA. The specificity of the probe was confirmed by sequencing. As a control for nonspecific hybridization, sections were hybridized with ^{35}S-labeled sense RNA from bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by Northern (Prosser et al, 1989) and by *in situ* hybridization (Saarialho-Kere et al, 1992). In addition, negative control probes for MMP-3, MMP-10, MMP-12, and MMP-14 transcribed in sense orientation were used.

Table 2. Probes for *in situ* hybridization

Probe (Study)	**Length bp/ Part of the seq.**	**Genbank accession no.**
MMP-3 (V)	217 (1584-1801)	XM_006271
MMP-7 (II,IV)	800 (14-813)	NM_002423
MMP-10 (V)	175 (1568-1743)	NM_002425
MMP-12 (I-V)	651 (600-1250)	NM_002426
Mouse MMP-12 (II)	800	NM_008605
MMP-13 (I, III)	511 (1532-2042)	NM_002427
MMP-14 (V)	405 (3´UTR)	NM_004995

4.3. *In situ* hybridization

Hybridizations were performed on 5-µm sections as described in detail (Prosser et al, 1989). All sections were pre-treated with 1 µg/ml (bone samples: 2,5 µg/ml) of proteinase K and washed in 0,1 M triethanolamine containing 0,25 % acetic acid. Sections were hybridized with ^{35}S-labeled probes (4-5 x 10^4 cpm/µl of hybridization buffer) at 50-55°C for at least 18 hours in a humidified chamber. Slides were then washed under stringent conditions, including treatment with RNAse A to remove unhybridised probe. After 20-45 days of autoradiography, the photographic emulsion was developed, and the slides were stained with hematoxylin and eosin. Samples previously positive for MMP-3 (cutaneous wounds), MMP-7 (sweat gland tumors), MMP-10 (wounds), MMP-12 (sarcoidosis), MMP-13 (skin cancer) and MMP-14 (breast cancer) were used as positive controls in each experiment. The slides were analyzed independently by two investigators.

4.4. Immunohistochemistry

Immunostaining was performed on sections parallel to those used for *in situ* hybridization by the avidin-biotin-peroxidase complex technique using Vectastain Elite ABC Kit (Vector Laboratories, Inc., Burlingame, CA) or Zymed Kit (Zymed Laboratories Inc., San Francisco, CA). Samples were deparaffinized, dehydrated and endogenous peroxidase was blocked with 0,3-0,6 % hydrogen peroxide. Treatment with normal serum was used to block non-specific staining. Primary antibodies (Table 3) were incubated for one hour at 37°C or overnight at 4°C in humidified atmosphere. Diaminobenzidine (DAB) or aminoethylcarbazole (AEC) were used as chromogenic substrates and Harris hematoxylin as counterstain, as described in detail (Saarialho-Kere et al, 1993). Sections were pretreated with trypsin (10 mg/ml), pepsin or microwaving, if necessary. Controls were performed with normal mouse or rabbit immunoglobulin or with pre-immune serum. Staining for revealing elastic fibers was performed with Weigert´s elastin stain (Weigert´s Resorcin-Fuchsin). Morphologic damage to collagen was assessed by van Gieson´s staining.

Table 3. List of antibodies used in immunohistochemistry

Antibody (Study)	Source	Dilution/pre-treatment
MMP-12 (I)	4D2, R&D Systems, Minneapolis, USA	1:20
MMP-12 (II, III)	Belaaouaj et al, 1995	1:300/trypsin
Cytokeratin (III-V)	A0575; Dako A/S, Glostrup, Denmark	1:200/trypsin
CD-68 (I, III-V)	KP-1, M814, Dako, Carpinteria, CA	1:300/trypsin
γ2-chain of laminin-5 (V)	Dr. Karl Tryggvason, Karolinska Institutet, Sweden	1:500/trypsin
TGF-β1 (I, V)	MAB1032, Chemicon International, Temecula, CA	1:500
MMP-2 (V)	IM33L, Calbiohem, Cambridge, MA	1:200/trypsin
Von Willebrandt factor/ Factor VIII-related antigen (IV)	A0082, Dako, Carpinteria, CA	1:500/pepsin
MMP-9 (IV)	GE213, Diabor, Oulu, Finland	1:500/trypsin
MMP-7 (II)	Busiek et al, 1992; Saarialho-Kere et al, 1995	1:800 or 1:1000
MMP-7 (II)	IM40L, Calbiochem, Cambridge, MA	1:50
tenascin (II)	No. 1927, Chemicon, Temecula, CA	1:1000/trypsin
versican (II)	no. 270428, Seigaku, Tokyo, Japan	1:500
Basic FGF (I)	Oncogene, Calbiochem, Boston, MA	1:1000/microwave

4.5. Quantitation of vessel density and macrophages

Areas of highest vascularization, so called “hot spots“, were screened at a low magnification (x40-x100). These areas typically occurred at tumor margins and, in some cases, inside the tumor but areas of tumor margins were focused on, if possible. *Vascular density* was defined as the mean vessel count of four areas considered to have the highest densities. Individual microvessel counts were made on a x160 field. Any clearly stained endothelial cell or cell cluster with or without lumen that was clearly separated from adjacent microvessels, other cells or connective tissue elements was counted as an individual vessel. The amount of *CD68 staining* was measured with image analysis software (Image-Pro® Plus, version 4.0 for Windows, Media Cybernetics, L.P.). Four areas containing the highest amounts of CD-68-positive macrophages were screened as above and selected per section. Positive staining was calculated on x200 fields as %-area/whole area and mean values were recorded. *Statistical analysis* (Kruskall-Wallis test with Dunn's post test and Spearman´s correlation) was performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

4.6. Cell cultures

Primary keratinocytes were established from adult abdominal or breast skin obtained at laparotomies or mammoplasties for non-malignant disease. Subcutaneous fat and deep dermis were removed, and the remaining tissue was incubated overnight at 0.25% trypsin in solution A (Gibco BRL, Life Technologies, Paisley, Scotland). After incubation, keratinocytes were scraped off from the epidermis and suspended in Keratinocyte Growth Medium (KGM, Gibco BRL, Life Technologies), containing 2% decalcified fetal calf serum (FCS) and supplemented with 5 ng/ml epidermal growth factor (EGF) and 50 μg/ml bovine pituitary extract (BPE). Keratinocytes were maintained in KGM supplemented with EGF and BPE.

A431-cells (epidermoid carcinoma cell line, ATCC) and *HaCaT cells* (Boukamp et al, 1988), transformed human epidermal keratinocytes, were cultivated in Dulbecco´s modified Eagle´s medium (DMEM, Gibco BRL) containing 10% FCS, 1% penicillin-streptomycin and 1% sodium

puryvate. Immortalized *human breast epithelial cells* (*MCF-10f,* ATCC) were cultivated in a mixture of DMEM and Ham´s F12 medium (1:1) (Sigma-Aldrich, Saint Louis, MO), containing 1% penicillin-streptomycin and 1% sodium puryvate, as well as insulin (10 µg/ml, Gibco), hydrocortisone (0,5 µg/ml, Sigma), cholera toxin (100 ng/ml, Gibco), EGF (20 ng/ml, Sigma) and reduced Ca^{2+}. Medium was supplemented with 5% horse serum, which had been treated with Chelex 100 (BioRad Laboratories, Richmond, CA) to remove divalent cations.

Forty-three *SCC cell lines* from primary and recurrent tumor and metastases of SCCs of the head and neck (skin, n = 7; larynx, n = 12; oral cavity, n = 22) were established at the time of operation in the Turku University Central Hospital (Johansson et al, 1997c). Human vulvar cell line *UT-SCV-3* was derived from primary grade II tumor (T2N2M0) (Johansson et al, 1999). Cells were cultivated in DMEM containing 10% FCS, 1% penicillin-streptomycin, 6 mmol/L glutamine and nonessential amino acids.

The well-differentiated human chondrosarcoma cells *HCS-2/8* (Takigawa et al, 1989) and more mesenchymal *HTB-94* (SW1353, ATCC) chondrosarcoma cells were cultivated in DMEM containing 10% FCS, 1% penicillin-streptomycin and 1% sodiumbicarbonate.

4.7. Cytokines, growth factors and matrices

To study the regulation of MMP-12 expression, equal amounts of cells were plated on 24-well tissue culture plates. The following day, cells were repeatedly washed with PBS and incubated for 12-24 hours in either serum-free medium without supplements or in medium containing 2 % serum. Analogously, all treatments were done either without serum (in 24 h and 48 h incubations) or with 2 % serum in 48 h incubations. After this, the cells were treated with different cytokines and growth factors (Table 4). Cells without any treatment were used as a control. The cells were also cultivated on different matrices, including collagen I, collagen IV, laminin, fibronectin and Matrigel (Becton-Dickinson, Bedford, MA). Matrigel was diluted 1:10 using serum-free medium according to the manufacturer's instructions. After 24 or 48 hours total RNA was extracted from the cells. All treatments were done in triplicates and positive results were confirmed at least in three different experiments.

Table 4. Cytokines and growth factors used in cell culture

Cytokine/growth factor (Study)	Concentration	Source
Basic FGF (I, IV)	10 ng/ml	Oncogene, Calbiochem, Boston, MA/ PeproTech EC Ltd., Rocky Hill, NJ
EGF (IV)	10-20 ng/ml	Sigma, St. Louis, MO
GM-CSF (IV)	10 ng/ml	R&D systems, Minneapolis
HGF (IV)	10-20 ng/ml	Sigma
IFN-γ (III-V)	20 ng/ml or 100 U/ml	Sigma/Promega, Madison, WI
IL-1β (IV)	10 ng/ml	Roche Molecular Biochemicals, Mannheim, Germany
KGF (IV, V)	10 ng/ml	Sigma/PeproTech EC Ltd.
PDGF (IV)	20 ng/ml	Sigma
TGF-α (V)	10 ng/ml	PeproTech EC Ltd.
TGF-β1 (I, III-V)	1-5 ng/ml	Sigma
TNF-α (I, III-V)	20 ng/ml	Sigma

4.8. Quantitative RT-PCR

PCR primers (Sigma Genosys, Cambridge, UK) and *probes* (PE Biosystems, Warrington, UK) for MMP-12 and MMP-7 were designed using the computer program Primer Express 1.0. (PE

Biosystems) (Table 5). As controls, primers and probes for MMP-1 and MMP-10 were also designed. Fluorogenic probes contained a reporter dye (FAM) covalently attached at the 5' end and a quencher dye (TAMRA) covalently attached at the 3' end. To quantitate the starting material, endogenous controls were amplified in the PCR reactions. Controls included human GAPDH and ribosomal RNA control (18S rRNA) (Pre-developed TaqMan® Control Reagents, PE Biosystems), both labeled with VIC™ reporter dye.

Table 5. Primers and probes for Taqman quantitative RT-PCR

MMP	Forward primer (nts)	Probe	Reverse primer (nts)
MMP-1	5´-CCACAACTGCCAAAT GGGCTTGA-3´(1032-1054)	TCCCTTTGAAAAACCGGAC TTCATCTCTGT (1107-1078)	5´-GTCCTTGGGGTATCC GTGTAG- -3´ (1157-1137)
MMP-7	5´-GCTGGACGGATGGTA GCA-3´ (630-647)	TCTAGGGATTAACTTCCTGT ATGCTGC (649-675)	5´-AAGAATGGCCAAGTT CATGAG-3´ (698-677)
MMP-10	5´-GACCTGGGCTTTATG GAGATATT-3´ (582-604)	TCCTCGTTGCTGCTCATGAA CTTGGC (668-693)	5´-GCTTCAGTGTTGGCTG AGTGAA-3´ (720-699)
MMP-12	5´-TGCTGATGACATACG TGGCA-3´ (765-784)	CAGTCCCTGTATGGAGAC CCAAAAGAG (786-812)	5´-AGGATTTGGCAAGCG TTGG-3´(834-816)

Total cellular RNA from cells was extracted by using RNeasy miniprep-kit (Qiagen, Chatsworth, CA) according to the manufacturer´s instructions. RNA was then reverse transcribed to cDNA with TaqMan® Multiscribe enzyme and random hexamers and used as a template in PCR reaction. Real-time quantitative PCR reactions were performed with the ABI PRISM® 7700 Sequence Detector System (PE Biosystems) as previously described (Rechardt et al, 2000). PCR amplifications were performed in a total volume of 20 µl, containing 5 µl cDNA sample, 1 µl human GAPDH endogenous control reagents, 100 nM of each primer and 100 nM of fluorogenic probe. The PCR was started with 2 min at 50°C and the initial 10 min denaturing temperature of 94°C, followed by a total of 40 cycles of 15 s of denaturing and 1 min of annealing and elongation at 60 °C. The same reactions were done by conventional PCR with the same primers but without fluorogenic probes. Finally, PCR products were analyzed in 3% low melting point agarose gel in the presence of 5 ng/ml ethidium bromide and visualized under UV light.

4.9. Northern analysis

Total cellular RNA was isolated using the single step method (Chomczynski and Sacchi, 1987). Aliquots of total RNA (10-20 µg) were fractionated on 0,8 % agarose gels containing 2,2 M formaldehyde, transferred to Zeta Probe filters (BioRad, Richmond, CA), and immobilized by heating at 80°C for 30 min. The filters were prehybridized for 2 h and hybridized for 20 h with cDNAs labeled with [α-^{32}P]dCTP using random priming. The filters were then washed, finally with 0,1 x SCC/0,1% SDS at 60°C and [^{32}P]-cDNA/mRNA hybrids were visualized by autoradiography. The mRNA levels were quantitated by scanning densitometry of the autoradiographs using MCID software (Imaging Research Inc., St Catharines, Ontario, Canada) and corrected for the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same samples. The MMP-12 probe was the same as used for *in situ* hybridizations. In addition, human MMP-13 cDNA fragments covering the coding region and part of 3'-untranslated region, altogether 1931 bp (Johansson et al, 1997a), a 2.0 kb human cDNA for human MMP-1 (Goldberg et al, 1986), and a 1.3 kb rat cDNA for GAPDH (Fort et al, 1985) were used for hybridizations.

4.10. Western blot analysis

To assay the production of MMP-12 protein, equal aliquots of the conditioned media of HaCaT cells were fractionated on 7.5% SDS-PAGE (III). Grade II chondrosarcoma (I) specimens were homogenized and suspended into lysisbuffer (Boehringer Mannheim). The amount of proteins was determined using Bradford's test (BioRad Protein Assay) and 30-60 µg of protein was loaded in the gel. Proteins were separated on 8-10% SDS-PAGE and transferred to a Hybond ECL filter

(Amersham). After blocking in PBS containing 5 % milk and 0,1 % Tween-20, the membrane was incubated with monoclonal antibody against human MMP-12 catalytic domain (R&D Systems, Minneapolis) or with monoclonal antibody against human recombinant MMP-12 (R&D Systems, Minneapolis) in concentration 1-1,5 μg/ml, followed by the detection of specifically bound primary antibodies with peroxidase-conjugated secondary antibodies (1:10000) visualized by enhanced chemiluminescence (ECL) (Amersham). Antibody against 72-kDa gelatinase (MMP-2; 1 μg/ml, Oncogene, Calbiochem, Cambridge, MA) was used as a control.

5. RESULTS

5.1. MMP-12 in fetal development and bone lesions (I)

To study the production of MMP-12 during human fetal development, we examined its tissue distribution in the fetal tissue samples obtained at 6-12, 15-16 and 19-22 weeks of gestation, using *in situ* hybridization. No signal was detected in embryos of 6-7 weeks, but beginning at week 8, signal for MMP-12 mRNA was found in the hypertrophic chondrocytes of fetal cartilage (I, Fig. 1 A,B). In addition, MMP-12 was expressed in the developing bone undergoing ossification, including extremities, ribs and limbs, while the remaining cartilaginous area with more primitive cells lacked signal for MMP-12 (I, Fig. 1 F,G). The signal was confined to chondrocytes in hypertrophic cartilage in most of the samples. In addition, in some samples MMP-12 was also expressed in the cells lining perichondrium next to developing bone (I, Fig. 1 C,D). At week 11, mRNA was also expressed in the mesenchymal tissue surrounding hypertrophic cartilage (I, Fig. 1 H-J). Two samples of fetal calvarial bones from weeks 15 and 16 were negative. In the samples examined, MMP-12 mRNA expression could not be detected in any other tissues such as the skin, lungs, kidneys, intestine and neural tissue. Furthermore, no signal could be detected in samples hybridized with the sense MMP-12 probe (I, Fig. 1E). Since we and others have previously characterized the expression and regulation of MMP-13 in fetal bone development (Johansson et al, 1997b; Ståhle-Bäckdahl et al, 1997), we were interested in comparing its expression with that of MMP-12. The expression pattern was very similar: MMP-12 and MMP-13 colocalized in hypertrophic chondrocytes in fetal cartilage and ossifying bones and also in the cells lining perichondrium (data not shown).

MMP-12 expression in malignant and pre-malignant bone lesions (I). As we observed the restricted expression of MMP-12 in developing bone, we also examined its expression in adult human bone and bone lesions. Of the benign and malignant bone lesions, MMP-12 mRNA was detected only in chondrosarcomas. It was confined to CD-68-positive macrophages surrounding the tumor in 8/19 samples (I, Fig. 2 A,B). Furthermore, MMP-12 was expressed in chondrosarcoma cells inside the tumor in 8/19 samples, where no macrophages were detected (I, Fig. 2 E,F; 3 A,C,c). Samples examined represented chondrosarcomas of different grades (I-III) from primary and recurrent tumors. The samples were also immunostained with the monoclonal MMP-12 antibody to confirm the expression pattern obtained by *in situ* hybridization, and the results were alike. Immunosignal for MMP-12 was seen both in macrophages and cancer cells and, thus, MMP-12 protein co-localized with the corresponding mRNA (I, Fig. 2 C-E). There was no correlation with MMP-12 mRNA expression or the aggressiveness of the tumor (I, Table I). However, MMP-12 mRNA was seen in the same regions as MMP-13, the expression of which was more abundant than that of MMP-12 (I, Fig. 3 A,B,E,F). All other benign and malignant tumors examined, e.g. osteoid osteoma, chondroma, osteochondroma, osteoblastoma and osteosarcoma, were negative for MMP-12, as were normal adult bone and cartilage.

TGF-β1 and bFGF immunostainings were done on all chondrosarcoma samples, since TGF-β1 upregulates MMP-13 in chondrocytes (Johansson et al, 1997b) and bFGF in chondrosarcoma (Uria et al, 1998). TGF-β1 was not, however, expressed in the same area as MMP-12. Basic FGF did co-localize with MMP-12 and MMP-13 (data not shown). Based on our cell culture studies, it is likely that bFGF induces production of MMP-13 but not MMP-12. To confirm our findings *in vivo*, we assayed the production of MMP-12 protein from homogenized grade II chondrosarcoma specimens by Western blot analysis. Immunoreactive MMP-12 protein was detected in two different patient samples (I, Fig. 4C) and the estimated molecular weight corresponded to the proMMP-12 (55 kDa). As a control, same samples were analyzed with monoclonal antibody against MMP-2 which detected immunoreactive protein with an estimated molecular weight of 79 kDa.

Induction of MMP-12 mRNA by TNF-α in HTB-94 chondrosarcoma cells. To clarify the physiological role of MMP-12 in chondrosarcomas, we studied its regulation in HTB-94 chondrosarcoma cell culture in the presence of bFGF, TGF-β1 and TNF-α. By conventional PCR, low levels of MMP-12 transcript were detected in both HTB-94 (I, Fig. 4A) and HCS-2/8 cells (data not shown). Total RNA from these cells was analyzed using quantitative real time-PCR method. Basal levels for MMP-12 mRNA expression were very low in control cells, but treatment with TNF-α (20 ng/ml) resulted in a marked induction of MMP-12 mRNA production after 24 hours of treatment (I, Fig. 4 A,B). The induction was detected already after 12 hours (about 40-fold induction compared to control cells) and was even higher after 48 hours (data not shown). Basic FGF and TGF-β1 did not upregulate MMP-12 in these cells (I, Fig. 4A,B).

