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Review

Matrix metalloproteinases: What do they not do? New substrates and biological roles identified by murine models and proteomics

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ABSTRACT

The biological roles of the matrix metalloproteinases (MMPs) have been traditionally associated with the degradation and turnover of most of the components of the extracellular matrix (ECM). This functional misconception has been used for years to explain the involvement of the MMP family in developmental processes, cell homeostasis and disease, and led to clinical trials of MMP inhibitors for the treatment of cancer that failed to meet their endpoints and cast a shadow on MMPs as druggable targets. Accumulated evidence from a great variety of post-trial MMP degradomics studies, ranging from transgenic models to recent state-of-the-art proteomics screens, is changing the dogma about MMP functions. MMPs regulate cell behavior through finely tuned and tightly controlled proteolytic processing of a large variety of signaling molecules that can also have beneficial effects in disease resolution. Moreover, net proteolytic activity relies upon direct interactions between the different protease and protease inhibitor families, interconnected in a complex protease web, with MMPs acting as key nodal components. Such complexity renders simple interpretation of *Mmp* knockout mice very difficult. Indeed, the phenotype of these models reveals the response of a complex system to the loss of one protease rather than necessarily a direct effect of the lack of functional activity of a protease. Such a shift in the MMP functional paradigm, together with the difficulties associated with current methods of studying proteases this highlights the need for new high content degradomics approaches to uncover and annotate MMP activities *in vivo* and identify novel interactions within the protease web. Integration of these techniques with specifically designed animal models for final validation should lay the foundations for the development of new inhibitors that specifically target disease-related MMPs and/or their upstream effectors that cause deleterious effects in disease, while sparing MMP functions that are protective.

1. MMPs and extracellular matrix remodeling

It has been almost 50 years since Gross and Lapiere reported an interesting collagenolytic activity present during metamorphosis in tadpoles [1]. The discovery of amphibian interstitial collagenase was the cornerstone for the eventual description of a large family of clinically relevant endopeptidases in man, later called matrix metalloproteinases (MMPs). Further studies then showed that beyond their collagen-degrading functions, MMPs degrade and process many other components of the extracellular matrix (ECM), including the basement membrane, and so were thought to be important for tumour spread and angiogenesis [2,3]. The ECM is a complex mesh of structural and signaling molecules that provides dynamic support to cells and tissues as well as harboring embedded information that modulates cell behavior [4,5]. Although high-throughput comprehensive MMP substrate discovery screens have only just begun and the most recently discovered MMPs have yet to be analyzed for their substrate specificities, it is generally stated that every component of the ECM can be cleaved by one or more of the 23

Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue-inhibitor of metalloproteinases; ECM, extracellular matrix; MMPI, matrix metalloproteinase inhibitor; ADAM, a disintegrin and metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin domains; VEGF, vascular endothelial growth factor; CTGF, connective tissue growth factor; HARP, heparin affin regulatory peptide; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; iTRAQ, isotope tags for relative and absolute quantitation; iCAT, isotope coded affinity tags; ESI, electrospray ionization; CRIM-1, cysteine-rich motor neuron-1; TGF, transforming growth factor; uPAR, urokinase-type plasminogen activator receptor; FGF, fibroblast growth factor; IL, interleukin; EGF, epidermal growth factor; LAP, latency-associated peptide; LTBP, latent transforming growth factor-β binding protein; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; HB-EGF, heparin-binding epidermal growth factor-like growth factor; TNF, tumour necrosis factor; LPS, lipopolysaccharide; PMN, polymorphonuclear leukocyte; GAG, glycosaminoglycan; RANKL, nuclear factor κB ligand

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members of the human MMP family and their orthologues [2,4,5]. We consider that the functional restriction for MMPs as mere ECM degradative enzymes has greatly stymied the unbiased examination of MMP activities *in vivo* for other, potentially more important, biological roles.

Numerous reports have shown that MMPs are directly implicated in almost every biological process involving matrix remodeling throughout the mammalian life span, from embryo implantation [6] to cell death or necrosis [7,8]. But at what level is their role? For instance, MMP-driven proteolysis has been linked to several morphogenetic events during development, including mammary gland ductal branching, bone ossification and blood vessel remodeling [5,9]. As well, MMPs play a primary role in normal tissue maintenance functions, including wound healing and repair, menstruation and reproductive processes, and innate immune defense [10–20]. As a consequence, altered expression and/or misregulation of MMP activities *in vivo* has been associated with the development of a wide range of pathologies, including chronic inflammatory diseases and cancer. In all these functions, the conventional dogma still insists that MMPs participate in these pleiotropic functions primarily through degradation of the ECM. Although such statements have been softened with evidence that the loss of ECM scaffold molecules can lead to the consequent release of a small number of bound growth factors and exposure of cryptic neoproteins such as endostatin and angiostatin [21–24] to regulate cell function [4,5]. In contrast proteomic and yeast two-hybrid screens for MMP substrates and other recent data have shown more complex roles for MMPs in the direct regulation of a host of signaling molecules, including most and likely all of the 54 chemokines in man, and hence the function of many cells of the stroma and innate immune system [25,26]. An important theme is also emerging that a key role of MMPs is to mobilize growth factors, not so much by release from degraded matrix, but by cleavage and release of cytokine masking carrier proteins that block growth factor function and activity [27,28]. These premises will underpin our review of MMP substrates and biological roles *in vivo*, in health and disease.