5.2. MMP-12 and MMP-7 in actinic damage (II)

Solar elastosis manifests sunlight-induced damage of the elastic fibers in the upper and mid-dermis. Immunosignal for MMP-12 was clearly seen in all 18 samples within the fragmented, distorted and thickened elastic fibers of the upper dermis stained with Weigert´s elastin stain (II, Fig. 1 A,D). MMP-7 protein was detected in thinner but also histologically abnormal elastic fibers, located deeper towards mid-dermis (II, Fig. 1 B,C,F). The morphology of collagen was better preserved in MMP-7 than MMP-12 -positive areas (data not shown). In 8/18 samples band-like staining under the BM for MMP-7 was also seen (II, Fig. 1E). Immunoreactivity for the MMP-7 substrate, versican, was detected in large areas of the upper and mid-dermis partly co-localizing with MMP-7 (II, Fig. 1 F,G). Tenascin was only detected as a linear band under BM, but not in any elastotic areas of the upper dermis (II, Fig. 1B). Elastotic areas did not show MMP-9 immunoreactivity (data not shown).

Solar keratosis shows actinically damaged fibers in upper dermis, whereas keratinocytes manifest with variable degrees of atypia. Like in solar elastosis, immunostaining for MMP-12 was seen in damaged, amorphous elastic fibers in upper dermis (II, Fig. 2 A,G), whereas MMP-7 protein was in mid-dermis (II, Fig. 2 C,D). However, only 1/8 samples demonstrated staining for MMP-7 under BM (II, Fig. 2 C,E). *In situ* hybridization did not reveal any positivity for MMP-12 or MMP-7 in either elastosis or keratosis (II, Fig. 2I). MMP-7 was occasionally seen in sweat glands and hair follicles (II, Fig. 1E, 2E), as previously described (Saarialho-Kere et al, 1995; Karelina et al, 1994). Staining for versican partly co-localized both with MMP-7 and -12 (II, Fig. 2 A-C). As in solar elastosis, tenascin was detected as a continuous line under BM, but did not co-localize with MMP-7 (II, Fig. 2 E,F).

UVA/UVB exposed human skin showed histologically slightly dilated capillaries, increase in the number of lymphocytes and melanocytes and parakeratosis. Shrinkage of keratinocytes was seen especially in UVB-irradiated skin. *In situ* hybridization revealed no MMP-12 expression after acute UVA or UVB provocation (data not shown). Immunosignal was seen in a few stromal fibroblast/macrophage-like cells, but not in the epidermis (II, Fig. 3 A,B). Both MMP-7 mRNA and protein was seen in sweat gland epithelium, but no positive stromal cells were detected (II, Fig. 3 C-E). The intensity of staining under BM after UVB provocation was clearly increased when compared to UVA irradiated samples (II, Fig. 3 C,D,G,K). This could be explained by the finding of MMP-7 immunopositive basal keratinocytes in 2 samples of frozen tissue obtained one day after UVB exposure (II, Fig. 3F). Versican was in the same area as MMP-7 under BM (II, Fig. 3 G,H,J). The amount of tenascin was increased after UVB compared to UVA provocation (II, Fig. 3 L,M).

Normal skin samples from different parts of the body and from patients of different ages (n = 17) were examined. No MMP-12 mRNA or protein was seen in the samples. Instead, MMP-7 was seen in sweat coils and the luminal surface of sweat ducts in all samples. Fibril-like staining below basal

keratinocytes was seen particularly in the samples of young adults (buttock, back) (II, Fig. 4D) and it became fainter in the specimens of children (II, Fig. 4A) and controls over 70 years of age. In normal skin, staining for versican and elastin was detected in MMP-7 positive areas under BM (II, Fig. 4 B,E,F). Tenascin was seen as a partly interrupted line immediately below basal keratinocytes in the papillary dermis, not co-localizing with MMP-7 (II, Fig. 4 D,G).

The hairless SKH-1 mouse has long been an experimental model to study the effects of chronic low-dose UVA and UVB irradiation on the histologic changes that occur during solar damage (Starcher et al, 1999). MME-positive stromal cells were infrequently detected in the stromal cells of the mid-dermis of age-matched non-UV-irradiated control mice (II, Fig. 5A). In samples taken 12 days after starting repeated UV administration, signal for MME was more frequently seen in the stromal cells of the mid-dermis in the vicinity of dermal cysts, characteristic of hairless mice (II, Fig. 5B). Abundant signal for MME was further observed in biopsies obtained 8 and 11 weeks after starting repeated UV irradiation. Signal was not detected in the epidermis or the epithelium of keratinizing cysts but in plump (II, Fig. 5A) macrophage-like or spindle fibroblast-like (II, Fig. 5C,D) stromal cells.

5.3. Expression of MMP-12 in epithelial cancers (III, IV)

SCC, BCC, and other skin lesions (III). Expression of MMP-12 mRNA was detected in 16/17 SCCs studied (III, Table I). The signal was found in epithelial cancer cells in 11/17 tumors (III, Fig. 1 A,F). The cell specific localization was confirmed using cytokeratin and CD-68 immunohistochemistry (III, Fig. 1 B,E). In 15/17 samples MMP-12 mRNA was also expressed by stromal macrophage-like cells (III, Fig. 1 A,F-H). Well-differentiated, non-invasive tumors (grade I SCCs) displayed considerably less positive cells than grade II and III tumors (III, Table I). In ulcerated SCCs, signal for MMP-12 mRNA was detected in macrophage-like cells just beneath the wound bed. MMP-12 protein expression was detected in all SCCs examined (n = 14) and it was found both in macrophages and cancer cells (III, Fig. 1 C,D,I) in the same region as mRNA -positive cells. Immunostaining for CD-68 was performed on serial sections in altogether 19 SCCs or BCCs to exclude the possibility that MMP-12 expressing cells in the middle of cancer nests would be macrophages. The staining demonstrated that part of the MMP-12-positive cells were macrophages, particularly those lying near the borders of tumor islands. However, MMP-12-positive cells were also detected inside cancer islands devoid of macrophages (III, Fig. 1 I,J).

MMP-12 mRNA was expressed also in BCCs in 10/19 samples studied (III, Table I), but the expression was focal and not as abundant as that of MMP-12 in SCCs (III, Fig. 2A). MMP-12 was generally expressed by macrophage-like cells, but in 4/19 samples it was also detected in cytokeratin-positive cancer cells within the tumor islands (III, Fig. 2 C,D). MMP-12 protein was expressed in the same area as MMP-12 mRNA either in cancer cells (III, Fig. 2 B,G) or macrophages. Like in SCCs, MMP-12 was expressed in the wound bed of ulcerated BCCs by macrophages (III, Fig. 2 E,F), based on the microscopical appearance of MMP-12-positive cells, their presence in cytokeratin-negative areas, and the lack of data on MMP-12 production by fibroblasts or endothelial cells.

Tumors representing Bowen's disease were negative for MMP-12 except for one sample. No MMP-12 expression was found in Clark grade II-IV melanomas (not shown). Normal skin has previously been shown to be negative for MMP-12 mRNA and protein (Vaalamo et al, 1999), and was also included as a negative control in the present *in situ* and immunostudies with similar results. No signal was detected using the sense MMP-12 cRNA probe.

SCC of the vulva (IV). To further elucidate the role of human metalloelastase in SCC progression and to investigate whether the pattern of its expression influences on tumor aggressiveness and patient outcome, we studied its expression in SCCs of the vulva. Expression of MMP-12 mRNA was observed in 31/33 SCC samples (IV; Table I). In 28 samples it was expressed in CD-68-positive macrophages (IV, Fig. 1 E,F), whereas in 10 samples in epithelial carcinoma cells (IV, Fig. 1 A-D). The epithelial origin of signal was assessed using CD-68 and cytokeratin stainings as well as histological examination. Generally, whenever MMP-12 mRNA was expressed in cancer cells, the samples were also histologically more aggressive and less differentiated (grade II/III tumors) than well-differentiated (grade I) tumors (P=0,0099) (IV, Table I). The presence of metastatic lymph nodes did not associate with MMP-12 expression in cancer cells, since in several such patients signal was only evident in macrophages. Macrophage-derived MMP-12 mRNA was more abundant in well-differentiated grade I tumors than in grade III tumors and, thus, correlated inversely to invasiveness (P=0,01). No MMP-12 expression was detected in intact vulvar epithelium or in the stroma under it (data not shown). The amount of signal for MMP-12, either in macrophages or cancer cells, did not correlate with lymph node metastasis or patient prognosis. Neither did the amount of macrophages differ between the grades. Furthermore, there was no correlation between macrophage-derived MMP-12 mRNA and a low amount of blood vessels. Overall microvessel density did not significantly vary between the grades, although microvessel density may serve as a prognostic factor in cancers (Weidner 1995).

5.4. Expression and regulation of MMP-12 in cultured epithelial cells (III, IV)

Expression of MMP-12 in HaCaTs and SCC cell lines by Northern analysis (III, IV). To confirm the ability of SCC cells to express MMP-12, we used Northern blot analysis to determine the expression of MMP-12 mRNA in four cell lines established from SCCs of the head and neck. Marked expression of MMP-12 mRNA was detected in UT-SCC-7 cells (III, Fig. 3A), established from metastasis of a cutaneous SCC and in UT-SCC-38 cells, established from primary SCC of glottic larynx. Both of these cell lines also expressed high basal levels of MMP-1 and MMP-13 mRNA (III, Fig. 3A). In addition, MMP-12 mRNA was detected in cell line UT-SCC-17, established from metastasis of a SCC of supraglottic larynx (not shown). Instead, no expression of MMP-12 mRNA was detected in UT-SCC-15, a primary SCC tongue cell line, which also expressed very low basal levels of MMP-1 and MMP-13 mRNA (III, Fig. 3A). We also examined the expression of MMP-12 in vulvar SCC cells. In agreement with vulvar SCC samples *in vivo*, MMP-12 was also expressed in SCC cells derived from grade II primary tumor (IV, Fig. 3 and Figure 7). In contrast to MMP-13, it was not induced by TNF-α in these cells, nor was it induced by TGF-β1 or IFN-γ.

We also assayed the amount of MMP-12 protein in the aliquots of conditioned media corresponding to an equal number of cells by Western blot analysis using a monoclonal anti-MMP-12 antibody. UT-SCC-7 cells from metastasis of cutaneous SCC produced immunoreactive proMMP-12 with an estimated molecular weight of 58 kDa, and its production was enhanced 2.6 and 7.5-fold by a 24-h treatment with TGF-β and TNF-α, respectively (III, Fig. 3B).

Marked expression of MMP-12 mRNA was also detected in the transformed HaCaT keratinocytes using Northern blot hybridizations, and its expression was slightly (1.8-fold) enhanced by TGF-β, but not by TNF-α, both of which markedly enhanced the expression of MMP-13 mRNA in the same cells (III, Fig. 4B). In HaCaT cells the basal expression of MMP-12 mRNA was suppressed by 40% with IFN-γ (100 U/ml) (III, Fig. 4B). However, IFN-γ slightly augmented the expression of MMP-12 mRNA in combination with TGF-β and TNF-α (III, Fig. 4B). In the same cells, IFN-γ potently inhibited the basal MMP-13 expression, as well as the up-regulation of MMP-13 mRNA levels by TGF-β and TNF-α (III, Fig. 4B). Interestingly, no expression of MMP-12 mRNA was detected in

normal epidermal keratinocytes treated with TNF-α, TGF-β, or IL-1 using Northern analysis (not shown).

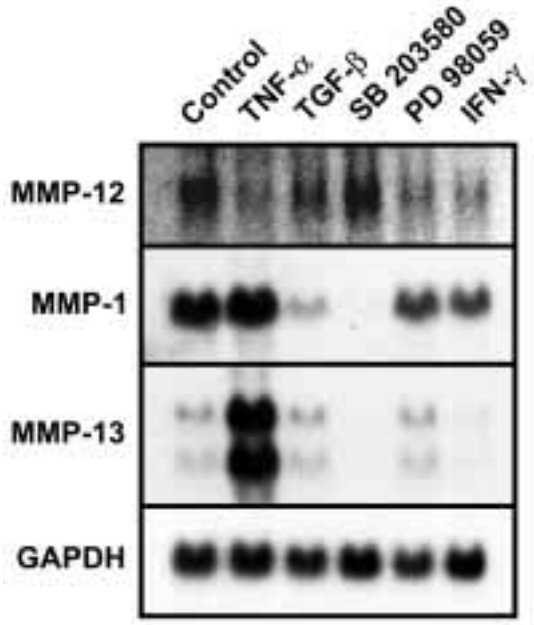


Figure 7. Expression of MMP-12 in vulvar carcinoma cell line UT-SCV-3. Cells were incubated for 24 h in serum-free DMEM alone (control) or in the presence of TNF-α (20 ng/ml), TGF-β1 (5 ng/ml), IFN-γ (100 U/ml), PD98059 (30 μM), a specific inhibitor of ERK1,2 kinases or SB203580 (10 μM), a selective inhibitor of p38 MAPK, prior to total RNA isolation. Twenty μg aliquots were analyzed for the level of MMP-12, MMP-1, MMP-13 and GAPDH mRNA by Northern hybridization.

Induction of MMP-12 mRNA in HaCaTs and epithelial MCF-10f cells by quantitative RT-PCR (IV). Since no clear data on MMP-12 regulation in epithelial cells exist, we cultured keratinocytes, HaCaT, epithelial MCF-10f and A431 cells on different matrices in the presence of various cytokines and growth factors. Total RNA from these cells was analyzed using the quantitative real time-PCR method (Rechardt et al, 2000). Basal levels for MMP-12 mRNA were practically undetectable in keratinocytes (III, Fig. 4A), as already shown *in vivo*, and A431 cells. After 24/48 hours of treatment with various cytokines and growth factors, there was no significant upregulation of MMP-12 (EGF, GM-CSF, IFN-γ, IL-1β, KGF, PDGF, TGF-β1 and TNF-α) in these cells.

In contrast, in MCF-10f cells, TGF-β1 (5 ng/ml) and TNF-α (20 ng/ml) induced the production of MMP-12 mRNA, but only after 48 hours of incubation. In MCF-10f cells basal levels of MMP-12 mRNA were very low and the treatment with TGF-β1 resulted in marked induction, which was about 50-fold on both plastic and matrigel and about 20-fold on fibronectin (IV, Fig. 4A and data not shown). Also TNF-α induced MMP-12 mRNA on plastic (20-fold) and fibronectin (7-fold) (IV, Fig. 4A). Results were similar with (2 %) or without serum. In HaCaTs, basal levels of MMP-12 mRNA were already high in agreement with our previous data, and treatment with TGF-β1 induced these levels about 6-fold on plastic (IV, Fig. 4A) and 2-fold on matrigel (data not shown). TNF-α did not seem to regulate MMP-12 in HaCaTs in accordance with the results from Northern analysis. As a control, regulation of MMP-1 was also studied. There was a strong upregulation of MMP-1 in HaCaT cells, when treated with EGF and cultured on collagen I (Pilcher et al, 1997) (data not shown).

5.5. Expression of MMP-7 and MMP-9 in SCC of the vulva (IV)

We also studied the expression of MMP-7 and MMP-9, other angiostatin-generating MMPs. MMP-7 mRNA was detected in 50% of the tumors. It was expressed by cancer cells in 14/33 specimens (IV, Fig 2 A-C) and in stromal cells surrounding cancer nests in 6/33 tumors (IV, Fig. 2 D-F) (Table I). Matrilysin expression tended to concentrate in grade II and III tumors. MMP-9 protein was usually detected in the stromal macrophages and neutrophils (IV, Fig. 1G). The amount of MMP-9 producing stromal cells was lower in grade III than grade I-II cancers. In 13/33 samples, MMP-9 was also detected in epithelial cancer cells at the invasive front (IV, Fig. 1G). Expression in the epithelium was more frequent in grade III than grade I tumors (IV, Table I). In most cases when tumor cells expressed MMP-12 they also expressed MMP-7 (6/9) or MMP-9 (5/9) or both (4/9).

The regulation of MMP-7 in HaCaT cells was studied (unpublished data), as, although several reports on MMP-7 regulation in adenocarcinoma and other cells exist (see Wilson and Matrisian 1998), little is known about its induction in transformed keratinocytes. After 24 and 48 hours of treatment with various cytokines and growth factors, TNF-α (20 ng/ml) clearly induced the production of MMP-7 mRNA in HaCaT cells (Figure 8). Instead, there was no significant upregulation of MMP-7 with other factors studied (bFGF, EGF, GM-CSF, IFN-γ, IL-1β, KGF, PDGF and TGF-β1) in these cells. Neither did direct contacts between cells and components of ECM induce the production of MMP-7, when cells were cultured on collagens I and IV, fibronectin, laminin-I or Matrigel.

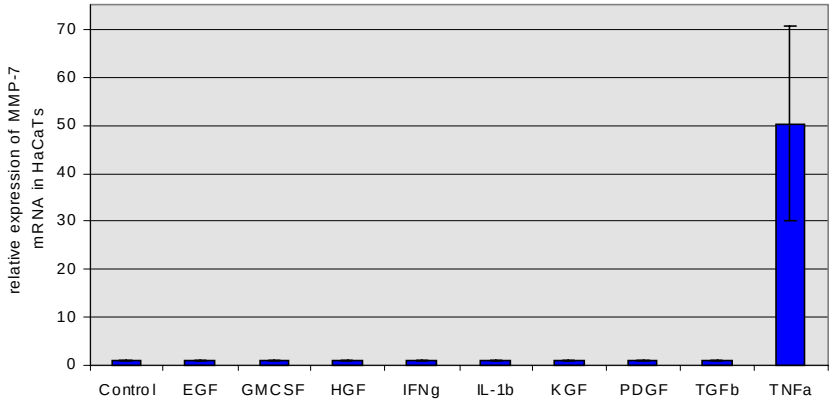


Figure 8. TNF-α induces MMP-7 in HaCaTs. Cells were cultured in DMEM and stimulated for 48 hours with various cytokines and growth factors (5-20 ng/ml) as described in Materials and methods. Cells without treatment were used as controls. Total RNA was extracted, reverse transcribed and analyzed by real-time quantitative RT-PCR (Taqman, PE Biosystems) using GAPDH as an endogenous control. Results are shown relative to mRNA levels from control cells.

5.6. Expression and regulation of MMP-10 in SCC cells (V)

MMP-10 was expressed in 13/21 SCC samples by epithelial cancer cells (V, Fig. 1 A,B,b;2 A,E). It was generally more abundantly expressed in tumors with substantial inflammation, near CD-68 positive inflammatory cells (V, Fig. 1 A,C). The tumors found negative were typically devoid of inflammation or necrosis (data not shown). There was no correlation between the number of cells expressing MMP-10 and the histological aggressiveness of the tumor (V, Table 1). However, highly differentiated tumors tended to be negative or showed only a weak signal for MMP-10 mRNA. Expression of MMP-3, a closely related enzyme to MMP-10, was also studied in a subset of SCC

samples (V, Table 1). Both stromal and tumor cells expressed MMP-3 mRNA (V, Fig. 2C), and only in 4/18 samples did it co-localize with MMP-10 (V, Fig. 2 A,C).

MMP-10 mRNA was also expressed in BCCs in 11/19 samples studied (V, Table 1). Of the positive tumors, 8/11 represented the fibrosing subtype, and in six of them mRNA was detected in cancer cells, while in two others the expression was connected with ulceration (V, Fig. 3 A,B,G). In general, the expression was not as abundant as in SCCs and was located near necrotic areas at the tumor surface. MMP-3 expression was more abundant in most samples than that of MMP-10, and it was more often expressed by stromal cells surrounding tumor islands than by cancer cells (V, Fig. 3 D,H,I,i; Table 1). Based on our cell culture studies, immunoanalysis for TGF-β1 was performed in cancers. Generally MMP-10 positive cells located in the vicinity of TGF-β1 positive areas (V, Fig. 3 E,F). All specimens of Bowen's disease and actinic keratosis were negative for MMP-10, the expression of which has not been found in normal intact skin (Saarialho-Kere et al, 1994). No specific signal was detected using the sense control probe (V, Fig. 1D).

Since laminin-5 has been associated with the invasive capacity of several carcinomas (Pyke et al, 1995), immunosignal for laminin-5 was examined in altogether 13 SCCs and 11 BCCs. MMP-10 positive cells showed intracellular staining for laminin-5 both in the areas of ulceration and at the borders of invasive tumor islands (V, Fig. 2 A,B,E,F). However, laminin-5 was clearly more abundantly expressed so that the edges of many tumor islands deeper in the dermis were laminin-5 but not MMP-10 positive. Gelatinase A, which is known to be able to degrade laminin-5 in epithelial cells (Giannelli et al, 1997), was not found in the same cells (n=14 SCCs, n=6 BCCs), but rather in fibroblasts surrounding cancer islands (data not shown).

Forty-three cell lines established from primary and recurrent tumors and metastases of SCCs of the head and neck (Johansson et al, 1997c) were examined for the expression of MMP-10. No basal MMP-10 mRNA expression was detected in any of the cell lines derived from skin (n = 7), while two cell lines from the larynx (n = 12) and two from the oral cavity (n = 22) showed some basal expression (V, Fig. 5A and data not shown). To examine the regulation of MMP-10 mRNA by Northern blot analysis, we chose the UT-SCC-7 cell line, established from metastasis of a cutaneous SCC. Cells were treated with TGF-α, FGF-2, and KGF, all potent stimulators of keratinocyte proliferation. Marked MMP-10 mRNA abundance was detected in UT-SCC-7 cells treated with TGF-α (10 ng/ml) and KGF (10 ng/ml), whereas FGF-2 (10 ng/ml) had no marked effect (V, Fig. 5A). The treatment of UT-SCC-7 cells with IFN-γ (100 U/ml) alone had no effect on MMP-10 mRNA levels, whereas treatment with TGF-β1 (5 ng/ml) and TNF-α (20 ng/ml) slightly enhanced MMP-10 mRNA expression (V, Fig. 5B). Interestingly, exposure of UT-SCC-7 cells to IFN-γ in combination with TGF-β1 or TNF-α resulted in marked induction of MMP-10 expression (V, Fig. 5B). Abundant expression of MMP-10 mRNA was also detected in HaCaT keratinocytes treated with TGF-β1, alone or in combination with IFN-γ (V, Fig. 5C). In contrast to UT-SCC-7 cells, TNF-α alone or in combination with IFN-γ had no marked effect on MMP-10 expression.

5.7. MT1-MMP expression in SCC and BCC (V)

The production of MT1-MMP was examined, firstly, since its expression in BCCs was poorly characterized and, secondly, since the expression of MT1-MMP may correlate with the invasiveness of the tumor samples under investigation (Mori et al, 1997; Gilles et al, 1996). MT1-MMP mRNA was expressed in 19/21 SCCs studied and in 11 of them the signal was detected in cancer cells (V, Fig. 4 B,C,c). Otherwise MT1-MMP was abundantly expressed by stromal fibroblasts (V, Fig. 4 D,E). With the exception of four samples (V, Fig. 4 A,B), MMP-10 and MT1-MMP had quite distinct expression patterns. In general, MT1-MMP was expressed deeper at invasive tumor islands (V, Fig. 3 A,C). In BCCs, MT1-MMP mRNA was detected more often than MMP-10 mRNA. It

was expressed in all fibrosing (11) and keratotic (3) BCC samples but not in adenoid BCCs. Unlike in SCCs, cells expressing MT1-MMP were not cancer cells, but stromal fibroblasts surrounding tumor islands (V, Fig. 3C; 4 F,G). Since MT1-MMP has been shown to degrade and co-localize with laminin-5 at least in colon and breast cancer (Koshikawa et al, 2000), we also compared its expression to laminin-5 immunopositivity. We found, altogether in 13 SCCs and 11 BCCs, that MT1-MMP mRNA was not expressed in the same cells as laminin-5 but rather in the stromal cells in the vicinity of laminin-5 positive cancer cells (V, Fig. 4 D,E).

6. DISCUSSION

6.1. MMP-12 in development and chondrosarcomas

Embryonic development requires coordinated programming of cellular processes such as cell migration, differentiation, apoptosis, angiogenesis, and ECM formation. MMPs and their inhibitors participate in the regulation of these fundamental processes as has been shown in many studies *in vitro* (see Vu and Werb, 2000). Expression of different human MMPs and TIMPs during fetal development has been shown *in vivo* in various tissues at specific time points (Karelina et al, 1994; McGowan et al, 1994; Terada et al, 1995; Airola et al, 1998; Robinson et al, 2001). We observed that MMP-12 is expressed during fetal development exclusively in the hypertrophic chondrocytes of the developing bone undergoing endochondral ossification, while the cartilaginous area with more primitive cells, and other tissues, lacked signal for MMP-12. In some samples MMP-12 was also expressed in the cells lining perichondrium next to developing bone.

Endochondral ossification is a process where avascular cartilage is turned into highly vascularized bone tissue. Cartilage is usually highly resistant to vascularization, except during bone formation when hypertrophic cartilage becomes a target for capillary invasion and angiogenesis (Werb et al, 1999). Mouse models implicate that several MMPs have an important role particularly in endochondral bone formation (see Werb and Chin, 1998). Both MMP-9 and MT1-MMP deficient mice have severe defects in endochondral ossification as well as in vascularization, although their defects are different (Vu et al, 1998; Holmbeck et al, 1999; Zhou et al, 2000). MMPs may play a role in generating biologically active fragments of ECM components, which could regulate early development. Resting hyaline cartilage has a lot of anti-angiogenic activities and it has been suggested that MMP-9 would regulate the release of an angiogenic factor from hypertrophic cartilage or inactivate an inhibitor (Werb et al, 1999). Thus, the lack of MMP-9 would lead to a decrease in vascular invasion. On the other hand, MMP-9, like MMP-12, has been suggested to be antiangiogenic, since it generates angiostatin from plasminogen (Dong et al, 1997; Patterson and Sang, 1997). Thus, MMP-12 might regulate angiogenesis during fetal bone formation as it does in wound healing (Madlener et al, 1998). Alternatively, MMP-12 may cleave matrix components of the bone anlage in order to initiate the formation of mature bone. MMP-12 can degrade heparan and chondroitin sulfate proteoglycans *in vitro* (Gronski et al, 1997), of which the latter, in particular, are present in cartilage. Furthermore, they are concentrated at sites where matrix calcifies during endochondral bone formation (see Poole et al, 1986). Interestingly, mouse cartilage proteoglycan degradation is mediated by macrophage metalloelastase (Janusz et al, 1999).

Our findings, together with previous reports, suggest that MMPs could mediate the development of human bone from cartilage in a cascade-like manner and may well have several functions. For example, if MMP-12 can activate MMP-2, as shown *in vitro* (Matsumoto et al, 1997), it could in turn activate MMP-13, since the expression patterns of MMP-12 and MMP-13 were remarkably similar in developing bone (Johansson et al, 1997b). Also MMP-13 was strongly expressed in chondrocytes of hypertrophic cartilage and in cells lining periosteum (Ståhle-Bäckdahl et al, 1997; Johansson et al, 1997b). It has been shown in a model system that MMP-13 is activated by MMP-2 during chondrocyte maturation and the combination of both proteinases is required to prepare cartilage matrix for subsequent calcification before endochondral ossification (D'Angelo et al, 2000). A very similar pattern has been shown for MMP-10, as it was expressed in chondrocytes of the growth plate in the fetal rib, while MMP-3 was in osteocytes in surrounding fibrous tissue. Furthermore, both were also expressed by osteoblasts at sites of bone formation (Bord et al, 1998). Theoretically, they are both able to activate MMP-13 (see Nagase 1998). No skeletal abnormalities have been described in the MMP-12 knock-out mice (Hautamäki et al, 1997), suggesting that other

MMPs can compensate for its functions. Its actual biological role during ossification awaits further studies.

Chondrosarcoma is the second most common malignant bone tumor, originating from cartilage cells. Primary chondrosarcoma arises *de novo* and has the ability to invade normal bone tissue. Patients often lack symptoms and tumors are large at the time of primary diagnosis. It is often difficult to predict the biological behavior of these tumors and thus, an objective prognostic marker would be extremely important (Unni 1996). Of all the bone lesions studied, MMP-12 was detected only in chondrosarcomas, both in malignant chondrosarcoma cells, and in macrophages, which is particularly interesting in the context of our findings in fetal cartilage. In agreement with our results, MMP-12 upregulation has been shown in chondrosarcoma using cDNA microarray technology (Hiller et al, 2000). Also MMP-1 and -9 proteins have been observed in the cells in the marginal areas of cartilaginous lobules (Kawashima et al, 1997), a pattern which is similar to that of MMP-12 in our study. Furthermore, expressions of MT1-MMP, MMP-2 and TIMP-2 demonstrate correlation with the tumor grade (Sakamoto et al, 1999). Similarly, a recent work from Söderström *et al.* (2000) suggests prognostic value for the same MMPs when determining aggressiveness of chondrosarcomas. In addition, the ratio of MMP-1/TIMP-1 gene expression may serve as a prognostic indicator in patients with chondrosarcoma (Scully et al, 1999). In our study, the expression of MMP-12 either in macrophages or in cancer cells did not significantly correlate with the tumor grade, although aggressive grade III tumors tended to have a smaller number of MMP-12 positive cells than better-differentiated tumors (V, Table I). The expression of MMP-12 may still be associated with malignant transformation of these tumors, since it is produced in chondrosarcomas by malignant cells, but not at all in benign bone lesions or normal bone or cartilage. Based on the *in vitro* substrate specificity of MMP-12 (Chandler et al, 1996; Gronski et al, 1997), it is possibly involved in the cleavage of aggrecan. This large proteoglycan is the major component of the ECM in the chondrosarcomas and MMPs are known to participate in its degradation in these tumors (Toriyama et al, 1998).

Like during development, MMP-12 and MMP-13 mRNAs were expressed in the same or adjacent areas in chondrosarcoma. Basic FGF enhances the expression of MMP-13 in chondrosarcoma cells *in vitro* (Uria et al, 1998; Balbín et al, 1999), but it did not induce MMP-12 in chondrosarcoma-derived HTB-94 cells in culture. Nor was MMP-12 upregulated by TGF-β1, which could still serve as the modulator of MMP-12 expression in chondrocytes during development, based on the similar expression patterns of MMP-12 and -13 in ossifying bone (Johansson et al, 1997b) and on the fact that it upregulates MMP-12 in other cells. In macrophages TGF-β1, however, inhibits cytokine-mediated induction of MMP-12 (Feinberg et al, 2000). Instead of bFGF and TGF-β1, TNF-α, an anti-tumor agent and an inflammatory mediator predominantly released by activated macrophages (see Fiers 1991), strongly upregulated MMP-12 mRNA in chondrosarcoma cells in time-dependent manner. Interestingly, TNF-α also stimulates MMP-9 production in HCS-2/8 cells leading to ECM degradation (Hattori et al, 2001). Furthermore, TNF-α is involved in macrophage-associated angiogenesis (see Ono et al, 1999). Besides chondrosarcoma cells, TNF-α is known to induce MMP-12 expression in macrophages (Feinberg et al, 2000). Macrophages surrounding chondrosarcoma lobules may secrete TNF-α and induce MMP-12 production in cancer cells of these tumors.

6.2. MMP-12 and MMP-7 in solar damage

Excessive exposure to sunlight is a crucial predisposing factor for actinic damage and malignancies of the skin. Photoageing causes accumulation of elastolytic material, i.e. elastin and versican (Bernstein et al, 1995a; Bernstein et al, 1995b) in the upper and mid-dermis. This is accompanied by the degeneration of collagenous matrix. Both in solar elastosis and keratosis, we observed abundant

expression for MMP-12 protein in the areas of abnormal elastotic fibers in the upper dermal region where UV-induced damage is expected to be most prominent. Since MMP-12 is the most effective MMP against elastin, it probably takes part in a reparative remodeling process by trying to cleave this abnormal elastotic material or fibrillin (Ashworth et al, 1999). It has been previously shown, that UVA and UVB induce MMP expression in dermal fibroblasts or epidermal keratinocytes. Expression of MMP-1, -2, -3, and -9 could lead to degradation of collagens and also noncollagenous ECM components, including proteoglycans and BM proteins (see Kähäri and Saarialho-Kere, 1997; Fisher and Voorhees, 1998). In aortic aneurysms, MMP-12 also localizes to residual elastic fibers but not in intact elastin (Curci et al, 1999), which is in accordance with our negative results in normal skin, anetoderma and mid-dermal elastolysis. GM-CSF is induced at least in keratinocytes by UV-light (Schwarz et al, 1989) and can upregulate MMP-12 expression in macrophages (Kumar et al, 1996); thus, it could be a candidate for inducing MMP-12 in solar damage. UV-radiation could also lead to the recruitment of macrophages that are a natural source of elastases. In the light of our results with SCCs, it is interesting that the majority of SCCs in light-exposed areas may arise from pre-existing solar keratoses (Marks et al, 1988).

MMP-7 protein was detected deeper in the dermis than MMP-12. The elastic bundles were less thickened and distorted and collagen better preserved in these areas. The epithelial nature of this enzyme suggests that dermal macrophages, rather than stromal fibroblasts, would be the source of it (Busiek et al, 1995; Halpert et al, 1996). MMP-7 can be induced by IL-1β and become activated by MMP-3, both of which are induced by UVB light. MMP-7 may enhance elastin degradation in photodamaged skin both as an elastase and as a leukocyte elastase inhibitor (Wilson and Matrisian 1998). *In vitro*, MMP-7 can degrade decorin, a small proteoglycan binding to collagen (Bernstein et al, 1995a), and MMP-7 is able to release TGF-β from this complex (Imai et al, 1997), thereby contributing to its activation. The immunoreactivity was always more intense after acute exposure to UVB than UVA. No MMP-7 mRNA or protein was seen in keratinocytes in samples collected three days after UV exposure. However, in frozen tissue taken one day after irradiation, the basal keratinocytes were repeatedly MMP-7-positive. This discrepancy may reflect either different sensitivity of the antibodies when working on frozen tissue versus paraffin samples or true time-dependent regulation. MMP-7 is able to cleave the β4-integrin present in hemidesmosomes (von Bredow et al, 1997) and thus, it could participate in the degradative changes occurring in the BM zone after UV exposure. The colocalization of versican and elastin has been shown (Zimmermann et al, 1994) as has the upregulation of versican in photoaged skin (Bernstein et al, 1995a). Versican and MMP-7 protein were expressed in the same areas of the middermis both in solar elastosis and keratosis and in the papillary layer of normal skin. Thus, it is possible that MMP-7 cleaves versican, as has earlier been shown in atherosclerotic lesions (Halpert et al, 1996). The expression of tenascin was also increased in solar elastosis and keratosis and in UVA/UVB irradiated skin in agreement with previous data (Tuominen et al, 1997; Filsell et al, 1999). Tenascin is an *in vitro* substrate of MMP-7, but due to their distinct expression patterns in our samples, they probably do not interact in solar damage.

Metalloelastase mRNA was upregulated in macrophage-like cells in UV-irradiated hairless mice skin. Repeated treatment of the mouse strain with UVB results in clinical and histologic changes similar to those observed in photodamaged human skin, such as inflammatory infiltrate and elastotic changes in the dermis (Kligman et al, 1982; Schwartz 1988). However, there are differences between mice and human skin in the amount and distribution of elastin that should be noted when extrapolating results between species. Most of the signal for MME was detected in mid-dermis, where elevated collagen mRNA levels and inflammatory cells have been reported (Starcher et al, 1999). No MMP-12 or MMP-7 mRNA was detected in human solar elastosis and keratosis specimens, suggesting that dermal macrophages secrete these enzymes over a long period of time and that the proteins accumulate after years of exposure to UV-light. MMPs are not usually

constitutively expressed, but upregulated in response to specific signals and, in general, secreted as soon as they are synthesized. No mRNA expression was detected in biopsies taken three days after acute UVA/UVB provocation. Based on the structure of MMP-12 and -7 promoter regions, UV-activated AP-1 can stimulate the transcription of both. Maximal induction of AP-1 occurs after 1 minimal erythema dose (Fisher et al, 1996), which is less than the dose given by us. A single UVB exposure can cause acute induction of MMP, which returns to basal levels after 48-72 hours. The induction of at least MMP-1, -2, and -9 is mediated by MAP-kinases, which are maximally activated within four hours after UV-irradiation, returning to baseline at 24 hours (Fisher and Voorhees, 1998). It is possible that, had we obtained our samples earlier than three days after photoprovocation, we would have seen increased mRNA expression of MMP-12 or -7 in the dermis. However, our data suggests that long-term exposure of the skin to sunlight is needed to induce these MMPs in the dermis.

6.3. Dual role of MMP-12 in epithelial cancers

The expression of MMP-12 has been shown mainly in macrophages, having a role, e.g., in macrophage-related proteolysis of elastin in various pathological conditions. Our study was the first to demonstrate MMP-12 expression in squamous and basal cell skin cancers. Interestingly, MMP-12 mRNA and protein were expressed both *in vivo* and *in vitro* not only by macrophages but also by transformed human keratinocytes (SCC cells and HaCaTs). 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 invasion process. Analogously, clinically more aggressive, fibrosing BCCs expressed MMP-12 more often than the keratotic or adenoid BCCs. Our findings were further substantiated by the fact that, in our previous studies on several benign skin disorders or wound healing, we had never detected MMP-12 mRNA in normal keratinocytes *in vivo* (Vaalamo et al, 1999) or in culture, indicating that MMP-12 expression is specific to transformed epithelial cells. MMP-12 mRNA expression had previously been detected in breast cancer (Heppner et al, 1996) in macrophage-like cells and, recently, in tumor-infiltrating macrophages in Barrett's esophageal adenocarcinoma (Salmela et al, 2001). Along with our findings in skin cancer, the only study demonstrating MMP-12 in cancer cells and not in macrophages *in vivo* was that of Gorrin-Rivas *et al*. (1998), in which MMP-12 was primarily expressed by epithelial hepatocellular carcinoma (HCC) cells. Later, MMP-12 has been described in colorectal carcinoma cells (Yang et al, 2001). Strong expression of MMP-12 has, furthermore, been described in immortalized mammary epithelial cell line (MCF-10f) by RT-PCR (Giambernardi et al, 1998). Expression of MMP-13, MMP-7 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 (Karelina et al, 1994; Airola et al, 1997; Johansson et al, 1997c). Thus, their expression serves as a marker for the transformation and invasion capacity of SCC cells. In the same way, the amount of MMP-12-expressing malignant keratinocytes could correlate with the invasiveness of SCCs.

Also in SCCs of the vulva, both macrophages and epithelial cancer cells expressed MMP-12 mRNA. MMP-12-positive cancer cells were, however, only detected in grade II and III tumors, therefore correlating with histological aggressiveness and dedifferentiation of the tumors (P=0,0099). Interestingly, MMP-12 can degrade plasminogen into angiostatin, which can inhibit angiogenesis and thus probably slow down tumor growth (Dong et al, 1997; Cornelius et al, 1998; Dong et al, 1998). Therefore, in contrast to our results in two subtypes of SCCs, it could be hypothesized that more invasive tumors express less MMP-12. Indeed, the hepatocellular carcinoma and colon carcinoma patients whose tumors did not express MMP-12 mRNA produced less angiostatin and had less favorable prognoses than those whose tumors expressed high amounts of MMP-12 (Gorrin-Rivas et al, 1998; Yang et al, 2001). In fact, MMP-12 gene transfer has recently been implicated in gene therapy in animal models of melanoma and colon carcinoma (Gorrin-Rivas

et al, 2000b; Gorrin-Rivas et al, 2001). These discrepant results suggest that the role of MMP-12 in cancer progression is complex and may vary between tumor (or cell) types. This goes for other MMPs too. It is evident that the expression of certain MMPs is very different in the epithelial than in adenocarcinomas. Besides, Northern blot hybridization, without the examination of the cellular source of MMP-12 except in very few samples, was performed in the reports by Gorrin-Rivas *et al*. (1998) and Yang *et al*. (2001). Angiogenesis inhibiting effect of MMP-12 in cancer seems, however, convincing at least in mouse models. In agreement with the results of other groups, macrophage-derived MMP-12 mRNA expression was more abundant in grade I tumors than grade III tumors (P=0,01), implicating that the more MMP-12 positive macrophages there are, the less aggressive the behavior of the tumors is. However, the amount of signal for MMP-12, either in macrophages or cancer cells, did not significantly correlate with other clinicopathological characteristics, including lymph node metastasis or patient survival. Furthermore, macrophage-derived MMP-12 mRNA did not correlate with low blood vessel density, as might be expected, if MMP-12 generates angiostatin in these tumors. It is entirely possible that other angiostatin producing MMPs, including MMP-3, -7, and -9, which are also expressed in SCCs (Karelina et al, 1994; Airola et al, 1997), may contribute to this process. The sensivity of tumors to angiostatin *in vivo* may also differ.