2. MMPs as key regulators of cell behavior and signaling pathways

2.1. MMP inhibition and failure of clinical trials. A paradigm shift for MMP functions

The physiological relevance of MMPs is highlighted by the direct relationship between altered MMP expression and disease development. Upregulation of MMPs in diverse important human pathologies, like cardiovascular diseases, rheumatoid arthritis, neurological disorders and cancer, prompted an early interest in the therapeutic potential of MMP inhibitors [7,29–33], but without considering the possibility that some MMP functions may be beneficial and hence those MMPs may be drug anti-targets [26]. The strong correlation between MMP overexpression and late-stage tumour progression and metastasis–usually associated with poor clinical outcome–has been reported as a common feature in transgenic and knockout cancer animal models, as well as in human tumour samples [7,26,33]. Hence, it was naturally assumed that such overexpression is causal or at least important in the progression of the disease. With the recent recognition that many MMPs are in fact protective in cancer, performing essential host functions aimed at mitigating the disease, this concept of elevated expression of a gene product being always associated with worsened disease outcome is discredited [26]. However, 25 years ago, it was reasonably assumed that connective tissue breakdown after extracellular and pericellular proteolysis by tumour and stromal secreted MMPs provided access to the vascular and lymphatic systems, promoting cell migration, dissemination and metastasis. This driving hypothesis was the basis for a large number of clinical trials for MMP inhibitors (MMPIs) as anticancer agents with some drugs progressing to phase III [26,33]. Unexpectedly, there was a global failure in meeting the predicted endpoints of increased life expectation. At best, the use of high concentrations of the peptidomimetic inhibitor Marimastat achieved similar levels of survival to conventional therapy, itself an accomplishment, but one that did not allow for declaration of clinical success [34]. Certain MMPIs generated side effects like musculoskeletal pain related to inflammation, while others even showed reduced survival rates for the patients receiving the MMPI compared to those treated with placebo [35]. Such disappointing outcomes resulted in the termination of most MMPI trials and prompted a reevaluation of the role of MMPs as relevant effectors of late cancer progression, invasion and metastasis. However, it has been generally overlooked that anti-MMP treatments showed little or no phenotypic effects that could be attributed to prevention of normal connective tissue degradation. This is an important consideration in defining the actual roles for MMPs *in vivo*. In concordance with the observations from the MMPI clinical trials, and with the exception of the *Mmp14*-knockout mice, most of the models showed mild phenotypic alterations with very little matrix remodeling defects. This indicates that MMPs–with the exception of MMP-14 which seems to be a major player in the extracellular fragmentation of collagen [36,37] and phagocytosis of collagen [38]–might not play such an essential role in ECM degradation and turnover as originally thought [2–5], a task that could be carried out by cathepsins via the lysosomal degradation pathway of phagocytosed collagen [39–41].

With hindsight, it seems clear that burdened with ignorance about the actual substrates of MMPs and hence their physiological functions [7,33,42], the MMPI trials were highly likely to fail. In addition, contributing factors to their failure included the treatment of late stage tumours only and the use of broad-spectrum MMPIs that also inhibit close-related metalloproteinases like the ADAMs (a disintegrin and metalloproteinase) and ADAMTSs (ADAMs with thrombospondin domains) [43,44].

The paradigm shift for MMP functions, from destructive enzymes to cell signaling regulators [25] and disease anti-targets [45] also suggests that the roles of MMPs in disease development are far more complex than originally thought. Hence, in cancer, MMP activities are probably more relevant during the early stages of tumour development, with MMP-dependent signaling more relevant biologically than ECM degradation. The identification of chemokine cleavage and the conversion of chemokine agonists to antagonists by MMPs [15] was the first anti-inflammatory role identified for MMPs. Other MMP anti-inflammatory activities that can be beneficial in disease resolution include the release of antiapoptotic or antiangiogenic epitopes from ECM, that also would be blocked by the use of broad-spectrum MMPIs [26]. Moreover, following the identification of MMP-8 as a cancer anti-target [45], MMPs-3, -9, -11, -12 and -19 have also been shown to play protective roles [46,47]. Thus, targeting proteases that are not effectors of pathology could generate secondary effects or worsening of the disease upon alteration of MMP-regulated pathways that can be either beneficial or essential.

Describing the substrate degradome of individual MMPs is one key to drug target validation that may ultimately lead to new clinical trials, in which altered deleterious MMP activity–the MMP targets–and host-protective MMP activity, the antitargets, are clearly defined so refining the rational development of specific new MMPIs.

2.2. Proteomics and pharmacoproteomics approaches to substrate discovery

Degradomics, the proteomics analysis of proteases, their substrates and inhibitors [48], is revolutionizing the understanding of protease function *in vivo* by revealing their substrate repertoire, also called the substrate degradome, and their interaction partners, the interactome [49]. Indeed, without knowledge of substrates, the biological roles of proteases can only be guessed. In addition to the

use of traditional biochemical and functional techniques, degradomics studies of MMPs have been complemented by the generation of many animal models based on genetic gain or loss of MMP function and by new high content analyses designed to unravel the complexity of MMP functions *in vivo*.

In the first application of the yeast two-hybrid system to find extracellular protein interactors [15] protease substrates were discovered using "Exosite Scanning" in which the MMP-2 carboxy-terminal hemopexin domain was used as bait. This led to the identification of the chemokines as a novel group of MMP substrates so establishing a direct role for MMPs, not just as effectors, but also as regulators of inflammation [14,15,17]. Scanning of peptide libraries was also used to identify cleavage-site motifs for six MMPs and to predict MMP substrates based on the preferred cleavage-site motifs identified [50]. However, despite the success of these two techniques, the number of substrates identified was low and the time consuming nature of these methods reduced their feasibility for widespread use to elucidate all MMP substrate degradomes. Such *in vitro* screens also have the added disadvantage of analyzing the proteinase or domains thereof in isolation, thus ignoring interactions with other molecules in the cellular or tissue context, that are critical for proteinase function, substrate access and cleavage [51].