The expression of MMPs is regulated by cytokines and growth factors secreted by both tumor and stromal cells. The regulation of MMP-12 has been studied mainly in macrophages, in which IL-1β, M-CSF, PDGF-BB, TNF-α and VEGF upregulated MMP-12 mRNA (Feinberg et al, 2000), while TGF-β1 inhibited this induction. Our present data indicate that MMP-12 is strongly upregulated by TGF-β1 in transformed keratinocytes (HaCaTs) and epithelial cells (MCF-10f). TGF-β1 is a multifunctional peptide that elicits many cellular events essential for cancer progression, including the modulation of angiogenesis and regulation of MMPs and other proteases (see Wright et al, 1993). Interestingly, it had biphasic action during skin carcinogenesis in mice, acting as a tumor suppressor at the early stages but later enhancing the malignant phenotype (Cui et al, 1996). TGF-β1 generally downregulates MMPs but may have the opposite effect depending on the cell type and state of transformation (Massague 1990). Also TNF-α induced MMP-12 mRNA in MCF-10f cells. TNF-α is a pleiotrophic anti-tumor and proinflammatory cytokine predominantly produced by macrophages (Fiers 1991), but it is also involved in processes that contribute to tumor progression. MMP-12 has been shown to have a capacity to process the TNF-α precursor (Gearing et al, 1994). However, MMP-12 transcription was not induced by TNF-α or TGF-β1 in the vulvar SCC cell line used in our Northern analysis (III, Figure 3). Although TNF-α and TGF-β1 generally enhanced MMP-13 expression in vulvar cancer cell lines, there was some variability in response to these factors, indicating that cell lines are heterogeneous (Johansson et al, 1999). Thus, TNF-α and TGF-β1 could still upregulate MMP-12 in SCCs *in vivo*, which is supported by the fact that both of them slightly enhanced the production of MMP-12 protein in cells from metastasis of cutaneous SCC as assessed by Western blot (II, Figure 3B). The induction of MMP-12 production with growth factors and cytokines happens through AP-1 site (Feinberg et al, 2000; Wu et al, 2000). Interestingly, ERK inhibitor PD90859 inhibited MMP-12 mRNA expression in vulvar SCC cells, while the inhibitor of p38 pathway, SB203580, had no effect (Figure 7, unpublished data). This implies that ERK1,2 pathway is involved in the regulation of MMP-12 transcription. Expression of MMP-1 and MMP-13 is dependent on p38 activity, in particular (Figure 7), which has also been shown in transformed keratinocytes (Johansson et al, 2000). Enhancement of MMP-9 expression was mediated by both p38 and ERK1,2 pathways (Johansson et al, 2000).

This and other studies, have pointed out some major roles for MMP-12. As originally suggested, it is involved in pathological conditions that involve excessive degradation of elastin, such as atherosclerosis, emphysema or solar elastosis (Halpert et al, 1996; Hautamäki et al, 1997), where it is secreted by macrophages recruited to the tissue. It is also involved in the macrophage-mediated

invasion of BM and matrix; these processes are impaired in MMP-12 deficient mice (Shipley et al, 1996b). In cancer, it may have a dual role. In cancer-associated macrophages it may function in host response and inhibit angiogenesis and tumor growth by generating angiostatin, most likely in the early phase of tumorigenesis. In cancer cells of SCC, it correlated with aggressiveness of the tumors and may actually promote ECM degradation and tumor growth in more invasive tumors, either directly or through MMP-activation cascades. Finally, it has a role during bone development, which may reflect its involvement in the regulation of capillary invasion and angiogenesis, degradation of matrix proteins to enable bone formation, or again, activation of other MMPs.

6.4. MMP-7 and MMP-9 in SCC of the vulva

Expression of MMP-7 mRNA in vulvar SCC cell lines has been previously shown, while its localization in these tumors hadn't been systematically studied (Johansson et al, 1999). The expression of MMP-7 in our vulvar SCCs seemed to concentrate in grade II and III tumors. Although MMP-7 was predominantly detected in carcinoma cells, in some samples it was also expressed in stromal cells, which were most likely macrophages (Busiek et al, 1995). It has been shown that integrin α1 knock-out mice have decreased vascularization due to angiostatin generated by MMP-7 and -9 (Pozzi et al, 2000). Although capable of generating angiostatin, MMP-7 clearly promotes tumor progression. Overexpression of MMP-7 led to enhanced tumorigenesis in the mouse breast cancer model (Rudolph-Owen et al, 1998) and MMP-7 deficient mice developed fewer tumors in the colon cancer model (Wilson et al, 1997). Its histological expression has previously been shown to correlate with an aggressive phenotype in many cancers, and it is predominantly found in tumor epithelium, e.g. in gastric and colorectal cancers, SCC of the skin, prostate cancer and breast cancer (McDonnell et al, 1991; Karelina et al, 1994; Heppner et al, 1996; Nelson et al, 2000). MMP-7 expression also correlates with poor prognosis in esophageal and colon carcinomas (Nelson et al, 2000; Salmela et al, 2001). It is overexpressed in colon adenocarcinomas, but also in benign polyps (Newell et al, 1994). Recently, MMP-7 was shown to induce angiogenesis *in vivo* in colon cancer model (Nishizuka et al, 2001). Thus, increased expression of MMPs may both confer invasiveness to the tumor cells and, paradoxically, lead to the production of molecules limiting their growth.

The regulation of MMP-7 in transformed keratinocytes has not been explored in much detail. It has been shown that TNF-α upregulates the expression of MMP-7 mRNA in vulvar SCC cell lines (Johansson et al, 1999). Similarly, our results show that TNF-α effectively induced the production of MMP-7 mRNA in HaCaTs (Figure 8, unpublished data). This has also been shown in mesangial cells (Marti et al, 1992). MMP-7 is also induced in premalignant intestinal epithelial cells by TNF-α and by IL-1β (Lopez-Boado et al, 2000). Instead, TNF-α was unable to induce MMP-7 in macrophages or in primary keratinocytes (Busiek et al, 1995; Lopez-Boado et al, 2000). Furthermore, TGF-β1 or other factors did not upregulate MMP-7 in HaCaTs or in vulvar SCC cell lines (Johansson et al, 1999), although TGF-β1 has been reported to induce it in the squamous cell line, II-4 (Borchers et al, 1997). Thus, the effect of different factors on MMPs clearly depends on the cell type.

Also MMP-9 expression has been described in SCC of the vulva (Johansson et al, 1999), where it co-localized with MMP-13 in some samples. Furthemore, MT1-MMP and MMP-2 colocalized with MMP-13 in these tumors, probably optimizing the conditions for cascade-like proteolysis, as they are known activators of proMMP-13, which in turn, can activate proMMP-9 (Knäuper et al, 1996c; Knäuper et al, 1997). In our studies of the MMP-9 expression pattern in a larger series of vulvar SCCs, it was mostly expressed in stromal neutrophils and macrophages. In histologically aggressive tumors it also appeared in epithelial cancer cells, resembling the pattern of MMP-12 to some extent. Stromal MMP-9 may perhaps have a role in host-defense, or angiostatin generation, although it has

been shown that MMP-9 supplied by reactive stromal cells also promoted skin carcinogenesis in a mouse model (Coussens et al, 2000). MMP-9 has been implicated in cancer invasion; it correlates with the malignant potential of many cancers and poor outcome of the patients (see Stetler-Stevenson et al, 1996; Nelson et al, 2000). Furthermore, activation of MMP-9 coincides with the 'angiogenic switch' in premalignant lesions during squamous epithelial carcinogenesis (Coussens et al, 1999) and MMP-9 promotes tumor angiogenesis in other mouse tumor models as well (Yu and Stamenkovich, 1999; Bergers et al, 2000b), despite its ability to generate angiostatin. Thus, findings on both MMP-7 and MMP-9 indirectly support our hypothesis of MMP-12 having a dual role in cancer depending on, for example, tumor type or stage of tumorigenesis.

6.5. MMP-10 and MMP-14 in skin cancer

MMP-10 has been considered to contribute to the invasive behavior of tumor cells. Prior to our study, there was practically no data concerning MMP-10 expression in human cancers *in vivo*, however. By *in situ* hybridization, MMP-3 and MMP-10 expression had been shown in tumor cells along disrupted BMs and in stromal cells in close contact to cancer cells in head and neck carcinoma (Polette et al, 1991). However, their probe did not make a difference between MMP-3 and -10. Furthermore, by Northern analysis, abundant expression of both stromelysin mRNAs has been shown in human lung and head and neck tumors with a more probable local invasiveness (Muller et al, 1991). According to our results, despite marked similarities in substrate specificity and structure, MMP-3 and MMP-10 were expressed with very distinct patterns, indicating different regulation and role in cancer. Although most MMPs are seen in cancer stroma, MMP-10 was expressed only by cancer cells in SCCs, as well as in BCCs. The epithelial origin of MMP-10 is further substantiated by novel results on Barrett`s adenocarcinomas, in which MMP-10 is only occasionally expressed in epithelial cells in conjunction with ulceration (Salmela et al, 2001). Instead, MMP-3 was more often expressed by stromal cells both in SCCs and BCCs and was clearly more abundant than MMP-10 in BCC. No signal for MMP-10 was detected in Bowen's disease or actinic keratoses, in agreement with data on MMP-3 (Tsukifuji et al, 1999). Differential expression patterns of MMP-3 and -10 have also been demonstrated in developing human bone (Bord et al, 1998), during cutaneous wound repair (Saarialho-Kere et al, 1994), and *in vitro*; human keratinocytes express MMP-10, while fibroblasts express MMP-3 (Windsor et al, 1993).

Previous studies have shown that the expression of MMP-10 is induced by phorbol ester, TGF-α and EGF in normal epidermal keratinocytes (Windsor et al, 1993) and by KGF, EGF, TNF-α and TGF-β1 in HaCaT keratinocytes (Madlener et al, 1996). Interestingly, most of the 43 SCC cell lines previously shown to be producing MMP-1 and -13 basally (Johansson et al, 1997c) were negative for MMP-10 mRNA. This suggests that malignant transformation *per se* is not enough to induce MMP-10, but rather inflammatory cytokines modify its expression. In our cell line from metastasis of cutaneous SCC (UT-SCC-7), TGF-α, KGF and TGF-β1 clearly upregulated MMP-10 transcription. IFN-γ in combination with TGF-β1 and TNF-α resulted in marked induction of MMP-10. Interestingly, the expression of two other MMPs relevant in cancer progression, MMP-1 and -13 are also upregulated by the same cytokines (Johansson et al, 1997c) but inhibited by IFN-γ (Ala-aho et al, 2000). Tumor-infiltrating lymphocytes in SCCs express IFN-γ, which can inhibit the proliferation of SCC cells. Furthermore, high expression of IFN-γ in SCCs correlates with favorable prognosis (Kim et al, 1995; Arany et al, 1997). This observation, in the context of our finding that IFN-γ stimulates SCC cell MMP-10 expression, suggests that MMP-10 is not involved in the invasion of SCC cells, not at least directly, but may rather be induced by several cytokines and growth factors functioning in ulcerative phenomena and inflammatory matrix remodeling associated with skin tumors.

In inactive tissues, laminin-5 is predominantly a static adhesive substrate, while in a changing environment it has a major role in epithelial cell motility. It aids the migration and invasion of tumor cells by establishing focal adhesions to the matrix (Pyke et al, 1995; Tani et al, 1996). Several studies on a variety of cancers have reported increased expression of laminin-5. For instance, γ2 chain of laminin-5 colocalizes with the invasive cells in cervical lesions and can serve as a marker for early invasion. It also correlates with poor prognosis in colon carcinoma and esophageal SCC (Skyldberg et al, 1999; Lenander et al, 2001; Yamamoto et al, 2001). However, the use of laminin-5 expression as a marker for the prognosis in skin cancers is complicated, since, also during wound healing, migrating keratinocytes express laminin-5, and cutaneous SCCs are often ulcerated. MMP-2 induces the migration of breast epithelial cells by cleaving and regulating the function of laminin-5 (Giannelli et al, 1997). Recent results suggest that expression of MT1-MMP is the primary trigger for migration over laminin-5, whereas MMP-2, activated by MT1-MMP, may play an additional role, perhaps by enhancing the MT1-MMP effects (Koshikawa et al, 2000). In this study, MMP-10 mRNA was expressed in SCCs by epithelial cells producing laminin-5, implicating a migratory status of these cells. However, laminin-5 was expressed more abundantly than MMP-10, particularly deeper in the invasive border of tumor islands. MT1-MMP and laminin-5 did not generally colocalize in the same cells, but MT1-MMP mRNA located in stromal cells next to laminin-5 expressing cancer cells. Thus, it is possible that either MT1-MMP or MMP-10, or both, might cleave laminin-5 during keratinocyte motility in skin cancer. MMP-2 protein did not colocalize with laminin-5 and apparently does not cleave it during skin carcinogenesis. Interestingly, TGF-β1 is able to induce both MMP-10 (Madlener et al, 1996) and laminin-5 in keratinocytes (Kainulainen et al, 1998).

MT-MMPs have an essential role in the pericellular activation cascades of MMPs. The activation of stromal proMMP-2 by MT1-MMP at the tumor cell surface is considered to be a critical event in cancer cell invasion (Sato et al, 1994; Knäuper et al, 1998). MT1-MMP mRNA has been detected both in cancer and stromal cells in various cancers (Okada et al, 1995; Gilles et al, 1996; Heppner et al, 1996; Ohtani et al, 1996; Mori et al, 1997; Harada et al, 1998). Our results are similar, since we found MT1-MMP mRNA expression both in cancer cells and in stromal fibroblasts surrounding SCCs. MT1-MMP-positive cancer cells were detected especially at the invasive edge of tumor islands. Although high levels of MT1-MMP expression have been shown to correlate with invasiveness in many cancer types, we found no significant difference in the intensity of MT1-MMP expression between poorly or well-differentiated cutaneous tumors. A dual role has been suggested for MT1-MMP too: its expression in cancer cell surface might relate to invasive growth, while in fibroblasts it may participate in tissue remodeling processes caused by invasive cancer cells (Ohtani et al, 1996). MT1-MMP can activate latent MMP-13 (Knäuper et al, 1996c) and, based on previous data (Airola et al, 1997), may be serving this function at the epithelial front. In contrast to SCCs, MT1-MMP expression was found generally in stromal fibroblasts surrounding tumor islands in BCCs. This suggests that MT1-MMP produced by fibroblasts in BCC might have another task than that produced by cancer cells in invasive SCCs. The limited invasion potential of BCC supports this result.

The expression of various MMPs and TIMPs in SCC and BCC of the skin *in vivo* is summarized in Table 6. Various MMPs might form a network, in which each MMP has a distinct role in the cleavage of 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 Kähäri and Saarialho-Kere, 1999; Curran and Murray, 2000). It seems that epithelial expression of MMPs is more common in SCCs than BCCs, which might be due to differences in the invasion capacity of these tumors. Low expression of TIMPs does not necessarily correlate with enhanced invasiveness, since malignant and metastatic tumors often express TIMPs *in vivo*. However, MMPs are usually

Table 6. Expression of MMPs and TIMPs in cutaneous SCCs and BCCs

	SCC		BCC	
MMP	**Epithelium**	**Stroma**	**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	-	+	+	+

expressed more abundantly, since overexpression of TIMPs can block tumor growth (Imren et al, 1996; Bian et al, 1996; Ahonen et al, 1998). Interestingly, MMP-12, MMP-9 and MT1-MMP were all expressed both in tumor and stromal cells in SCCs so that epithelial expression was more frequent in invasive tumors when compared to less aggressive SCCs (or BCCs).This suggests that individual MMPs may have different roles depending on a cell type or state of transformation. 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, such as regulatory or even protective role in host defence. The expanding knowledge on the role of MMPs in cancer suggests that their role is much more complex than initially thought.

7. SUMMARY AND CONCLUSIONS

Coordinated breakdown, synthesis and remodeling of the ECM are crucial events in normal physiological situations, such as embryonic development. On the other hand, excessive matrix degradation occurs in pathological conditions including tumor invasion and metastasis. Numerous studies have provided evidence for the importance of matrix metalloproteinases (MMPs) during these processes. In this study, we examined the expression and regulation of metalloelastase (MMP-12), stromelysin-2 (MMP-10), and matrilysin (MMP-7) in epithelial cancers. Furthermore, we studied the role of MMP-12 during human development, where a restricted expression of MMP-12 was detected in hypertrophic chondrocytes in developing bone. Of the bone lesions, MMP-12 was detected only in chondrosarcomas both in macrophages and chondrosarcoma cells. Its expression in cultured chondrosarcoma cells was induced in response to TNF-α stimulation.

MMP-12 expression was detected in BCCs and SCCs of the skin and vulva both in epithelial cancer cells and macrophages, as well as in SCC and transformed HaCaT cells in culture, whereas pre-malignant tumors and primary keratinocytes were negative. However, MMP-7 and -12 proteins were also upregulated in macrophages in areas of elastolysis in solar damage and actinic keratosis, a condition that can develop into SCC. In vulvar SCCs, MMP-12 mRNA was expressed in cancer cells only in histologically more aggressive grade II/III samples, thereby correlating with aggressiveness and dedifferentiation of the tumors. In contrast, macrophage-derived MMP-12 mRNA was most abundant in grade I tumors, thus predicting less aggressive behavior of the tumors. The amount of signal for MMP-12, regardless of its origin, did not significantly correlate with lymph node metastasis, amount of blood vessels or outcome of the patients. The production of MMP-12 was induced by TGF-β1 and TNF-α in cells from metastasis of cutaneous SCC as well as in epithelial MCF-10f cells, while only by TGF-β1 in HaCaTs. MMP-7 mRNA was mainly expressed by epithelial tumor cells and was strongly induced by TNF-α in HaCaT cells. MMP-10 mRNA was expressed in SCCs and BCCs only in epithelial laminin-5 positive cancer cells with a pattern totally different from its close homologue MMP-3. MMP-10 was upregulated in cutaneous SCC cells by TGF-α and KGF, and by IFN-γ in combination with TGF-β1 and TNF-α both in SCC and HaCaT cells.

In conclusion, this study shows for the first time that MMP-12 can be expressed *in vivo* and *in vitro* by transformed epithelial cells. The level of carcinoma derived MMP-12 correlates with histological aggressiveness, while that produced by macrophages predicts a less aggressive behavior of the tumors suggesting a dual role for MMP-12 in tumor progression. Our results suggest that MMP-12 mRNA expression in macrophages might serve as a new prognostic marker in SCC patients. MMP-10 expression does not seem to correlate with the invasive behavior of SCCs, but may be induced by the wound healing and inflammatory matrix remodeling events associated with skin tumors. Furthermore, MMP-12 plays an important role in ECM remodeling during fetal bone development and is induced when chondrocytes undergo malignant transformation.