New rapid techniques that could identify and quantify proteinase substrates in more complex biological samples and on a system-wide basis were required. These came with the development of quantitative mass-spectrometry (MS) based proteomic techniques such as isotope-coded affinity tag (ICAT) labeling [52]. ICAT labeling involves differential tagging by reductive alkylation of proteins containing cysteine residues with chemically identical biotin-tags that differ in isotopic composition and therefore mass. This allows MS quantification of the relative abundance of the labeled proteins within two samples. ICAT was used in a cell-based substrate discovery screen of MMP-14 and led to the identification of fourteen novel MMP-14 candidate substrates, only two of which were ECM proteins [53]. The remaining proteins encompassed cytokines, chemokines, cell receptors and serine proteinase inhibitors. A similar approach was used to examine the effect of the MMP inhibitor Prinomastat on MMP-14 expressing MDA-MB-231 cells. The membrane and secreted proteomes of cells were analyzed giving insight into the effect of both MMP-14 and Prinomastat on cell membrane ectodomain shedding. Over twenty-five known MMP and MMP-14 substrates were identified validating the technique, as well as over forty novel substrates with diverse functions, twenty of which were biochemically validated in the same study [27]. ICAT analysis has proven to be a powerful tool for revealing new functions for MMPs in processes such as angiogenesis. Aside from facilitating angiogenesis through ECM degradation, identification of novel MMP-2 substrates uncovered the role of MMP-2 in the proteolytic release of proangiogenic vascular endothelial growth factor (VEGF) from inhibitory complexes with connective tissue growth factor (CTGF) and Heparin Affin Regulatory Peptide (HARP) that led to stimulation of neovascularization [28]. The main disadvantage of ICAT is that only proteins containing cysteines are analyzed and the number of cysteine containing peptides per protein is limited, reducing the confidence of protein identification and quantification. To circumvent these problems, an alternative labeling approach, isotope tags for relative and absolute quantification (iTRAQ) was developed [54]. In iTRAQ analysis, proteins from complex biological samples are digested with trypsin and free-amines are labeled with isobaric tags, thus ensuring that all proteins within the sample can potentially be represented and also improving the peptide coverage of those proteins. Tandem mass-spectrometry (MS/MS) analysis provides both sequence identification of peptides and quantification via the unique mass signature of the tags. An added advantage of this technique is that there are now eight unique iTRAQ tags, allowing eight different conditions to be analyzed in a single experiment. iTRAQ analysis was used to further uncover the diverse substrate degradome of MMP-2 in a cellular context. With the increased peptide coverage it was possible to perform "peptide-mapping" in order to predict the location of cleavage sites within the native cellular substrate for six of the twenty-three novel MMP-2 substrates identified [55].

Alternative quantitative proteomic analysis of cell-based MMP substrate screens includes a label-free technique involving ultraperformance liquid chromatography electrospray ionization (LC-ESI)-high/low collision energy MS used to analyze a metastatic prostate cancer cell line in which MMP-9 expression was knocked-down by RNA interference [56]. Relative quantification between samples was achieved by comparing peak intensity, with each mass peak defined by exact mass and retention time (EMRT). Of the twenty novel MMP-9 potential substrates identified, only four were ECM related. Interestingly there was significant overlap of potential MMP-9 substrates identified by this label-free approach and the MMP substrates identified by proteomic techniques using isotope tags. In a second paper for MMP-9 substrates [57] the proteome of a macrophage cell line over-expressing MMP-9 was compared to control cells and proteins that were selectively enriched or depleted were identified by spectral counting, a quantitation technique that is less accurate than isotopic labeling but one that is readily adaptable to many proteomics laboratories. A peptide mapping procedure similar to that described before [55] was used to infer cleavage sites *in vivo*, however, validation was performed employing nested synthetic peptides instead of native proteins. This approach could lead to wrong conclusions, as the exact cleavage sites might differ in the folded protein state. Other proteomic screens for MMP substrates in complex biological fluids have utilized 2-dimensional polyacrylamide gel electrophoresis and in-gel digest. These approaches are easily used by many laboratories, but lack throughput and sensitivity and have low discovery rates. A screen of plasma proteins digested with MMP-14 identified apolipoproteins thought to play a role in atherosclerosis and several proteinase inhibitors as substrates [58,59]. Two-dimensional differential in gel electrophoresis (DIGE) of bronchoalveolar (BAL) fluid from *Mmp2/9*$^{-/-}$ mice compared to wild type mice identified three MMP substrates that were chemotactic proteins involved in lung inflammation (see below) [60].

The application of these novel approaches to MMP substrate discovery has greatly accelerated the rate of novel substrate identification, with a diverse range of proteins implicated (Fig. 1). The following sections summarize the rich catalogue of MMP substrates recently identified by degradomics analyses, many through proteomics-based screens.

2.3. MMP substrates in developmental processes, cellular homeostasis and disease

2.3.1. Bone remodeling

Skeletal development requires the precisely orchestrated processing of specialized cartilage and bone matrices through the interplay of many different cell types. Moreover, once formed, bones still undergo dynamic tissue remodeling for homeostasis and repair [5]. As confirmed from the analysis of several MMP knockout mouse models, MMPs play a major role in bone metabolism. Indeed *Mmp14*$^{-/-}$ mice, the only MMP knockout mouse to show a lethal phenotype, have gross connective tissue and skeletal abnormalities and do not survive more than 13 weeks *postpartum* [61,62]. Surprisingly, the loss of MMP activity resulted in increased bone resorption rather than increased bone deposition and delayed formation of secondary ossification centers due to impaired vascularization of the hyaline cartilage, MMP-14 being essential for this angiogenic process, but with no substrates identified, the mechanism remains unknown [61]. Osteoblasts from *Mmp14*-mutant mice showed impaired osteogenic and collagenolytic activities [61], thus validating the hypothesis proposed on the basis of *in vitro* data that MMP-14 is a physiologically relevant collagenase [63].

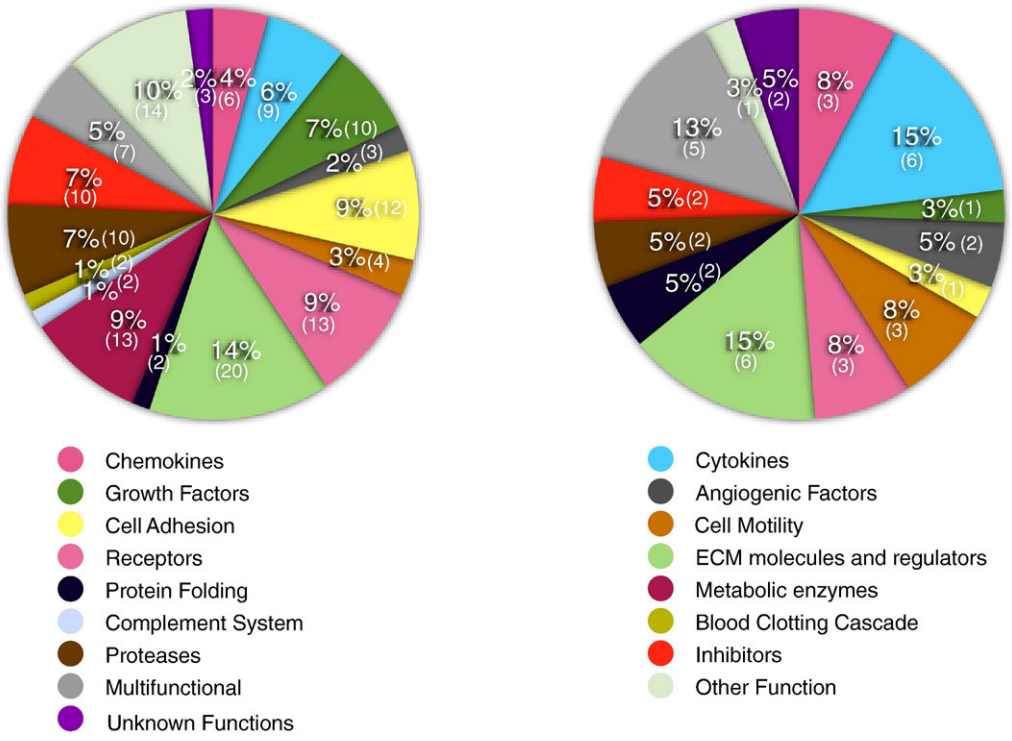

Fig. 1. MMP substrates identified in complex biological samples by proteomic analysis. (A) The diverse range of novel MMP substrates identified using a variety of proteomic techniques and (B) Proteins identified in proteomic screens that were biochemically validated as MMP substrates. The number of proteins identified or validated for each group is indicated in parentheses.

MMPs are also involved in endochondral ossification of long bones mediating the release and processing of apoptotic and angiogenic modulators. *Mmp9*-mutant mice have abnormal expansion of the zone of hypertrophic chondrocytes caused by delayed apoptosis and vascularization [64]. Galectin-3, an antiapoptotic lectin that is cleaved by MMPs-2, -9 and -14 *in vitro* [65], is enriched in the growth plate of *Mmp9*-deficient mice. Indeed, galectin-3-knockout mice show premature apoptosis of hypertrophic chondrocytes [66], whereas addition of exogenous full-length galectin-3 phenocopies MMP-9 deficiency in culture [67]. Hence, these studies demonstrate regulated chondrocyte apoptosis in growth plates by specific MMP-9 processing of an anti-apoptotic molecule rather than a phenotype due to perturbed ECM degradation. In addition, recent proteomics screens have uncovered another member of this family, galectin-1, as an MMP-2 and MMP-14 substrate [27,55].

The link between MMP-9 and hypertrophic cartilage angiogenesis was demonstrated upon delayed bone fracture repair in *Mmp9*-mutant mice [68]. Loss of MMP-9 activity in the mutant bone cartilage decreases VEGF bioavailability, resulting in impaired angiogenesis-dependent remodeling at the fracture site, although the actual substrate of MMP-9 was not identified [68]. Based on the finding that the closely related protease MMP-2 mobilizes VEGF by cleavage and release of inhibitory CTGF and HARP carrier proteins [28] from their complexes with VEGF [69] (Fig. 2), these are likely candidate substrates that may mechanistically explain these mutant mouse studies. Similarly, *Mmp13*-mutant mice also showed expansion of the zone of hypertrophic chondrocytes, but in this case, MMP-13 function in the growth plate of long bones seems to be related to the processing of type II collagen and the proteoglycan aggrecan, which are the major components of cartilage at the cartilage-bone interface. In normal endochondral ossification, MMP-9 and MMP-13 might synergistically cleave aggrecan prior to MMP-13 proteolysis of type II collagen for proper cartilage-to-bone transition [70,71].