Interactions between tumor and its microenvironment result in the production of degrading enzymes crucial for each step of tumor progression, including invasion, angiogenesis and metastasis. MMPs not only degrade ECM components in cancer, but it is now becoming evident that they actually have multiple roles in carcinogenesis. Although the number of MMPs and their expression levels are generally increasing along with cancer progression, the correlation between the levels of various MMPs and malignant potential of cancers clearly varies between tumor types. Thus, the actual role and contribution of individual MMP members to the development of different cancers still need to be better understood in order to get information on which MMPs could be used as prognostic markers in directing cancer therapy and, especially, on which MMPs would be the best targets for inhibition with minimal side effects.

8. ACKNOWLEDGEMENTS

This study was carried out at the Department of Dermatology, Helsinki University Central Hospital and Biomedicum Helsinki during the years 1998-2001. It could not have been completed without the help and support from many people. First of all, I would like to express my gratitude to professor Annamari Ranki for providing good research facilities within the Department.

My sincerest gratitude is owed to my supervisor, Docent Ulpu Saarialho-Kere, for introducing me to the field of MMPs and guiding me during my first years as a scientist. I deeply appreciate her devotion to my work and willingness for discussions. Her vast knowledge, enthusiasm, and efficiency are something to aim for.

Docent Ilmo Leivo and Professor Veli-Jukka Uitto are gratefully acknowledged for their constructive and supportive critisism on my thesis. I am also grateful to Selja Saarialho for the excellent work on revising the language of my manuscript.

I wish to thank all co-authors for their invaluable contribution, especially docent Veli-Matti Kähäri and Risto Ala-aho from the MediCity research laboratory in Turku for rewarding collaboration during many years. Doctors Arja-Leena Kariniemi, Leila Jeskanen, Pekka Klemi and Tom Böhling are greatly acknowledged for their irreplaceable expertise in pathology.

My warmest thanks are due to the past and present members of our research group; Alli, Maarit, Mikko, Oona, Sari, Katja and Ulla for being what they are; friendly, cheerful and supportive. Because of them, the atmosphere in the lab has been great and coming to work hardly ever unpleasant. Many legendary ideas have been developed during these years, some of them also scientific.. Hilarious moments have been shared also outside the laboratory. Special thanks go to Alli for skillful assistance, which goes beyond “normal” laboratory work and Maarit for guiding me in the beginning of my work.

I want to thank colleagues and ladies in the Histopathology and DNA laboratory, Department of Dermatology for their enjoyable company during lunch and coffee-breaks, for being interested in my work, and for being so nice and helpful throughout this process. Also all my friends, colleagues and other personnel from the Department of Dermatology, Haartman Institute and Biomedicum Helsinki are warmely thanked, especially Anu for sharing many delightful and strengthening lunch-breaks as well as sport events with me.

I wish to warmely thank Juha Kere and all the friendly people working in his lab for always making me feel welcome in their lab (and outside-lab activities) and patiently sharing the corner of the table with me, even when it was very crowded. It was important to be a part of that scientific community and to discuss scientific problems, as well as other things. I especially want to thank Siru, Kati P and Outi for sharing their knowledge and helping me with various problems and techniques. Mikko deserves warm thanks for his invaluable help with the computers as well as his friendship.

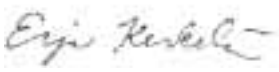
Special thanks and hugs go to Nina for her existence and for being my true friend. Our discussions about life, as well as science, have been crucial for my “spiritual well-being”.

I have been very fortuned to have such great friends around Finland, who I also want to thank for many relaxing and delightful moments and for having supportive attitude towards my research.

I am most grateful to my mother, sister Sari, Amira, and Jukka for their love and encouragement during my thesis work. Finally, I thank Marko for sharing everyday-life with me, for his love and understanding, in good times and bad. And for always having the faith in me, even when I didn´t.

This study was supported by grants from Helsinki University Central Hospital Research Foundation (EVO), Turku University Central Hospital, Academy of Finland, the Sigrid Jusélius Foundation, Finska Läkaresällskapet, and my personal grants from The Finnish Cultural Foundation, The Biomedicum Helsinki Foundation, the Cancer Research Foundation of Finland, University of Helsinki and The Maud Kuistila´s Foundation.

Helsinki, November 2001



9. REFERENCES

Abe, M, Kawamoto, K, Okamoto, H, Horiuchi, N: Induction of collagenase-2 (matrix metalloproteinase-8) gene expression by interleukin-1beta in human gingival fibroblasts. J Periodontal Res (2001) **36**: 153-159.

Agnihotri, R, Crawford, HC, Haro, H, Matrisian, LM, 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.

Ahmad, A, Marshall, JF, Basset, P, Anglard, P, Hart, IR: Modulation of human stromelysin 3 promoter activity and gene expression by human breast cancer cells. Int J Cancer (1997) **73**: 290-296.

Ahonen, M, Baker, AH, Kähäri, VM: Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells**.** Cancer Res (1998) **58**: 2310-2315.

Airola, K, Johansson, N, Kariniemi, AL, Kähäri,VM, Saarialho-Kere, UK: Human collagenase-3 is expressed in malignant squamous epithelium of the skin. J Invest Dermatol. (1997) **109**: 225-231.

Airola, K, Ahonen, M, Johansson, N, Heikkilä, P, Kere, J, Kähäri, VM, Saarialho-Kere, UK: Human TIMP-3 is expressed during fetal development, hair growth cycle, and cancer progression. J Histochem Cytochem (1998) **46**: 437-447.

Airola, K, Karonen, T, Vaalamo, M, Lehti, K, Lohi, J, Kariniemi, AL, Keski-Oja, J, Saarialho-Kere, UK: Expression of collagenases-1 and -3 and their inhibitors TIMP-1 and -3 correlates with the level of invasion in malignant melanomas. Br J Cancer (1999) **80**: 733-743.

Ala-aho, R, Johansson, N, Grénman, R, Fusenig, NE, López-Otín, C, Kähäri,VM: Inhibition of collagenase-3 (MMP-13) expression in transformed human keratinocytes by interferon-γ is associated with activation of extracellular signal-regulated kinase-1,2 and STAT1. Oncogene (2000) **19**: 248-257.

Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K, Watson, JD: The extracellular matrix of animals. In: *Molecular Biology of the Cell.* Garland Publishing, New York, USA. (1994a), pp. 971-995.

Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K, Watson, JD: Differentiated cells and the maintenance of the tissues. In: *Molecular Biology of the Cell.* Garland Publishing, New York, USA. (1994b), p. 1140.

Anglard, P, Melot, T, Guerin, E, Thomas, G, Basset, P: Structure and promoter characterization of the human stromelysin-3 gene. J Biol Chem (1995) **270**: 20337-20344.

Arany, I, Fleischmann,CM, Tyring, SK, Fleischmann, WR Jr: Lack of mda-6/WAF1/CIP1-mediated inhibition of cyclin-dependent kinases in interferon-alpha resistant murine B16 melanoma cells. Cancer Lett (1997) **119**: 237-240.

Archer, C: Functions of the skin. In: Champion, R., Burton, JL, Burns, DA, Breathnach, SM (Eds.) *Textbook of Dermatology.* Blackwell Science, Oxford. (1998), pp. 113-122.

Armstrong, P, Armstrong, MT: Intercellular invasion and the organizational stability of tissues: a role for fibronectin. Biochim Biophys Acta (2000) **1470**: 9-20.

Asch, P, Basset, P, Roos, M, Grosshans, E, Bellocq, JP, Cribier, B: Expression of stromelysin 3 in keratoacanthoma and squamous cell carcinoma. Am J Dermatopathol (1999) **21**: 146-150.

Ashworth, J, Murphy, G, Rock,MJ, Sherratt, MJ, Shapiro, SD, Shuttleworth, CA, Kielty, CM: Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodelling. Biochem J (1999) **340**: 171-181.

Aumailley, M, Gayraud, B: Structure and biological activity of the extracellular matrix. J Mol Med (1998) **76**: 253-265.

Baker, A, Zaltsman, AB, George, SJ, Newby, AC: Divergent effects of tissue inhibitor of metalloproteinase-1, -2, or -3 overexpression on rat vascular smooth muscle cell invasion, proliferation, and death in vitro. TIMP-3 promotes apoptosis. J Clin Invest (1998) **101**: 1478-1487.

Balbín, M, Pendas, AM, Uria, JA, Jimenez, MG, Freije, JP, Lopez-Otin, C: Expression and regulation of collagenase-3 (MMP-13) in human malignant tumors. APMIS (1999) **107**: 45-53.

Baragi, V, Fliszar, CJ, Conroy, MC, Ye, QZ, Shipley, JM, Welgus, HG: Contribution of the C-terminal domain of metalloproteinases to binding by tissue inhibitor of metalloproteinases. C-terminal truncated stromelysin and matrilysin exhibit equally compromised binding affinities as compared to full-length stromelysin. J Biol Chem (1994) **269**: 12692-12697.

Basset, P, Bellocq, JP, Wolf, C, Stoll, I, Hutin, P, Limacher, JM, Podhajcer, OL, Chenard, MP, Rio, MC, Chambon, P: A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature (1990) **348**: 699-704.

Belkin, A, Akimov, SS, Zaritskaya, LS, Ratnikov, BI, Deryugina, EI, Strongin, AY: Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. J Biol Chem (2001) **276**: 18415-18422.

Benbow, U, Brinckerhoff, CE: The AP-1 site and MMP gene regulation: what is all the fuss about? Matrix Biol (1997) **15**: 519-526.

Bergers, G, Coussens, LM: Extrinsic regulators of epithelial tumor progression: metalloproteinases. Curr Opin Genet Dev (2000) **10**: 120-127.

Bergers, G, Brekken, R, McMahon, G, Vu, TH, Itoh, T, Tamaki, K, Tanzawa, K, Thorpe, P, Itohara, S, Werb, Z, Hanahan, D: Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol (2000) **2**: 737-744.

Bernstein, E, Chen, YQ, Tamai, K, Shepley, KJ, Resnik, KS, Zhang, H, Tuan, R, Mauviel, A, Uitto, J: Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. J Invest Dermatol (1994) **103**: 182-186.

Bernstein, E, Fisher, LW, Li, K, LeBaron, RG, Tan, EM, Uitto, J: Differential expression of the versican and decorin genes in photoaged and sun-protected skin. Comparison by immunohistochemical and northern analyses. Lab Invest (1995a) **72**: 662-669.

Bernstein, E, Brown, DB, Urbach, F, Forbes, D, Del Monaco, M, Wu, M, Katchman, SD, Uitto, J: Ultraviolet radiation activates the human elastin promoter in transgenic mice: a novel in vivo and in vitro model of cutaneous photoaging. J Invest Dermatol (1995b) **105**: 269-273.

Bernstein, E, Chen, YQ, Kopp, JB, Fisher, L, Brown, DB, Hahn, PJ, Robey, FA, Lakkakorpi, J, Uitto, J: Long-term sun exposure alters the collagen of the papillary dermis. Comparison of sun-protected and photoaged skin by northern analysis, immunohistochemical staining, and confocal laser scanning microscopy. J Am Acad Dermatol (1996a) **34**: 209-218.

Bernstein, S, Lim, KK, Brodland, DG: The many faces of squamous cell carcinoma. Dermatol Surg (1996b) **22**: 243-254.

Bian, J, Wang, Y, Smith, MR, Kim, H, Jacobs, C, Jackman, J, Kung, HF, Colburn, NH, Sun, Y: Suppression of in vivo tumor growth and induction of suspension cell death by tissue inhibitor of metalloproteinases (TIMP)-3. Carcinogenesis (1996) **17**: 1805-1811.

Bigg, H, Shi, YE, Liu, YE, Steffensen, B, Overall, CM: Specific, high affinity binding of tissue inhibitor of metalloproteinases-4 (TIMP-4) to the COOH-terminal hemopexin-like domain of human gelatinase A. TIMP-4 binds progelatinase A and the COOH-terminal domain in a similar manner to TIMP-2. J Biol Chem (1997) **272**: 15496-15500.

Birkedal-Hansen, H, Moore, WG, Bodden, MK, Windsor, LJ, Birkedal-Hansen, B, DeCarlo, A, Engler, A: Matrix metalloproteinases: a review. Crit Rev Oral Biol Med (1993) **4**: 197-250.

Birkedal-Hansen, H: Proteolytic remodeling of extracellular matrix. Curr Opin Cell Biol (1995) **7**: 728-735.

Biswas, C, Zhang, Y, DeCastro, R, Guo, H, 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.

Borchers, A, Steinbauer, H, Schafer, BS, Kramer, M, Bowden, GT, Fusenig, NE: Fibroblast-directed expression and localization of 92-kDa type IV collagenase along the tumor-stroma interface in an in vitro three-dimensional model of human squamous cell carcinoma. Mol Carcinog (1997) **19**: 258-266.

Bord, S, Horner, A, Hembry, RM, Compston, JE: Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) expression in developing human bone: potential roles in skeletal development. Bone (1998) **23**: 7-12.

Borradori, L, Sonnenberg, A: Structure and function of hemidesmosomes: more than simple adhesion complexes. J Invest Dermatol (1999) **112**: 411-418.

Boukamp, P, Petrussevska, RT, Breitkreutz, D, Hornung, J, Markham, A, Fusenig, NE: Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol (1988) **106**: 761-771.

Boulay, A, Masson, R, Chenard, MP, El Fahime, M, Cassard, L, Bellocq, JP, Sautes-Fridman, C, Basset, P, Rio, MC: High cancer cell death in syngeneic tumors developed in host mice deficient for the stromelysin-3 matrix metalloproteinase. Cancer Res (2001) **61**: 2189-2193.

von Bredow, D, Nagle, RB, Bowden, GT, Cress, AE: Cleavage of beta 4 integrin by matrilysin. Exp Cell Res (1997) **236**: 341-345.

Brew, K, Dinakarpandian, D, Nagase, H: Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biopys Acta (2000) **1477**: 267-283.

Brooks, P, Silletti, S, von Schalscha, TL, Friedlander, M, Cheresh, DA: Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. Cell (1998) **92**: 391-400.

Brown, P: Clinical studies with matrix metalloproteinase inhibitors. APMIS (1999) **107**: 174-180.

Burgeson, R, Christiano,AM: The dermal-epidermal junction. Curr Opin Cell Biol (1997) **9**: 651-658.

Busiek, D, Baragi, V, Nehring, LC, Parks, WC, Welgus, HG: Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones. J Immunol (1995) **154**: 6484-6491.

Butler, G, Apte, SS, Willenbrock, F, Murphy, G: Human tissue inhibitor of metalloproteinases 3 interacts with both the N- and C-terminal domains of gelatinases A and B. Regulation by polyanions. J Biol Chem (1999) **274**: 10846-10851.

Carmeliet, P, Moons, L, Lijnen, R, Baes, M, Lemaitre, V, Tipping, P, Drew, A, Eeckhout, Y, Shapiro, S, Lupu, F, Collen, D: Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet (1997) **17**: 439-444.

Chan, LS: Human skin basement membrane in health and in autoimmune diseases. Front Biosci (1997) **2**: d343-352.

Chandler, S, Cossins, J, Lury, J, Wells, G: Macrophage metalloelastase degrades matrix and myelin proteins and processes a tumour necrosis factor-alpha fusion protein. Biochem Biophys Res Commun (1996) **228**: 421-429.

Chandler, S, Miller, KM, Clements, JM, Lury, J, Corkill, D, Anthony, DC, Adams, SE, Gearing, JA: Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. J Neuroimmunol (1997) **72**: 155-161.

Chapman, H, Riese, RJ, Shi, GP: Emerging roles for cysteine proteases in human biology. Annu Rev Physiol (1997) **59**: 63-88.

Chomczynski, P, Sacchi, N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem (1987) **162**: 156-159.

Cole, A, Chubinskaya, S, Schumacher, B, Huch, K, Szabo, G, Yao, J, Mikecz, K, Hasty, KA, Kuettner, KE: Chondrocyte matrix metalloproteinase-8. Human articular chondrocytes express neutrophil collagenase. J Biol Chem (1996) **271**: 11023-11026.

Cornelius, L, Nehring, LC, Harding, E, Bolanowski, M, Welgus, HG, Kobayashi, DK, Pierce, RA, Shapiro, SD: Matrix metalloproteinases generate angiostatin: effects on neovascularization. J Immunol (1998) **161**: 6845-6852.

Cossins, J, Dudgeon, TJ, Catlin, G, Gearing, AJ, Clements, JM: Identification of MMP-18, a putative novel human matrix metalloproteinase. Biochem Biophys Res Commun (1996) **228**: 494-498.

Coussens, L, Raymond, WW, Bergers, G, Laig-Webster, M, Behrendtsen, O, Werb, Z, Caughey, GH, Hanahan, D: Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev (1999) **13**: 1382-1397.

Coussens, L, Tinkle, CL, Hanahan, D, Werb, Z: MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell (2000) **103**: 481-490.

Coussens, L, Werb, Z: Inflammatory cells and cancer: think different! J Exp Med (2001) **193**: F23-26.

Crawford, H, Matrisian, LM: Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. Enzyme Protein (1996) **49**: 20-37.

Crawford, H, Fingleton, B, Gustavson, MD, Kurpios, N, Wagenaar, RA, Hassell, JA, Matrisian, LM: The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrilysin transcription in intestinal tumors. Mol Cell Biol (2001) **21**: 1370-1383.

Cui, W, Fowlis, DJ, Bryson, S, Duffie, E, Ireland, H, Balmain, A, Akhurst, RJ: TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. Cell (1996) **86**: 531-542.

Curci, J, Liao, S, Huffman, MD, Shapiro, SD, Thompson, RW: Expression and localization of macrophage elastase (matrix metalloproteinase-12) in abdominal aortic aneurysms. J Clin Invest (1999) **102**: 1900-1910.

Curran, S, Murray, GI: Matrix metalloproteinases: molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer (2000) **36**: 1621-1630.
D'Angelo, M, Yan, Z, Nooreyazdan, M, Pacifici, M, Sarment, DS, Billings, PC, Leboy, PS: MMP-13 is induced during chondrocyte hypertrophy. J Cell Biochem (2000) **77**: 678-693.
D'Armiento, J, DiColandrea, T, Dalal, SS, Okada, Y, Huang, MT, Conney, AH, Chada, K: Collagenase expression in transgenic mouse skin causes hyperkeratosis and acanthosis and increases susceptibility to tumorigenesis. Mol Cell Biol (1995) **15**: 5732-5739.
de Coignac, A, Elson, G, Delneste, Y, Magistrelli, G, Jeannin, P, Aubry, JP, Berthier, O, Schmitt, D, Bonnefoy, JY, Gauchat, JF: Cloning of MMP-26. A novel matrilysin-like proteinase. Eur J Biochem (2000) **267**: 3323-3329.
Debelle, L, Tamburro, AM: Elastin: molecular description and function. Int J Biochem Cell Biol (1999) **31**: 261-272.
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.
Dong, Z, Kumar, R, Yang, X, Fidler, IJ: Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell (1997) **88**: 801-810.
Dong, Z, Yoneda, J, Kumar, R, Fidler, IJ: Angiostatin-mediated suppression of cancer metastases by primary neoplasms engineered to produce granulocyte/macrophage colony-stimulating factor. J Exp Med (1998) **188**: 755-763.
d'Ortho, M, Will, H, Atkinson, S, Butler, G, Messent, A, Gavrilovic, J, Smith, B, Timpl, R, Zardi, L, Murphy, G: Membrane-type matrix metalloproteinases 1 and 2 exhibit broad-spectrum proteolytic capacities comparable to many matrix metalloproteinases. Eur J Biochem (1997) **250**: 751-757.
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.
Dunsmore, S, Saarialho-Kere, UK, Roby, JD, Wilson, CL, Matrisian, LM, Welgus, HG, Parks, WC: Matrilysin expression and function in airway epithelium. J Clin Invest (1998) **102**: 1321-1331.
Eckert, R: Structure, function, and differentiation of the keratinocyte. Physiol Rev (1989) **69**: 1316-1346.
Edwards, D, Beaudry, PP, Laing, TD, Kowal, V, Leco, KJ, Leco, PA, Lim, MS: The roles of tissue inhibitors of metalloproteinases in tissue remodelling and cell growth.1996 Mar;20 Suppl 3:S9-15. Review. Int J Obes Relat Metab Disord (1996) .
Ellis, L, Fidler, IJ: Angiogenesis and metastasis. Eur J Cancer (1996) **32A**: 2451-2460.
English, W, Puente, XS, Freije, JM, Knäuper, V, Amour, A, Merryweather, A, Lopez-Otin, C, Murphy, G: 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.
English, W, Velasco, G, Stracke, JO, Knäuper, V, Murphy, G: Catalytic activities of membrane-type 6 matrix metalloproteinase (MMP25). FEBS Lett (2001) **491**: 137-142.
Etoh, T, Inoue, H, Yoshikawa, Y, Barnard, GF, 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.
Feinberg, M, Jain, MK, Werner, F, Sibinga, NE, Wiesel, P, Wang, H, Topper, JN, Perrella, MA, Lee, ME: Transforming growth factor-beta 1 inhibits cytokine-mediated induction of human metalloelastase in macrophages. J Biol Chem (2000) **275**: 25766-25773.
Fiers, W: Tumor necrosis factor. Characterization at the molecular, cellular and in vivo level. FEBS Lett (1991) **285**: 199-212.
Filsell, W, Rudman, S, Jenkins, G, Green, MR: Coordinate upregulation of tenascin C expression with degree of photodamage in human skin. Br J Dermatol (1999) **140**: 592-599.
Fingleton, B, Heppner Goss KJ, Crawford, HC, Matrisian, LM: Matrilysin in early stage intestinal tumorigenesis. APMIS (1999) **107**: 102-110.
Fisher, G, Datta, SC, Talwar, HS, Wang, ZQ, Varani, J, Kang, S, Voorhees, JJ: Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature (1996) **379**: 335-339.
Fisher, G, Voorhees, JJ: Molecular mechanisms of photoaging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin in vivo. J Investig Dermatol Symp Proc (1998) **3**: 61-68.
Foda, H, Zucker, S: Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. Drug Discov Today (2001) **6**: 478-482.
Folkman, J: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med (1995) **1**: 27-31.
Fort, P, Marty, L, Piechaczyk, M, El Sabrouty, S, Dani, C, Jeanteur, P, Blanchard, JM: Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res (1985) **13**: 1431-1441.
Fortunato, S, Menon, R, Lombardi, SJ: Expression of a progelatinase activator (MT1-MMP) in human fetal membranes. Am J Reprod Immunol (1998) **39**: 316-322.
Freije, J, Diez-Itza, I, Balbin, M, Sanchez, LM, Blasco, R, Tolivia, J, Lopez-Otin, C: Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J Biol Chem (1994) **269**: 16766-16773.
Gearing, A, Beckett, P, Christodoulou, M, Churchill, M, Clements, J, Davidson, AH, Drummond, AH, Galloway, WA, Gilbert, R, Gordon, JL, et al: Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature (1994) **370**: 555-557.
Giambernardi, T, Grant, GM, Taylor, GP, Hay, RJ, Maher, VM, McCormick, JJ, Klebe, RJ: Overview of matrix metalloproteinase expression in cultured human cells. Matrix Biol (1998) **16**: 483-496.
Giancotti, F, Ruoslahti, E: Integrin signaling. Science (1999) **285**: 1028-1032.
Giannelli, G, Falk-Marzillier, J, Schiraldi, O, Stetler-Stevenson, WG, Quaranta, V: Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science (1997) **277**: 225-228.
Gilles, C, Polette, M, Piette, J, Munaut, C, Thompson, EW, Birembaut, P, Foidart, JM: High level of MT-MMP expression is associated with invasiveness of cervical cancer cells. Int J Cancer (1996) **65**: 209-213.

Giudice, G, Emery, DJ, Diaz, LA: Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. J Invest Dermatol (1992) **99**: 243-250.

Goldberg, G, Wilhelm, SM, Kronberger, A, Bauer, EA, Grant, GA, Eisen, AZ: Human fibroblast collagenase. Complete primary structure and homology to an oncogene transformation-induced rat protein. J Biol Chem (1986) **261**: 6600-6605.

Goldberg, G, Marmer, BL, Grant, GA, Eisen, AZ, Wilhelm, S, He, CS: Human 72-kilodalton type IV collagenase forms a complex with a tissue inhibitor of metalloproteases designated TIMP-2. Proc Natl Acad Sci U S A (1989) **86**: 8207-8211.

Goldberg, G, Strongin, A, Collier,IE, Genrich, LT, Marmer, BL: 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.

Goldman, G: Squamous Cell Carcinoma. In: Taylor RS, W.M., Farley M, Gelfand JM, Elston DM *eMedicine Journal, Dermatology, Malignant Neoplasms*. © Copyright 2001, eMedicine.com, Inc. (2001).

Gomez, D, Alonso, DF, Yoshiji, H, Thorgeirsson, UP: Tissue inhibitors of metalloproteinases: structure, regulation and biological function. Eur J Cell Biol (1997) **74**: 111-122.

Gomis-Rüth, F, Maskos, K, Betz, M, Bergner, A, Huber, R, Suzuki, K, Yoshida, N, Nagase, H, Brew, K, Bourenkov, GP, Bartunik, H, Bode, W: Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. Nature (1997) **389**: 77-81.

Gorrin-Rivas, M, Arii, S, Furutani, M, Harada, T, Mizumoto, M, Nishiyama, H, Fujita, J, Imamura, M: Expression of human macrophage metalloelastase gene in hepatocellular carcinoma: Correlation with angiostatin generation and its clinical significance. Hepatology (1998) **28**: 986-993.

Gorrin-Rivas, M, Arii, S, Furutani, M, Mizumoto, M, Mori, A, Hanaki, K, Maeda, M, Furyama, H, Kondo, Y, Imamura, M: Mouse macrophage metalloelastase gene transfer into a murine melanoma suppresses primary tumor growth by halting angiogenesis. Clin Cancer Res (2000b) **6**: 1647-1654.

Gorrin-Rivas, M, Arii, S, Mori, A, Kaneda, Y, Imamura, M: Mouse macrophage metalloelastase gene delivery by HVJ-cationic liposomes in experimental antiangiogenic gene therapy for murine CT-26 colon cancer. Int J Cancer (2001) **93**: 731-735.

Grant, G, Giambernardi, TA, Grant, AM, Klebe, RJ: Overview of expression of matrix metalloproteinases (MMP-17, MMP-18, and MMP-20) in cultured human cells. Matrix Biol (1999) **18**: 145-148.

Gray, S, Wilkins, RJ, Yun, K: Interstitial collagenase gene expression in oral squamous cell carcinoma. Am J Pathol (1992) **141**: 301-306.

Greene, J, Wang, M, Liu, YE, Raymond, LA, Rosen, C, Shi, YE: Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem (1996) **27**: 30375-30380.

Gronski, TJ, Martin, RL, Kobayashi, DK, Walsh, BC, Holman, MC, Huber, M, Van Wart, HE, Shapiro, SD: Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage metalloelastase. J Biol Chem (1997) **272**: 12189-12194.

Gross, J, Lapière, CM: Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc Natl Acad Sci USA (1962) **48**: 1014-1022.

Halpert, I, Sires, UI, Roby, JD, Potter-Perigo, S, Wight, TN, Shapiro, SD, Welgus, HG, Wickline, SA, Parks, WC: 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.

Hanahan, D, Folkman, J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell (1996) **86**: 353-364.

Hanemaaijer, R, Sorsa, T, Konttinen, YT, Ding, Y, Sutinen, M, Visser, H, van Hinsbergh, VW, Helaakoski, T, Kainulainen, T, Ronka, H, Tschesche, H, Salo, T: 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.

Harada, T, Arii, S, Mise, M, Imamura, T, Higashitsuji, H, Furutani, M, Niwano, M, Ishigami, S, Fukumoto, M, Seiki, M, Sato, H, Imamura, M: Membrane-type matrix metalloproteinase-1 (MT1-MTP) gene is overexpressed in highly invasive hepatocellular carcinomas. J Hepatol (1998) **28**: 231-239.

Hasan, T, Nyberg, F, Stephansson, E, Puska, P, Häkkinen, M, Sarna, S, Ros, AM, Ranki, A: Photosensitivity in lupus erythematosus, UV photoprovocation results compared with history of photosensitivity and clinical findings. Br J Dermatol (1997) **135**: 699-705.

Hasegawa, S, Koshikawa, N, Momiyama, N, Moriyama, K, Ichikawa, Y, Ishikawa, T, Mitsuhashi, M, Shimada, H, Miyazaki, K: Matrilysin-specific antisense oligonucleotide inhibits liver metastasis of human colon cancer cells in a nude mouse model. Int J Cancer (1998) **76**: 812-816.

Hasty, K, Hibbs, MS, Kang, AH, Mainardi, CL: Secreted forms of human neutrophil collagenase. J Biol Chem (1986) **261**: 5645-5650.

Hasty, K, Jeffrey, JJ, Hibbs, MS, Welgus, HG: The collagen substrate specificity of human neutrophil collagenase. J Biol Chem (1987) **262**: 10048-10052.

Hasty, K, Pourmotabbed, TF, Goldberg, GI, Thompson, JP, Spinella, DG, Stevens, RM, Mainardi, CL: Human neutrophil collagenase. A distinct gene product with homology to other matrix metalloproteinases. J Biol Chem (1990) **265**: 11421-11424.

Hattori, T, Kubota, S, Yutani, Y, Fujisawa, T, Nakanishi, T, Takahashi, K, Takigawa, M: Change in cellular localization of a rheumatoid arthritis-related antigen (RA-A47) with downregulation upon stimulation by inflammatory cytokines in chondrocytes. J Cell Physiol (2001) **186**: 168-181.

Hautamäki, R, Kobayashi, DK, Senior, RM, Shapiro, SD: Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. Science (1997) **277**: 2002-2004.

Henriet, P, Blavier, L, Declerck, YA: Tissue inhibitors of metalloproteinases (TIMP) in invasion and proliferation. APMIS (1999) **107**: 111-119.

Heppner, K, Lynn, MM, Jensen, RA, Rodgers, WH: Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol (1996) **149**: 273-282.

Hernandez-Barrantes, S, Shimura, Y, Soloway, PD, Sang, QA, Fridman, R: Differential roles of TIMP-4 and TIMP-2 in pro-MMP-2 activation by MT1-MMP. Biochem Biophys Res Commun (2001) **281**: 126-130.

Hidalgo, M, Eckhardt, SG: Matrix metalloproteinase inhibitors: how can we optimize their development? Ann Oncol (2001) **12**: 285-287.

Hiller, O, Lichte, A, Oberpichler, A, Kocourek, A, Tschesche, H: Matrix Metalloproteinases Collagenase-2, Macrophage Elastase, Collagenase-3, and Membrane Type 1-Matrix Metalloproteinase Impair Clotting by Degradation of Fibrinogen and Factor XII. J Biol Chem (2000) **275**: 33008-33013.

Hiraoka, N, Allen, E, Apel, IJ, Gyetko, MR, Weiss, SJ: Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell (1998) **95**: 365-377.

Hoegl, L, Korting, HC, Klebe, G: Inhibitors of aspartic proteases in human diseases: molecular modeling comes of age. Pharmazie (1999) **54**: 319-329.

Holmbeck, K, Bianco, P, Caterina, J, Yamada, S, Kromer, M, Kuznetsov, SA, Mankani, M, Robey, PG, Poole, AR, Pidoux, I, Ward, JM, Birkedal-Hansen, H: MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell (1999) **99**: 81-92.

Illman, SA, Keski-Oja, J, Lohi, J: Promoter characterization of the human and mouse epilysin (MMP-28) genes. Gene (2001) **275**:185-194.

Imai, K, Hiramatsu, A, Fukushima, D, Pierschbacher, MD, Okada, Y: Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. Biochem J (1997) **322**: 809-814.

Imamura, T, Ohshio, G, Mise, M, Harada, T, Suwa, H, Okada, N, Wang, Z, Yoshitomi, S, Tanaka, T, Sato, H, Arii, S, Seiki, M, Imamura, M: Expression of membrane-type matrix metalloproteinase-1 in human pancreatic adenocarcinomas. J Cancer Res Clin Oncol (1998) **124**: 65-72.

Imren, S, Kohn, DB, Shimada, H, Blavier, L, DeClerck, YA: Overexpression of tissue inhibitor of metalloproteinases-2
retroviral-mediated gene transfer in vivo inhibits tumor growth and invasion. Cancer Res (1996) **56**: 2891-2895.

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.

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.

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.

Janusz, M, Hare, M, Durham, SL, Potempa, J, McGraw, W, Pike, R, Travis, J, Shapiro, SD: Cartilage proteoglycan degradation by a mouse transformed macrophage cell line is mediated by macrophage metalloelastase. Inflamm Res (1999) **48**: 280-288.

Johansson, N, Westermarck, J, Leppä, S, Häkkinen, L, Koivisto, L, Lopez-Otin, C, Peltonen, J, Heino, J, Kähäri, VM: Collagenase-3 (MMP-13) gene expression by HaCaT keratinocytes is enhanced by tumor necrosis factor-α and transforming growth factor-β. Cell Growth Differ (1997a) **8**: 243-250.

Johansson, N, Saarialho-Kere, U, Airola, K, Herva, R, Nissinen, L, Westermarck, J, Vuorio, E, Heino, J, Kähäri, VM: Collagenase -3 (MMP-13) is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts during human fetal bone development. Dev Dyn (1997b) **208**: 387-397.

Johansson, N, Airola, K, Grénman, R, Kariniemi, AL, Saarialho-Kere, U, Kähäri, VM: Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. Am J Pathol (1997c) **15**: 499-508.

Johansson, N, Vaalamo, M, Grénman, S, Hietanen, S, Klemi, P, Saarialho-Kere, U, Kähäri, VM: Collagenase-3 (MMP-13) is expressed by tumor cells in invasive vulvar squamous cell carcinomas. Am J Pathol (1999) **154**: 469-480.

Johansson, N, Kähäri, VM: Matrix metalloproteinases in squamous cell carcinoma. Histol Histopathol (2000) **15**: 225-237.

Johansson, N, Ala-aho, R, Uitto, V, Grenman, R, Fusenig, NE, Lopez-Otin, C, Kähäri, VM: Expression of collagenase-3 (MMP-13) and collagenase-1 (MMP-1) by transformed keratinocytes is dependent on the activity of p38 mitogen-activated protein kinase. J Cell Sci (2000) **113**: 227-235.

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.

Jones, F, Jones, PL: The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn (2000) **218**: 235-259.

Jormsjo, S, Ye, S, Moritz, J, Walter, DH, Dimmeler, S, Zeiher, AM, Henney, A, Hamsten, A, Eriksson, P: Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circ Res (2000) **86**: 998-1003.

Joseph, I, Isaacs, JT: Macrophage role in the anti-prostate cancer response to one class of antiangiogenic agents. J Natl Cancer Inst. (1998) **90**: 1648-1653.

Kainulainen, T, Häkkinen, L, Hamidi, S, Larjava, K, Kallioinen, M, Peltonen, J, Salo, T, Larjava, H, Oikarinen, A: Laminin-5 expression is independent of the injury and the microenvironment during reepithelialization of wounds. J Histochem Cytochem (1998) **46**: 353-360.

Kajita, M, Kinoh, H, Ito, N, Takamura, A, Itoh, Y, Okada, A, Sato, H, Seiki, M: Human membrane type-4 matrix metalloproteinase (MT4-MMP) is encoded by a novel major transcript: isolation of complementary DNA clones for human and mouse mt4-mmp transcripts. FEBS Lett (1999) **457**: 353-356.

Karelina, T, Hruza, GJ, Goldberg, GI, Eisen, AZ: Localization of 92-kDa type IV collagenase in human skin tumors: comparison with normal human fetal and adult skin. J Invest Dermatol (1993) **100**: 159-165.

Karelina, T, Goldberg, GI, Eisen, AZ: Matrilysin (PUMP) correlates with dermal invasion during appendageal development and cutaneous neoplasia. J Invest Dermatol (1994) **103**: 482-487.

Karelina, T, Goldberg, GI, Eisen, AZ: Matrix metalloproteinases in blood vessel development in human fetal skin and in cutaneous tumors. J Invest Dermatol (1995) **105**: 411-417.

Karelina, T, Bannikov, GA, Eisen, AZ: Basement membrane zone remodeling during appendageal development in human fetal skin. The absence of type VII collagen is associated with gelatinase-A (MMP2) activity. J Invest Dermatol (2000) **114**: 371-375.

Karin, M, Liu, ZG, Zandi, E: AP-1 function and regulation. Curr Opin Cell Biol (1997) **9**: 240-246.

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.

Kawamata, H, Uchida, D, Hamano, H, Kimura-Yanagawa, T, Nakashiro, KI, Hino, S, Omotehara, F, Yoshida, H, Sato, M: Active-MMP2 in cancer cell nests of oral cancer patients: correlation with lymph node metastasis. Int J Oncol (1998) **13**: 699-704.

Kawashima, A, Okada, Y, Nakanishi, I, Ueda, Y, Iwata, K, Roessner, A: Immunolocalization of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human chondrosarcomas. Gen Diagn Pathol (1997) **142**: 129-137.

Kim, J, Modlin, RL, Moy, RL, Dubinett, SM, McHugh, T, Nickoloff, BJ, Uyemura, K: IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T cell immune response. J Immunol (1995) **155**: 2240-2247.

Kligman, L, Akin, FJ, Kligman, AM: Prevention of ultraviolet damage to the dermis of hairless mice by sunscreens. J Invest Dermatol (1982) **78**: 181-189.

Knäuper, V, Lopez-Otin, C, Smith, B, Knight, G, Murphy, G: Biochemical characterization of human collagenase-3. J Biol Chem (1996a) **271**: 1544-1550.

Knäuper, V, Murphy, G, Tschesche, H: Activation of human neutrophil procollagenase by stromelysin 2. Eur J Biochem (1996b) **235**: 187-191.

Knäuper, V, Will, H, Lopez-Otin, C, Smith, B, Atkinson, SJ, Stanton, H, Hembry, RM, Murphy, G: 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 (1996c) **271**: 17124-17131.

Knäuper, V, Smith, B, Lopez-Otin, C, Murphy, G: Activation of progelatinase B (proMMP-9) by active collagenase-3 (MMP-13). Eur J Biochem (1997) **248**: 369-373.

Knäuper, V, Murphy, G: Membrane-type matrix metalloproteinases and cell-surface-associated activation cascades for matrix metalloproteinases. In: Parks, W., Mecham, RP *Matrix metalloproteinases.* Academic Press, San Diego. (1998), 199-218.

Koivukangas, V, Kallioinen, M, Autio-Harmainen, H, Oikarinen, A: UV irradiation induces the expression of gelatinases in human skin in vivo. Acta Derm Venereol (1994) **74**: 279-282.

Koivunen, E, Arap, W, Valtanen, H, Rainisalo, A, Medina, OP, Heikkilä, P, Kantor, C, Gahmberg, CG, Salo, T, Konttinen, YT, Sorsa, T, Ruoslahti, E, Pasqualini, R: Tumor targeting with a selective gelatinase inhibitor. Nat Biotechnol (1999) **17**: 768-774.

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.

Kolb, C, Mauch, S, Krawinkel, U, Sedlacek, R: Matrix metalloproteinase-19 in capillary endothelial cells: expression in acutely, but not in chronically, inflamed synovium. Exp Cell Res (1999) **250**: 122-130.

Koolwijk, P, Sidenius, N, Peters, E, Sier, CF, Hanemaaijer, R, Blasi, F, van Hinsbergh, VW: Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. Blood (2001) **97**: 3123-3131.

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.

Kricker, A, Armstrong, BK, English, DR: Sun exposure and non-melanocytic skin cancer. Cancer Causes Control (1994) **5**: 367-392.

Kumar, R, Dong, Z, Fidler, IJ: Differential regulation of metalloelastase activity in murine peritoneal macrophages by granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor. J Immunol (1996) **157**: 5104-5111.