Recently, a pharmacoproteomics screen of MMP-14-transfected breast cancer cells performed by Butler and colleagues [27] has uncovered new links between MMP proteolysis and bone metabolism after the identification of the Wnt signaling pathway inhibitor dickkopf-1 and cysteine-rich motor neuron-1 (CRIM-1) as new MMP-14 substrates. Dickkopf-1 regulates bone mass through inhibition of bone formation and induction of resorption and promotes proliferation and blockage of the differentiation in mesenchymal stem cells. In disease, dickkopf-1 is strongly related to joint pathology in rheumatoid arthritis and ankylosing spondylitis, osteolysis in multiple myeloma and metastatic breast cancer, and bone metastasis in prostate cancer [72]. On the other hand, CRIM-1 can regulate the functions of bone morphogenetic proteins (BMPs) by reducing their effective levels as secreted active growth factors [73]. Further functional studies are underway to assess the physiological consequences of dickkopf-1 and CRIM-1 proteolytic processing by MMP-14 *in vivo*.

2.3.2. Vascular development and angiogenesis

The complex repertoire of signaling functions modulated by MMPs is reflected in the way this protease family plays a wide range of pro- and anti-angiogenic roles in blood vessel development and homeostasis. Not surprisingly, deregulated MMP expression and activity is linked to vascular pathology, such as the previously described defects in impaired bone remodeling and during tumour angiogenesis.

One of the main mechanisms utilized by MMPs in this process is the regulation of the bioavailability of angiogenic factors that are sequestered by the ECM or the basement membrane. Such is the case of ECM-tethered VEGF, which is mobilized by MMPs-1, -3, -7, -9, -16 and -19 cleavage of the VEGF-binding ECM proteins [68,74,75]. Similarly VEGF forms an inhibitory complex with the heparin-binding growth factor HARP [69] and is released upon MMP-2 cleavage of HARP [28] (Fig. 2). In addition, VEGF also forms a latency complex with a known MMP substrate, CTGF, and MMPs-1, -2, -3, -7 and -13 can release active VEGF from this complex [76] (Fig. 2). In a different mechanism, VEGF-A transcriptional activation can be specifically increased by MMP-14 overexpression. This VEGF-A up-regulation,