Kurman, R: Blaustein´s Pathology of the Female Genital Tract. 4th edn. Springer-Verlag, New York, 1994.

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.

Kähäri, V, Saarialho-Kere, U: Matrix metalloproteinases in skin. Exp Dermatol (1997) **6**: 199-213.

Kähäri, V, Saarialho-Kere, U: Matrix metalloproteinases and their inhibitors in tumour growth and invasion. Ann Med (1999) **31**: 34-45.

Langton, K, Barker, MD, 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.

Leco, K, Khokha, R, Pavloff, N, Hawkes, SP, Edwards, DR: 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.

Lenander, C, Habermann, JK, Ost, A, Nilsson, B, Schimmelpenning, H, Tryggvason, K, Auer, G: Laminin-5 gamma2 chain expression correlates with unfavorable prognosis in colon carcinomas. Anal Cell Pathol (2001) **22**: 201-209.

Lengyel, E, Gum, R, Juarez, J, Clayman, G, Seiki, M, Sato, H, Boyd, D: Induction of M(r) 92,000 type I V collagenase expression in a squamous cell carcinoma cell line by fibroblasts. Cancer Res (1995) **55**: 963-967.

Lever, W, Schaumburg-Lever, G: Histology of the skin. In: *Histopathology of the skin.* J.B. Lippincott Company, Philadelphia, USA. (1990a), pp. 9-43.

Lever, W, Schaumburg-Lever, G: Tumors and cysts of the epidermis. Solar keratosis. In: *Histopathology of the skin.* J.B. Lippincott Company, Philadelphia, USA. (1990b), pp. 542-546.

Lever, W, Schaumburg-Lever, G: Tumors of the epidermal appendages. Basal cell epithelioma. In: *Histopathology of the skin.* J.B. Lippincott Company, Philadelphia, USA. (1990c), pp. 622-634.

Lichtinghagen, R, Helmbrecht, T, Arndt, B, Boker, KH: Expression pattern of matrix metalloproteinases in human liver. Eur J Clin Chem Clin Biochem (1995) **33**: 65-71.

Liotta, L, Tryggvason, K, Garbisa, S, Hart, I, Foltz, CM, Shafie, S: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature (1980) **284**: 67-68.

Liu, Z, Shipley, JM, Vu, TH, Zhou, X, Diaz, LA, Werb, Z, Senior, RM: Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. J Exp Med (1998) **188**: 475-482.

Llano, E, Pendas, AM, Knäuper, V, Sorsa, T, Salo, T, Salido, E, Murphy, G, Simmer, JP, Bartlett, JD, Lopez-Otin, C: Identification and structural and functional characterization of human enamelysin (MMP-20). Biochemistry (1997) **36**: 15101-15108.

Llano, E, Pendas, AM, Freije, JP, Nakano, A, Knäuper, V, Murphy, G, Lopez-Otin, C: 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.

Lohi, J, Lehti, K, Westermarck, J, Kähäri, VM, Keski-Oja, J: Regulation of membrane type metalloproteinase-1 expression by growth factors and phorbol 12-myristate 13-acetate. Eur J Biochem (1996) **239**: 239-247.

Lohi, J, Lehti, K, Valtanen, H, Parks, WC, Keski-Oja, J: Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. Gene (2000) **242**: 75-86.

Lohi, J, Wilson, CL, Roby, JD, Parks, WC: 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.

Lopez-Boado, Y, Wilson, CL, Hooper, LV, Gordon, JI, Hultgren, SJ, Parks, WC: Bacterial exposure induces and activates matrilysin in mucosal epithelial cells. J Cell Biol (2000) **148**: 1305-1315.

Lund, L, Romer, J, Thomasset,N, Solberg, H, Pyke, C, Bissell, MJ, Dano, K, Werb, Z: Two distinct pha ses of apoptosis in mammary gland involution: proteinase-independent and -dependent pathways. Development (1996) **122**: 181-193.

Madlener, M, Mauch, C, Conca, W, Brauchle, M, Parks, WC, Werner, S: Regulation of the expression of stromelysin-2 by growth factors in keratinocytes: implications for normal and impaired wound healing. Biochem J (1996) **320**: 659-664.

Madlener, M, Parks, WC, Werner, S: Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs) are differentially expressed during excisional skin wound repair. Exp Cell Res (1998) **242**: 201-210.

Majmudar, G, Nelson, BR, Jensen, TC, Johnson, TM: 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.

Marchenko, G, Ratnikov, BI, Rozanov, DV, Godzik, A, Deryugina, EI, Strongin, AY: Characterization of matrix metalloproteinase-26, a novel metalloproteinase widely expressed in cancer cells of epithelial origin. Biochem J (2001a) **356**: 705-718.

Marchenko, G, Strongin, AY: MMP-28, a new human matrix metalloproteinase with an unusual cysteine-switch sequence is widely expressed in tumors. Gene (2001b) **265**: 87-93.

Marks, R, Rennie, G, Selwood, T: The relationship of basal cell carcinomas and squamous cell carcinomas to solar keratoses. Arch Dermatol (1988) **124**: 1039-1042.

Marti, H, McNeil, L, Thomas, G, Davies, M, Lovett, DH: Molecular characterization of a low-molecular-mass matrix metalloproteinase secreted by glomerular mesangial cells as PUMP-1. Biochem J (1992) **285**: 899-905.

Massague, J: The transforming growth factor-beta family. Annu Rev Cell Biol (1990) **6**: 597-641.

Masson, R, Lefebvre, O, Noel, A, Fahime, ME, Chenard, MP, Wendling, C, Kebers, F, LeMeur, M, Dierich , A, Foidart, JM, Basset, P, Rio, MC: In vivo evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. J Cell Biol (1998) **140**: 1535-1541.

Massova, I, Kotra, LP, Fridman, R, Mobashery, S: Matrix metalloproteinases: structures, evolution, and diversication. FASEB J (1998) **12**: 1075-1095.

Matsumoto, S, Katoh, M, Saito, S, Watanabe, T, Masuho, Y: Identification of soluble type of membrane-type matrix metalloproteinase-3 formed by alternatively spliced mRNA. Biochim Biophys Acta (1997) **1354**: 159-170.

Mauch, C, Krieg, T: Collagens: their structure and metabolism. In: Lapiére, C., Krieg, T (Eds.) *Connective tissue diseases of the skin.* Marcel Decker, Inc., New York. (1993), pp. 1-16.

Mazzieri, R, Masiero, L, Zanetta, L, Monea, S, Onisto, M, Garbisa, S, Mignatti, P: Control of type IV collagenase activity by components of the urokinase-plasmin system: a regulatory mechanism with cell-bound reactants. EMBO J (1997) **16**: 2319-2332.

McCawley, L, Matrisian, LM: Matrix metalloproteinases: multifunctional contributors to tumor progression. Mol Med Today (2000) **6**: 149-156.

McCawley, L, Matrisian, LM: Tumor progression: defining the soil round the tumor seed. Curr Biol (2001) **11**: R25-27.

McCawley L, Matrisian LM: Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol (2001) **13**: 534-540

McDonnell, S, Navre, M, Coffey, RJ Jr, Matrisian, LM: Expression and localization of the matrix metalloproteinase pump-1 (MMP-7) in human gastric and colon carcinomas. Mol Carcinog (1991) **4**: 527-533.

McGowan, K, Bauer, EA, Smith, LT: Localization of type I human skin collagenase in developing embryonic and fetal skin. J Invest Dermatol (1994) **102**: 951-957.

McLean, W, Pulkkinen, L, Smith, FJ, Rugg, EL, Lane, EB, Bullrich, F, Burgeson ,RE, Amano, S, Hudson, DL, Owaribe, K, McGrath, JA, McMillan, JR, Eady, RA, Leigh, IM, Christiano, AM, Uitto, J: Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organization. Genes Dev (1996) **10**: 1724-1735.

Mechan, R, Heuser, JE: The elastic fiber. In: Hay, E.E. *Cell biology of extracellular matrix.* Plenum Press, New York. (1991), pp. 79-109.

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.

Monsky, W, Kelly, T, Lin, CY, Yeh, Y, Stetler-Stevenson, WG, Mueller, SC, Chen, WT: Binding and localization of M(r) 72,000 matrix metalloproteinase at cell surface invadopodia. Cancer Res (1993) **53**: 3159-3164.

Mori, M, Mimori, K, Shiraishi, T, Fujie, T, Baba, K, Kusumoto, H, Haraguchi, M, Ueo, H, Akiyoshi, T: Analysis of MT1-MMP and MMP2 expression in human gastric cancers. Int J Cancer (1997) **74**: 316-321.

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.

Muller, D, Breathnach, R, Engelmann, A, Millon, R, Brenner, G, Flesch, H, Dumont, P, Eber, M, Abecassis, J: Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. Int J Cancer (1991) **48**: 550-556.

Muller, D, Wolf, C, Abecassis, J, Millon, R, Engelmann, A, Bronner, G, Rouyer, N, Rio, MC, Eber, M, Methlin, G, 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.

Murphy, G, Houbrechts, A, Cockett, MI, Williamson, RA, O'Shea, M, Docherty, JA: The N-terminal domain of tissue inhibitor of metalloproteinases retains metalloproteinase inhibitory activity. Biochemistry (1991a) **30**: 8097-8102.

Murphy, G, Cockett, MI, Ward, RV, Docherty, AJP: Matrix metalloproteinase degradation of elastin, type IV collagen and proteoglycan. A quantitative comparison of the activities of 95 kDa and 72 kDa gelatinases, stromelysins-1 and -2 and punctuated metalloproteinase (PUMP). Biochem J (1991b) **277**: 277-279.

Murphy, G, Nguyen, Q, Cockett ,MI, Atkinson, SJ, Allan, JA, Knight, CG, Willenbrock, F, Docherty, JA: Assessment of the role of the fibronectin-like domain of gelatinase A by analysis of a deletion mutant. J Biol Chem (1994) **269**: 6632-6636.

Murphy, G, Knäuper, V: Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? Matrix Biol (1997) **15**: 511-518.

Murray, G, Duncan, ME, O'Neil, P, McKay, JA, Melvin, WT, Fothergill, JE: Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J Pathol (1998) **185**: 256-261.

Määttä, M, Soini, Y, Liakka, A, Autio-Harmainen, H: Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: implications for tumor progression and clinical prognosis. Clin Cancer Res (2000) **6**: 2726-2734.

Nagase, H: Stromelysins 1 and 2. In: Parks, W, Mecham, RP (Eds.) *Matrix Metalloproteinases*. Academic Press, San Diego. (1998), pp. 43-84.

Nagase, H, Woessner, JF: Matrix metalloproteinases. J Biol Chem (1999) **274**: 21491-21494.

Nelson, A, Fingleton, B, Rothenberg, ML, Matrisian, LM: Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol (2000) **18**: 1135-1149.

Newell, K, Witty, JP, Rodgers, WH, Matrisian, LM: Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. Mol Carcinog (1994) **10**: 199-206.

Nicholson, R, Murphy, G, Breathnach, R: Human and rat malignant-tumor-associated mRNAs encode stromelysin-like metalloproteinases. Biochemistry (1989) **28**: 5195-5203.

Nishizuka, I, Ichikawa, Y, Ishikawa, T, Kamiyama, M, Hasegawa, S, Momiyama, N, Miyazaki, K, Shimada, H: Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis in vivo. Cancer Lett (2001) **173**: 175-182.

Nomura, H, Sato, H, Seiki, M, Mai, M, Okada, Y: Expression of membrane-type matrix metalloproteinase in human gastric carcinomas. Cancer Res (1995) **55**: 3263-3266.

Ogata, Y, Itoh, IY, Nagase, H: Steps involved in activation of the pro-matrix metalloproteinase 9 (progelatinase B)-tissue inhibitor of metalloproteinases-1 complex by 4-aminophenylmercuric acetate and proteinases. J Biol Chem (1995) **270**: 18506-18511.

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.

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.

Ohuchi, E, Imai, K, Fujii, Y, Sato, H, Seiki, M, Okada, Y: Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem (1997) **272**: 2446-2451.

Okada, A, Bellocq, JP, Rouyer, N, Chenard, MP, Rio, MC, Chambon, P, Basset, P: 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.

Ono, M, Torisu, H, Fukushi, J, Nishie, A, Kuwano, M: Biological implications of macrophage infiltration in human tumor angiogenesis. Cancer Chemother Pharmacol (1999) **43**: S69-71.

Park, H, Ni, J, Gerkema, FE, Liu, D, Belozerov, VE, Sang, QX: Identification and characterization of human endometase (Matrix metalloproteinase-26) from endometrial tumor. J Biol Chem (2000) **275**: 20540-20544.

Patterson, B, Sang, QA: Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J Biol Chem (1997) **272**: 28823-28825.

Pei, D, Weiss, SJ: Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature (1995) **375**: 244-247.

Pei, D: Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. J Biol Chem (1999a) **274**: 8925-8932.

Pei, D: Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. Cell Res (1999b) **9**: 291-303.

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.

Pendas, A, Knäuper, V, Puente, XS, Llano, E, Mattei, MG, Apte, S, Murphy, G, Lopez-Otin, C: 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.

Pendas, A, Uria, JA, Jimenez, MG, Balbin, M, Freije, JP, 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.

Petruzzelli, L, Takami, M, Humes, HD: Structure and function of cell adhesion molecules. Am J Med (1999) **106**: 467-476.

Pihlajaniemi, T, Rehn, M: Two new collagen subgroups: membrane-associated collagens and types XV and XVII. Prog Nucleic Acid Res Mol Biol (1995) **50**: 225-262.

Pilcher, B, Dumin, JA, Sudbeck, BD, Krane, SM, Welgus, HG, Parks, WC: The activity of collagenase -1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol (1997) **137**: 1445-1457.

Plow, E, Herren, T, Redlitz, A, Miles, LA, Hoover-Plow, JL: The cell biology of the plasminogen system. FASEB J (1995) **9**: 939-945.

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.

Polette, M, Nawrocki, B, Gilles, C, Sato, H, Seiki, M, Tournier, JM, Birembaut , P: MT-MMP expression and localisation in human lung and breast cancers. Virchows Arch (1996) **428**: 29-35.

Poole, A: Proteoglycans in health and disease: structures and functions. Biochem J (1986) **236**: 1-14.

Pozzi, A, Moberg, PE, Miles, LA, Wagner, S, Soloway, P, Gardner, HA: 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.

Prikk, K, Maisi, P, Pirilä, E, Sepper, R, Salo, T, Wahlgren, J, Sorsa, T: In vivo collagenase-2 (MMP-8) expression by human bronchial epithelial cells and monocytes/macrophages in bronchiectasis. J Pathol (2001) **194**: 232-238.

Prockop, D, Kivirikko, KI: Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem (1995) **64**: 403-434.

Prosser, I, Stenmark, KR, Suthar, M, Crouch, EC, Mecham, RP, Parks, WC: Regional heterogeneity of elastin and collagen gene expression in intralobar arteries in response to hypoxic pulmonar hypertension as demonstrated by in situ hybridization. Am J Pathol (1989) **135**: 1073-1088.

Puente, X, Pendas, AM, 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.

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.

Pyke, C, Ralfkiaer, E, Tryggvason, K, Dano, K: Messenger RNA for two type IV collagenases is located in stromal cells in human colon cancer. Am J Pathol (1993) **142**: 359-365.

Pyke, C, Salo, S, Ralfkiaer, E, Romer, J, Dano, K, Tryggvason, K: Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. Cancer Res (1995) **55**: 4132-4139.

Quondamatteo, F, Knittel, T, Mehde, M, Ramadori, G, Herken, R: Matrix metalloproteinases in early human liver development. Histochem Cell Biol (1999) **112**: 277-282.

Ramsey, M: Basal Cell Carcinoma. In: Taylor, R., Wells, MJ, Chan, EF, Quirk, C, Elston, DM (Eds.) *eMedicine Journal, Dermatology, Malignant Neoplasms*. © Copyright 2001, eMedicine.com, Inc. (2001).

Ravanti, L, Häkkinen, L, Larjava, H, Saarialho-Kere, U, Foschi, M, Han, J, Kähäri, VM: 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.

Ravanti, L, Kähäri, VM: Matrix metalloproteinases in wound repair (review). Int J Mol Med (2000) **6**: 391-407.

Rechardt, O, Elomaa, O, Vaalamo, M, Pääkkonen, K, Jahkola, T, Höök-Nikanne,J, Hembry, RM, Häkkinen, L, Kere, J, Saarialho-Kere, U: Stromelysin-2 is upregulated during normal wound repair and is induced by cytokines. J Invest Dermatol (2000) **115**: 778-787.

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.

Robinson, L, Sznajder, NA, Riley, SC, Anderson, RA: Matrix metalloproteinases and tissue inhibitors of metalloproteinases in human fetal testis and ovary. Mol Hum Reprod (2001) **7**: 641-648.

Rodgers, W, Matrisian, LM, Giudice, LC, Dsupin, B, Cannon, P, Svitek, C, Gorstein, F, Osteen, KG: Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones. J Clin Invest (1994) **94**: 946-953.

Romanic, A, Burns-Kurtis, CL, Ao, Z, Arleth, AJ, Ohlstein, EH: Upregulated expression of human membrane type-5 matrix metalloproteinase in kidneys from diabetic patients. Am J Physiol Renal Physiol (2001) **281**: F309-317.

Rosenbloom, J, Abrams, WR, Mecham, R: Extracellular matrix 4: the elastic fibre. FASEB J (1993) **7**: 1209-1218.

Rousselle, P, Lunstrum, GP, Keene, DR, Burgeson, RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol (1991) **114**: 567-576.

Rousselle, P, Keene, DR, Ruggiero, F, Champliaud, MF, Rest, M, Burgeson, RE: Laminin 5 binds the NC-1 domain of type VII collagen. J Cell Biol (1997) **138**: 718-728.

Rouyer, N, Wolf, C, Chenard, MP, Rio, MC, Chambon, P, Bellocq, JP, Basset, P: Stromelysin-3 gene expression in human cancer: an overview. Invasion Metastasis (1994) **14**: 269-275.

Rudolph-Owen, L, Chan, R, Muller, WJ, Matrisian, LM: The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. Cancer Res (1998) **58**: 5500-5506.

Ruoslahti, E: Fibronectin and its integrin receptors in cancer. Adv Cancer Res (1999) **76**:1-20

Saarialho-Kere, U, Chang, ES, Welgus, HG, Parks, WC: Distinct localization of collagenase and TIMP expression in wound healing associated with ulcerative pyogenic granuloma. J Clin Invest (1992) **90**: 1952-1957.

Saarialho-Kere, U, Kovacs, SO, Pentland, AP, Olerud, JE, Welgus, HG, Parks, WC: Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. J Clin invest (1993) **92**: 2858-2866.

Saarialho-Kere, U, Pentland, AP, Birkedal-Hansen, H, Parks, WC, Welgus, HG: Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. J Clin Invest (1994) **94**: 79-88.

Saarialho-Kere, U, Crouch, EC, Parks, WC: Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. J Invest Dermatol (1995) **105**: 190-196.

Saarialho-Kere, U, Vaalamo, M, Puolakkainen, P, Airola, K, Parks, WC, Karjalainen-Lindsberg, ML: Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. Am J Pathol (1996) **148**: 519-526.

Saarialho-Kere, U: Patterns of matrix metalloproteinase and TIMP expression in chronic ulcers. Arch Dermatol Res (1998) **294**: S47-54.

Sakai, L, Keene, DR, Engvall, E: Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol (1986) **103**: 2499-2509.

Sakamoto, A, Oda, Y, Iwamoto, Y, Tsuneyoshi, M: Expression of membrane type 1 matrix metalloproteinase, matrix metalloproteinase 2 and tissue inhibitor of metalloproteinase 2 in human cartilaginous tumors with special emphasis on mesenchymal and dedifferentiated chondrosarcoma. J Cancer Res Clin Oncol (1999) **125**: 541-548.

Salmela, M, Karjalainen-Lindsberg, ML, 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.

Sanchez-Lopez, R, Alexander, CM, Behrendtsen, O, Breathnach, R, Werb, Z: Role of zinc-binding- and hemopexin domain-encoded sequences in the substrate specificity of collagenase and stromelysin-2 as revealed by chimeric proteins. J Biol Chem (1993) **268**: 7238-7247.

Sato, H, Takino, T, Okada, Y, Cao, J, Shinagawa, A, Yamamoto, E, Seiki, M: A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature (1994) **370**: 61-65.

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.

Scharffetter, K, Wlaschek, M, Hogg, A, Bolsen, K, Schothorst, A, Goerz, G, Krieg, T, Plewig, G: UVA irradiation induces collagenase in human dermal fibroblasts in vitro and in vivo. Arch Dermatol Res (1991) **283**: 506-511.

Schoenermark, M, Mitchell, TI, Rutter, JL, Reczek, PR, Brinckerhoff, CE: Retinoid-mediated suppression of tumor invasion and matrix metalloproteinase synthesis. Ann N Y Acad Sci (1999) **30**: 466-486.

Schwartz, E: Connective tissue alterations in the skin of ultraviolet irradiated hairless mice. J Invest Dermatol (1988) **91**: 158-161.

Schwartz, N: Biosynthesis and regulation of expression of proteoglycans. Front Biosci (2000) **5**: D649-55.

Schwarz, T, Luger, TA: Effect of UV irradiation on epidermal cell cytokine production. J Photochem Photobiol B (1989) **4**: 1-13.

Scully, S, Berend, KR, Qi, WN, Harrelson, JM: Collagenase specificity in chondrosarcoma metastasis. Braz J Med Biol Res (1999) **32**: 885-889.

Sedlacek, R, Mauch, S, Kolb, B, Schatzlein, C, Eibel, H, Peter, HH, Schmitt, J, Krawinkel, U: 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.

Seiki, M: Membrane-type matrix metalloproteinases. APMIS (1999) **107**: 137-143.

Shapiro, S, Griffin, GL, Gilbert, DJ, Jenkins, NA, Copeland, NG, Welgus, HG, Senior, RM, Ley, TJ: Molecular cloning, chromosomal localization, and bacterial expression of a murine macrophage metalloelastase. J Biol Chem (1992) **267**: 4664-4671.

Shapiro, S, Kobayashi, D, Ley, TJ: Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. J Biol Chem (1993) **268**: 23824-23829.

Shapiro, S: Matrix metalloproteinase degradation of extracellular matrix: biological consequences. Curr Opin Cell Biol (1998) **10**: 602-608.

Shima, I, Sasaguri, Y, Kusukawa, J, Yamana, H, Fujita, H, Kakegawa, T, Morimatsu, M: Production of matrix metalloproteinase-2 and metalloproteinase-3 related to malignant behavior of esophageal carcinoma. A clinicopathologic study. Cancer (1992) **70**: 2747-2753.

Shimada, T, Nakamura, H, Ohuchi, E, Fujii, Y, Murakami, Y, Sato, H, Seiki, M, Okada, Y: Characterization of a truncated recombinant form of human membrane type 3 matrix metalloproteinase. Eur J Biochem (1999) **262**: 907-914.

Shimada, T, Nakamura, H, Yamashita, K, Kawata, R, Murakami, Y, Fujimoto, N, Sato, H, Seiki, M, Okada, Y: Enhanced production and activation of progelatinase A mediated by membrane-type 1 matrix metalloproteinase in human oral squamous cell carcinomas: implications for lymph node metastasis. Clin Exp Metastasis (2000) **18**: 179-188.

Shipley, J, Doyle, GA, Fliszar, CJ, Ye, QZ, Johnson, LL, Shapiro, SD, Welgus, HG, Senior, RM: The structural basis for the elastolytic activity of the 92-kDa and 72-kDa gelatinases. Role of the fibronectin type II-like repeats. J Biol Chem (1996a) **271**: 4335-4341.

Shipley, J, Wesselschmidt, RL, Kobayashi, DK, Ley, TJ, Shapiro, SD: Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. Proc Natl Acad Sci U S A (1996b) **93**: 3942-3946.

Shofuda, K, Yasumitsu, H, Nishihashi, A, Miki, K, Miyazaki, K: Expression of three membrane-type matrix metalloproteinases (MT-MMPs) in rat vascular smooth muscle cells and characterization of MT3-MMPs with and without transmembrane domain. J Biol Chem (1997) **272**: 9749-9754.

Shofuda, K, Nagashima, Y, Kawahara, K, Yasumitsu, H, Miki, K, Miyazaki, K: Elevated expression of membrane-type 1 and 3 matrix metalloproteinases in rat vascular smooth muscle cells activated by arterial injury. Lab Invest (1998) **78**: 915-923.

Sires, U, Griffin, GL, Broekelmann, TJ, Mecham, RP, Murphy, G, Chung, AE, Welgus, HG, Senior, RM: Degradation of entactin by matrix metalloproteinases. Susceptibility to matrilysin and identification of cleavage sites. J Biol Chem (1993) **268**: 2069-2074.

Sirum, K, Brinckerhoff, CE: Cloning of the genes for human stromelysin and stromelysin 2: differential expression in rheumatoid synovial fibroblasts. Biochemistry (1989) **28**: 8691-8698.

Skyldberg, B, Salo, S, Eriksson, E, Aspenblad, U, Moberger, B, Tryggvason, K, Auer, G: Laminin-5 as a marker of invasiveness in cervical lesions. J Natl Cancer Inst (1999) **91**: 1882-1887.

Smith, D, Barclay, DL: Premalignant & Malignant Disorders of the Vulva & Vagina. Chapter 46. In: DeCherney, A., Pernoll, ML *Current Obstetric & Gynecologic Diagnosis & Treatment*. McGraw-Hill Professional Publishing. (1994), 1426.

Sonnenberg, A, Calafat, J, Janssen, H, Daams, H, van der Raaij-Helmer, LM, Falcioni, R, Kennel, SJ, Aplin, JD, Baker, J, Loizidou, M, et al: Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. J Cell Biol (1991) **113**: 907-917.

Springman, E, Angleton, EL, Birkedal-Hansen, H, Van Wart, HE: Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of a Cys73 active-site zinc complex in latency and a "cysteine switch" mechanism for activation. Proc Natl Acad Sci U S A (1990) **87**: 364-368.

Stanley, J, Tanaka, T, Mueller ,S, Klaus-Kovtun, V, Roop, D: Isolation of complementary DNA for bullous pemphigoid antigen by use of patients' autoantibodies. J Clin Invest. (1988) **82**: 1864-1870.

Starcher, B, Pierce, R, Hinek, A: UVB irradiation stimulates deposition of new elastic fibers by modified epithelial cells surrounding the hair follicles and sebaceous glands in mice. J Invest Dermatol (1999) **112**: 450-455.

Stepp, M, Spurr-Michaud, S, Tisdale, A, Elwell, J, Gipson, IK: Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci U S A (1990) **87**: 8970-8974.

Sternlicht, M, Lochter, A, Sympson, CJ, Huey, B, Rougier, JP, Gray, JW, Pinkel, D, Bissell, MJ, Werb, Z: The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell (1999) **98**: 137-146.

Stetler-Stevenson, W, Hewitt, R, Corcoran, M: Matrix metalloproteinases and tumor invasion -from correlation and causality to the clinic. Semin. Cancer Biol (1996) **7**: 147-1545.

Stetler-Stevenson, W: Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest (1999) **103**: 1237-1241.

Stetler-Stevenson, W, Yu, AE: Proteases in invasion: matrix metalloproteinases. Semin Cancer Biol (2001) **11**: 143-153.

Stracke, J, Hutton, M, Stewart, M, Pendas, AM, Smith, B, Lopez-Otin, C, Murphy, G, Knäuper, V: 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 (2000a) **275**: 14809-14816.

Stracke, J, Fosang, AJ, Last, K, Mercuri, FA, Pendas, AM, Llano, E, Perris, R, Di Cesare, PE, Murphy, G, Knäuper, V: Matrix metalloproteinases 19 and 20 cleave aggrecan and cartilage oligomeric matrix protein (COMP). FEBS Lett (2000b) **478**: 52-65.

Ståhle-Bäckdahl, M, Sandstedt, B, Bruce, K, Lindahl, A, Jimenez, MG, Vega, JA, Lopez-Otin, C: Collagenase-3 (MMP-13) is expressed during human fetal ossification and re-expressed in postnatal bone remodeling and in rheumatoid arthritis. Lab Invest (1997) **76**: 717-728.

Stöcker, W, Grams, F, Baumann, U, Reinemer, P, Gomis-Ruth,FX, McKay, DB, Bode, W: The metzincins--topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases. Protein Sci (1995) **4**: 823-840.

Sutinen, M, Kainulainen, T, Hurskainen, T, Vesterlund, E, Alexander, JP, Overall, CM, Sorsa, T, Salo, T: 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.

Söderström, M, Aro, HT, Ahonen, M, Johansson, N, Aho, A, Ekfors, T, Böhling, T, Kähäri, VM, Vuorio, E: Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human chondrosarcomas. APMIS (2001) **109**: 305-315.

Takigawa, M, Tajima, K, Pan, HO, Enomoto, M, Kinoshita, A, Suzuki, F, Takano, Y, Mori, Y: Establishment of a clonal chondrosarcoma cell line with cartilage phenotypes. Cancer Res (1989) **49**: 3996-4002.

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.

Tani, T, Karttunen, T, Kiviluoto, T, Kivilaakso, E, Burgeson, RE, Sipponen, P, Virtanen, I: Alpha 6 beta 4 integrin and newly deposited laminin-1 and laminin-5 form the adhesion mechanism of gastric carcinoma. Continuous expression of laminins but not that of collagen VII is preserved in invasive parts of the carcinomas: implications for acquisition of the invading phenotype. Am J Pathol (1996) **149**: 781-793.

Terada, T, Okada,Y, Nakanuma, Y: Expression of matrix proteinases during human intrahepatic bile duct development. A possible role in biliary cell migration. Am J Pathol (1995) **147**: 1207-1213.

Thewes, M, Worret, WI, Engst, R, Ring, J: Stromelysin-3 (ST-3): immunohistochemical characterization of the matrix metalloproteinase (MMP)-11 in benign and malignant skin tumours and other skin disorders. Clin Exp Dermatol (1999) **24**: 122-126.

Toriyama, M, Rosenberg, AE, Mankin, HJ, Fondren, T, Treadwell, BV, Towle, CA: Matrix metalloproteinase digestion of aggrecan in human cartilage tumours. Eur J Cancer (1998) **34**: 1969-1973.

Trojanowska, M, LeRoy, EC, Eckes, B, Krieg, T: Pathogenesis of fibrosis: type 1 collagen and the skin. J Mol Med (1998) **76**: 266-274.

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.

Tuominen, H, Pollanen, R, Kallioinen, M: Multicellular origin of tenascin in skin tumors--an in situ hybridization study. J Cutan Pathol (1997) **24**: 590-596.

Uitto, VJ, Airola, K, Vaalamo, M, Johansson, N, Putnins, EE, Firth, JD, Salonen, J, Lopez-Otin, C, Saarialho-Kere, U, Kähäri, VM: Collagenase-3 (matrix metalloproteinase-13) expression is induced in oral mucosal epithelium during chronic inflammation. Am J Pathol (1998) **152**:1489-1499.

Unni, K: Chondrosarcoma (primary, secondary, dedifferentiated, and clear cell). In: Unni, K.E. *Dahlin´s Bone Tumours: general aspects and data on 11,087 cases.* Lippincott-Raven, Philadelphia. (1996), pp. 71-108.

Uria, J, Balbin, M, Lopez, JM, Alvarez, J, Vizoso, F, Takigawa, M, Lopez-Otin, C: Collagenase-3 (MMP-13) expression in chondrosarcoma cells and its regulation by basic fibroblast growth factor. Am J Pathol (1998) **153**: 91-101.

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.

Vaalamo, M, Mattila, L, Johansson, N, Kariniemi, AL, Karjalainen-Lindsberg, ML, Kähäri, VM, Saarialho-Kere, U: Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. J Invest Dermatol (1997) **109**: 96-101.

Vaalamo, M, Karjalainen-Lindsberg, ML, 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.

Vaalamo, M, Kariniemi, A-L, Shapiro, SD, Saarialho-Kere, U: Enhanced expression of human metalloelastase (MMP-12) in cutaneous granulomas and macrophage migration. J Invest Dermatol (1999) **112**: 499-505.

Wagner, S, Ockenfels, HM, Wagner, C, Soyer, HP, Goos, M: Differential expression of tissue inhibitor of metalloproteinases-2 by cutaneous squamous and basal cell carcinomas. J Invest Dermatol (1996) **106**: 321-326.

Wahl, L, Kleinman, HK: Tumor-associated macrophages as targets for cancer therapy. J Natl Cancer Inst (1998) **90**: 1583-1584.

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.

Wang, Y, Johnson, AR, Ye, QZ, Dyer, RD: Catalytic activities and substrate specificity of the human membrane type 4 matrix metalloproteinase catalytic domain. J Biol Chem (1999) **274**: 33043-33049.

Varani, J, Hattori, Y, Chi, Y, Schmidt, T, Perone, P, Zeigler, ME, Fader, DJ, Johnson, TM: 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.

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.

Weidner, N: Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol (1995) **147**: 9-19.

Velasco, G, Pendas, AM, 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.

Velasco, G, Cal, S, Merlos-Suarez, A, Ferrando, AA, Alvarez, S, Nakano, A, Arribas, J, Lopez-Otin, C: Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. Cancer Res (2000) **60**: 877-882.

Welgus, H, Jeffrey, JJ, Eisen, AZ: The collagen substrate specificity of human skin fibroblast collagenase. J Biol Chem (1981) **256**: 9511-9515.

Werb, Z, Chin, JR: Extracellular matrix remodeling during morphogenesis. Ann N Y Acad Sci (1998) **857**: 110-118.

Werb, Z, Vu, TH, Rinkenberger, JL, Coussens, LM: Matrix-degrading proteases and angiogenesis during development and tumor formation. APMIS (1999) **107**: 11-18.

Wernert, N, Raes, MB, Lassalle, P, Dehouck, MP, Gosselin, B, Vandenbunder, B, Stehelin, D: C-ets1 proto-oncogene is a transcription factor expressed in endothelial cells during tumor vascularization and other forms of angiogenesis in humans. Am J Pathol (1992) **140**: 119-127.

Wernert, N: The multiple roles of tumour stroma. Virchows Arch (1997) **430**: 433-443.

Westermarck, J, Kähäri, VM: Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J (1999) **13**: 781-792.

Wight, T, Heinegård, DK, Hascall, VC: Proteoglycans, structure and function. In: Hay, E.E. (Ed.) *Cell biology of extracellular matrix.* Plenum Press, New York. (1991), pp. 45-78.

Wilhelm, S, Collier, IE, Marmer, BL, Eisen, AZ, Grant, GA, Goldberg, GI: SV40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which is identical to that secreted by normal human macrophages. J Biol Chem (1989) **264**: 17213-17221.

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.

Will, H, Atkinson, SJ, Butler, GS, 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.

Wilson, C, Heppner, KJ, Rudolph, LA, Matrisian, LM: The metalloproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. Mol Biol Cell (1995) **6**: 851-869.

Wilson, C, Heppner, KJ, Labosky, PA, Hogan, BL, Matrisian, LM: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A (1997) **94**: 1402-1407.

Wilson, C, Matrisian, LM: Matrilysin. In: Parks, W., Mecham, RP (Eds.) *Matrix Metalloproteinases.* Academic Press, San Diego, USA. (1998), pp. 149-184.

Vincenti, M: The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase genes. Transcriptional and posttranscriptional regulaion, signal transduction and cell-type specific expression. In: Clarck, I.E. *Matrix metalloproteinase Protocols.* Humana Press Inc., Totowa, NJ. (2001), pp. 121-145.

Windsor, L, Grenett, H, Birkedal-Hansen, B, Bodden, MK, Engler, JA, Birkedal-Hansen, H: Cell type-specific regulation of SL-1 and SL-2 genes. Induction of the SL-2 gene but not the SL-1 gene by human keratinocytes in response to cytokines and phorbolesters. Biol Chem (1993) **268**: 17341-17347.

Winyard, P, Zhang, Z, Chidwick, K, Blake, DR, Carrell, RW, Murphy, G: Proteolytic inactivation of human alpha 1 antitrypsin by human stromelysin. FEBS Lett (1991) **279**: 91-94.

Witty, J, Wright, JH, Matrisian, LM: Matrix metalloproteinases are expressed during ductal and alveolar mammary morphogenesis, and misregulation of stromelysin-1 in transgenic mice induces unscheduled alveolar development. Mol Biol Cell (1995) **6**: 1287-1303.

Wright, J, Turley, EA, Greenberg, AH: Transforming growth factor beta and fibroblast growth factor as promoters of tumor progression to malignancy. Crit Rev Oncog (1993) **4**: 473-492.

Wu, L, Fan, J, Matsumoto, S, Watanabe, T: Induction and regulation of matrix metalloproteinase-12 by cytokines and CD40 signaling in monocyte/macrophages. Biochem Biophys Res Commun (2000) **269**: 808-815.

Vu, T, Shipley, JM, Bergers, G, Berger, JE, Helms, JA, Hanahan, D, Shapiro, SD, Senior, RM, Werb, Z: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell (1998) **93**: 411-422.

Vu, T, Werb, Z: Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev (2000) **14**: 2123-2133.

Yamada, K: Adhesive recognition sequences. J Biol Chem (1991) **266**: 12809-12812.
Yamada, K, Clark, RA: Provisional matrix. In: Clarck, RA (Ed.). *The Molecular and Cellular Biology of Wound Repair*. Plenum press, New York and London. (1996), 78-88.
Yamamoto, H, Itoh, F, Iku, S, Hosokawa, M, Imai, K: Expression of the gamma(2) chain of laminin-5 at the invasive front is associated with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Clin Cancer Res (2001) **7**: 896-900.
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.
Yang, W, Arii, S, Gorrin-Rivas, MJ, Mori, A, Onodera, H, Imamura, M: Human macrophage metalloelastase gene expression in colorectal carcinoma and its clinicopathologic significance. Cancer (2001) **91**: 1277-1283.
Yoshizaki, T, Sato, H, Maruyama, Y, Murono, S, Furukawa, M, Park, CS, Seiki, M: Increased expression of membrane type 1-matrix metalloproteinase in head and neck carcinoma. Cancer (1997) **79**: 139-144.
Yu, A, Hewitt, RE, Kleiner, DE, Stetler-Stevenson, WG: Molecular regulation of cellular invasion--role of gelatinase A and TIMP-2. Biochem Cell Biol (1996) **74**: 823-831.
Yu, Q, Stamenkovic, I: Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes Dev (1999) **13**: 35-48.
Yu, Q, Stamenkovic, I: Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev (2000) **14**: 163-176.
Zhang, K, Kramer, RH: Laminin 5 deposition promotes keratinocyte motility. Exp Cell Res (1996) **227**: 309-322.
Zhang, Z, Winyard, PG, Chidwick, K, Murphy, G, Wardell, M, Carrell, RW, Blake, DR: Proteolysis of human native and oxidised alpha 1-proteinase inhibitor by matrilysin and stromelysin. Biochim Biophys Acta (1994) **1199**: 228-228.
Zhou, Z, Apte, SS, Soininen, R, Cao, R, Baaklini, GY, Rauser, RW, Wang, J, Cao, Y, Tryggvason, K: Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci (2000) **97**: 4052-4057.
Ziegler, A, Jonason, AS, Leffell, DJ: Sunburn and p53 in the onset of skin cancer. Nature (1994) **372**: 773-776.
Zimmermann, D, Dours-Zimmermann, MT, Schubert, M, Bruckner-Tuderman, L: Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. J Cell Biol (1994) **124**: 817-825.
Zucker, S, Drews, M, Conner, C, Foda, HD, DeClerck, YA, Langley, KE, Bahou, WF, Docherty, AJ, Cao, J: Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP). J Biol Chem (1998) **273**: 1216-1222.
Zucker, S, Cao, J, Chen, WT: Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. Oncogene (2000) **19**: 6642-6650.