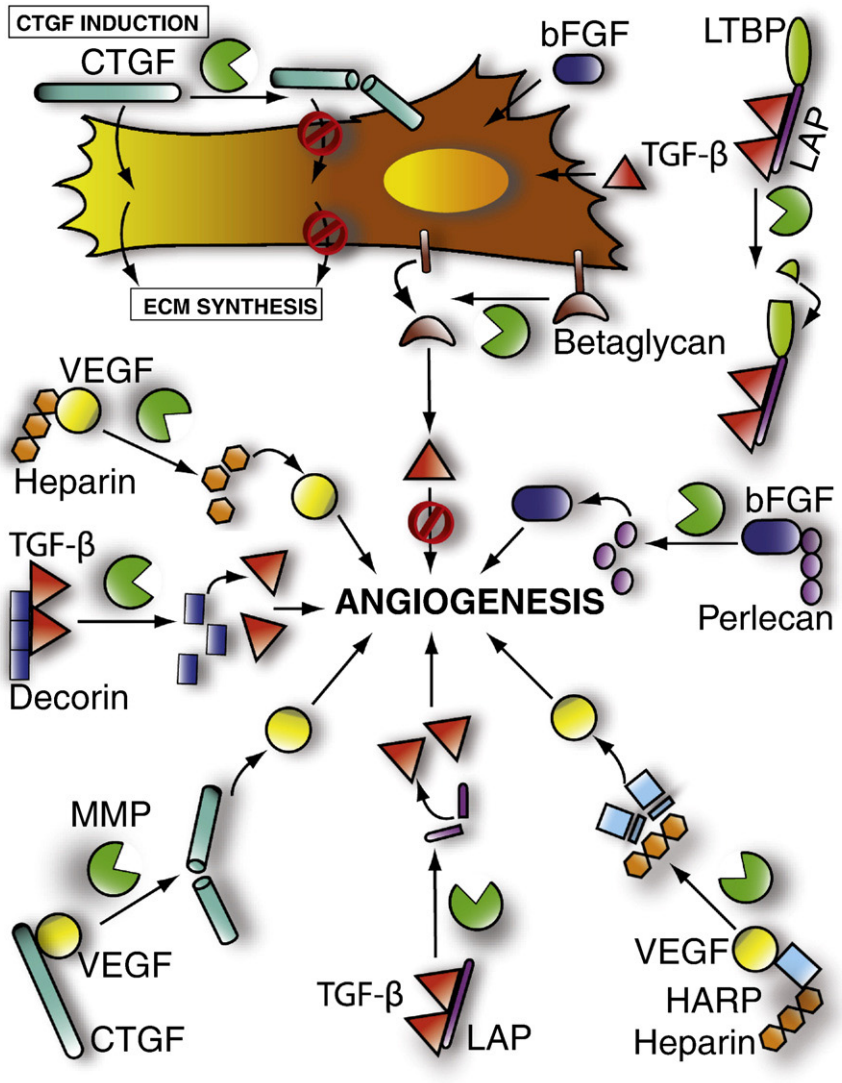

Fig. 2. MMPs modulate growth factor functions. CTGF induces secretion of ECM proteins by fibroblasts and associates with VEGF to form a VEGF latency complex. Other proangiogenic growth factors can be stored in a latent state through interactions with ECM components. When expressed, MMPs can cleave CTGF to eliminate the ECM synthesis stimulus. The subsequent reduction in matrix deposition can be an alternative MMP-dependent mechanism that has the same outcome as ECM degradation. In addition, MMP processing can release ECM-tethered growth factors to induce angiogenesis and other cellular functions. By this means, degradation of perlecan renders soluble bFGF; VEGF is released following proteolytic disruption of the complexes formed with either CTGF, heparin or HARP; TGF-β big latency complex can be solubilized upon cleavage of the LTBP protein, and further activated by processing of the LAP peptide. Degradation of TGF-β-bound decorin also increases active growth factor levels in the extracellular milieu. Finally, MMP-shed betaglycan can interact and inactivate circulating TGF-β, thus inhibiting pro-angiogenic processes. Again, whereas MMP activity is suggested to enhance angiogenesis by basement membrane remodeling, the unmasking of angiogenic factors is another route that also potently increases or blocks angiogenesis. Hence, the importance of MMP activity in physiological processes traditionally thought to be the result of ECM degradation may in fact be due to other reasons, often representing higher order control of information circuits that are critical to tissue homeostasis or the process.

that can be blocked by MMP-14 inhibitors, is driven through an Src tyrosine kinases-dependent signal transduction pathway [77].

The *in vivo* relevance of MMP modulation of VEGF bioavailability was demonstrated by the RIP1-Tag murine pancreatic insulinoma model. These mice switch on angiogenesis in a discrete step during cancer progression, despite constitutive expression of VEGF and its receptors. When RIP1-tag mice were crossed with *Mmp9*-mutant mice the angiogenic switch was prevented, showing that VEGF availability by MMP-9-mediated release is pivotal for the development of the angiogenic phenotype [74]. However, in this case, the release from degraded ECM was thought to account for VEGF mobilization. Degradation of ECM/basement-membrane by MMPs can release several other pro-angiogenic factors. Proteolysis of the heparan-sulfate proteoglycan perlecan by MMP-1 and MMP-3 in endothelial cells detaches basic fibroblast growth factor (bFGF) [78] and as discussed in Section 2.3.4, MMPs also release and activate transforming growth factor -β (TGF-β), another potent proangiogenic factor (Fig. 2).

Many other processes are included in the functional repertoire of MMP-dependent control of vascularization and angiogenesis. For instance, MMP-9 cleaves the cytokine interleukin-8 (CXCL8/IL-8) [16], enhancing its pro-angiogenic activity tenfold, and degrades the angiogenesis inhibitor platelet factor-4 [79]. Similarly, MMPs-1, -8, -13 and -14 also activate CXCL8/IL-8 [19]. During blood vessel development, MMP-14 plays a regulatory role via proteolytic processing affecting signaling downstream of the platelet derived growth factor receptor β (PDGFRβ) [80]. In a very recent paper, the multifunctional LDL receptor-related protein LRP1 has been uncovered as the link between MMP-14 activity and the platelet derived growth factor receptor BB (PDGF-BB)-PDGFRβ signaling axis in vascular smooth muscle cells dedifferentiation, proliferation and invasion [81].

2.3.3. Exposure of cryptic sites with biological activity and release of neoproteins from ECM

MMPs cleave ECM/basement membrane proteins to expose cryptic sites or to release fragments that act as neo-epitopes. This is the case of the generation of pro-angiogenic $\alpha_v\beta_3$ integrin binding sites upon cleavage of collagen IV by the gelatinases [82,83]. Interestingly, several ECM proteolytic fragments are anti-angiogenic factors, like endostatin, which is generated upon the cleavage of the NC1 domain of collagen by different proteases, including MMPs-3, -9, -12, -13 and -14 [21]. Another angiogenesis inhibitory fragment, called tumstatin, is a cleavage product from the NC1 domain of the α3 chain of collagen IV. Tumstatin is released upon MMP-9 cleavage *in vitro*. Moreover, *Mmp9*-null mice show decreased levels of circulating tumstatin associated with an increase in tumour-associated pathological angiogenesis in a Lewis lung carcinoma model [84]. However, whether this is due directly to tumstatin is unclear as it is uncertain whether high enough levels of these fragments can be generated and maintained to be biologically significant. Repression of angiogenesis can be achieved as well by plasminogen hydrolysis and release of the potent angiogenesis inhibitor angiostatin by MMPs-2, -7, -9 and -12 [22–24].

The sheddase activities of MMPs can also be linked to either promotion or inhibition of angiogenesis. Several MMPs are able to cleave and shed the D1 domain of the urokinase-type plasminogen activator receptor (uPAR) *in vitro* [85,86]. In an experimental angiogenesis model investigating the role of uPAR, inhibition of MMPs promoted angiogenesis, likely due to an increase in functionally available uPAR [87]. On the other hand, by releasing the soluble ectodomain of the fibroblast growth factor receptor 1 (FGFR1), MMP-2 abrogates signal transduction even when its FGF binding capacity is maintained [88]. The shedding of the transmembrane proangiogenic semaphorin 4D by MMP-14 in head and neck squamous carcinoma cells induces endothelial cell chemotaxis *in vitro* and blood vessel growth *in vivo* [89].

Lastly, Macotela and colleagues [90] detected MMP-specific cleavage of the hormone prolactin in chondrocytes, which releases a 16-kDa N-terminal fragment that inhibits angiogenesis by blocking epidermal growth factor (EGF)-induced endothelial cell proliferation. Interestingly, after hyaline bone formation, cartilage remains an avascular tissue, and induction of angiogenesis is usually linked to bone remodeling or tissue destruction in osteoarthritis, thus reflecting the exquisite ability of MMPs to maintain a delicate homeostatic equilibrium through completely unexpected–and apparently conflicting–activities.

2.3.4. Control of ECM synthesis

MMP-mediated inactivation of the mitogen CTGF results in impairment of ECM deposition by fibroblasts [91]. Importantly, this mechanism may represent an alternative pathway regulated by MMPs that induces phenotypic outcomes resembling connective tissue degradation [28] (Fig. 2). In addition, another protein closely related to CTGF, Cyr61 is cleaved by MMP-14 [27]. Cyr61 is a multifunctional protein that has been reported to promote cell adhesion, migration, proliferation, differentiation and angiogenesis. It is also related to ECM deposition and tumourigenesis, and shows dual cell type-specific anti- and pro-apoptotic activities [92]. The functional consequences of MMP-14 proteolytic processing of Cyr61 are unknown, but likely also result in inactivation, modification or even release of free Cyr61, as it binds to integrins and heparan sulfate for signal transduction [27].

The latent form of transforming growth factor β (TGF-β) can be sequestered within the matrix through the interaction of its latency-associated peptide (LAP) and the ECM component latent TGF-β binding protein (LTBP). Activation of latent TGF-β through cleavage of LAP (Fig. 2) has been reported for MMPs-2, -9, -13 and -14 [93–95] and processed LTBPs are consistently revealed in proteomics analyses of MMPs [27,53]. MMP-2 and MMP-3 cleave and release ECM-tethered LTBP [96,97] and MMPs-2, -3 and -7 degrade the proteoglycan decorin to release latent TGF-β1 [98]. TGF-β activity might be also regulated through MMP-14 and/or MMP-16-dependent shedding of the TGF-β-binding betaglycan, a coreceptor that, when released, can block TGF-β interaction with its signaling receptors *in vitro* [99] (Fig. 2).

MMPs participate in elaborate feedback loops in the control of ECM synthesis and remodeling [3]. Rather than assuming that ECM degradation accounts for tissue remodeling following MMP expression and activation, the reduced synthesis of ECM by decapitation of CTGF anabolic pathways leading to ECM synthesis and accumulation also results in a similar phenotype but through a different, more controlled mechanism [49] (Fig. 2). This is fine-tuned and counterbalanced through TGF-β repression of MMP synthesis and expression, and further strengthened by the MMP-dependent release of latent TGF-β and its activation by cleavages in the LAP and LTBP (Fig. 2). On the other hand, tissue transglutaminase, another MMP substrate, has been reported to be required for TGF-β activation in some cellular systems [100]. Transglutaminase is necessary for the crosslinking of latent TGF-β to the ECM, which promotes TGF-β activation [101]. MMP-dependent degradation of transglutaminase could therefore reduce this TGF-β activation pathway, as an additional level of control of ECM synthesis.

Collagen binding to cell surface integrins also upregulates MMP-14 expression [102,103], and so MMP-14 degradation of cellular collagen would feedback to reduce this integrin-activated expression of MMP-14, allowing native collagen to accumulate. In this dynamic, MMP-14 degradation of β1-integrin-ligated pericellular collagen would release proMMP-2 from its type I collagen binding site for activation by MT1-MMP [104]. Thus, the degradation of cellular collagen would reduce the integrin-activated expression of MMP-14, allowing native collagen to accumulate [3]. In these diverse ways, MMPs can maintain homeostasis of the ECM in the pericellular environment by positively and negatively regulating the cytokines that control ECM synthesis, with substrates, such as collagen, controlling the activation and activity of the MMPs required for their remodeling.

2.3.5. MMP substrates in cell migration

MMPs have traditionally been considered as major facilitators of cellular migration via the breakdown of ECM barriers. However, even when present, these degrading functions are likely of secondary importance to more complex, higher order processes that modulate signal transduction pathways such as proteolysis of extracellular mediators of cell-ECM or cell-cell adhesion, ectodomain shedding, receptor cleavage or exposure of cryptic sites leading to cell motility and invasion. For example MMP-2 and MMP-14 cleave the γ2 chain of laminin-5 to release domain III, exposing a cryptic epitope that induces epithelial cell migration [105,106]. Laminin-5 γ2 fragment levels are reduced in the *Mmp14*-null mouse [107] and it has been shown that MMPs-3, -8, -12, -13 and -20 can cleave laminin-5 γ2 *in vitro* [108]. Another strategy to facilitate cell motility involves cleavage of adherens junction components. More specifically, the transmembrane protein E-cadherin seems to be a primary target for the MMP-dependent disruption of cell-cell contacts. MMP-7 shedding of E-cadherin promoted lung epithelium cell migration and repair [109]. The observed shedding of the 80-kDa soluble ectodomain sE-cadherin by MMP-3 and MMP-7 facilitated invasion and metastasis *in vitro* in a paracrine manner [110,111]. Furthermore, triggering of signal transduction by concomitant release of β-catenin can exacerbate additional proteolysis upon induction of the expression of MMPs-2, -9 and -14 [112,113]. E-cadherin cleavage by MMPs is also linked to the induction of the epithelial-to-mesenchymal transition [110], a transformation associated with aggressive malignant behavior [114]. In addition to E-cadherin, MMP-7 can cleave VE-cadherin,

inducing proliferation of human umbilical vein endothelial cells in culture [115].

Integrins are also pivotal elements for cell adhesion, migration and invasion. These cell-surface proteins mediate cell-cell and cell-matrix adhesion and are involved in molecular signaling and crosstalk processes [87,116]. A controversial paper from the Cheresh group proposed that MMP-2 binds the $\alpha_v\beta_3$ integrin through the hemopexin domain [117]. However, this was later shown to be an experimental artefact, due to a non-specific interaction that was only detected in the presence of BSA-containing ELISA blocking buffer [118], so casting doubt on other studies [119,120]. Nonetheless, the MMP-2 activator MMP-14 participates in the proteolytic maturation of some integrin subunits, such as the components of the $\alpha_v\beta_3$ integrin. Such processing enhances integrin-mediated signal transduction, leading to increased adhesion and migration on the specific $\alpha_v\beta_3$ ligand vitronectin [120], and regulates crosstalk between signals that are mediated by different integrins. On the contrary, tumour-expressed MMP-7 displays an interesting anti-metastatic activity that is dependent on the cleavage of the β_4 subunit, with the consequent abolition of $\alpha_6\beta_4$ integrin binding to the lung endothelial protein CLCA2 and the blockage of tumour cell adhesion and migration [121].

2.3.6. MMP substrates in cellular invasion and metastasis

The transmembrane proteoglycan CD44 binds the hemopexin domain of MMP-2, as well as for several other MMPs, via its extracellular portion, locating the proteases at the migrating front [122-125]. CD44 is an MMP substrate itself, and its proteolytic processing promotes cell mobility through altered cell adhesion functions [125,126]. Non-physiological CD44 fragments are often shed by MMP-14 in malignant tumours, and CD44-mediated anchoring of MMP-2 promotes angiogenesis and invasion by facilitating localized MMP-2 dependent TGF-β activation [127].

MMP-14 and MMP-16 cleave the transmembrane heparan sulfate proteoglycan syndecan-1, which has been associated with inhibition of invasive and migratory properties in the HT1080 fibrosarcoma cell line. MMP-14 is the main candidate for the observed syndecan-1 ectodomain shedding, which correlates with the stimulation of cell migration on a collagen matrix [128]. Shedding of the syndecan-3 ectodomain in cultured rat peripheral nervous system Schwann cells is blocked by various MMP inhibitors, thus enhancing cell adhesion to collagen. This MMP-dependent shedding has been also reported to occur *in vivo* in the peripheral nerve tissue of newborn rats [129].

The surface-associated tissue transglutaminase is essential for the maintenance of integrin dependent cell-ECM adhesion and spreading on fibronectin in both normal and tumour cells. Transglutaminase has been shown to be cleaved in culture by MMP-2 and MMP-14 and by MMPs -15 and MMP-16 *in vitro* [130,131]. Importantly, transglutaminase degradation is dependent on MMP-14 overexpression in glioma and fibrosarcoma cells, and impairs adhesion and migration on fibronectin [131]. The biological effects of MMP activities through transglutaminase appear to be rather complex, as cell mobility in collagen matrices is increased upon transglutaminase degradation. Further investigation is needed in order to assess the consequences of the loss of transglutaminase activities in stroma and cancer cells at tumour leading edges under different conditions. In addition, transglutaminase is involved in stabilization of ECM by enzymatic crosslinking and polymerization of matrix components, making them resistant to proteolysis and different types of stresses [132]. MMP cleavage could then regulate ECM stability and rigidity through controlled transglutaminase inactivation.

The physiological consequences of the degradation of the low-density lipoprotein receptor-related protein (LRP) are a perfect example of the pleiotrophic effects that can be derived from targeted MMP-proteolysis. LRP exerts multiple functions that modulate cell behavior, either as a cargo protein that mediates endocytosis of a wide range of ligands including ECM proteins, growth factors and proteases or as a signal transducer that regulates cell migration [133]. Several membrane-type MMPs degrade LRP, and the degradation of this receptor is associated with MMP-14 overexpression in breast cancer and fibrosarcoma cells [134]. In addition to alterations in cell signaling, loss of LRP enhances invasiveness by blocking endocytosis-mediated degradation of several matrix components and extracellular proteinases, including MMPs-2, -9, -13, urokinase- and tissue-type plasminogen activators. In this way, MMP-14 overexpression exerts a dual role in tumours by activating extracellular pro-MMP-2 and impeding its clearance [135,136]. Protease activated receptor 1 (PAR1) is another cell-surface receptor in which expression levels and transduction pathways are correlated with metastatic potential *in vitro* and *in vivo*. Proteolytic activation of PAR1 by MMP-1 has been demonstrated in PAR-1-transfected breast cancer cells, providing an explanation for the reduction in tumour growth observed in mice treated with MMP-1 specific inhibitors [137,138].

During prostate cancer metastasis development in bone, tumour cells induce the secretion of the transmembrane receptor activator of the nuclear factor κB ligand (RANKL) by osteoblasts, stromal or other malignant cells. When in close contact, these RANKL expressing cells can activate osteoclast-precursor cells via the RANKL receptor RANK. As a consequence, the activated and differentiated osteoclasts degrade the bone matrix during metastasis through the expression of several extracellular proteases. MMP-7 mediated shedding of an active soluble form of RANKL (sRANKL) into the stroma facilitates this activation process by eliminating the need for a close cell-to-cell contact [139]. sRANKL levels and metastasis-related osteolysis were found to be highly reduced in the tumour-bone interface of *Mmp7*-deficient mice when compared with the wild type [140]. Moreover, MMP-7 might not be the only mediator in these processes, as RANKL can be also shed by MMP-14 in cell culture [141].

Many other secreted and soluble molecules are subjected to MMP processing that have the potential to regulate cellular functions, like the tumour suppressor protein KiSS, which is processed to generate the soluble decapeptide known as metastin. Metastin is involved in signaling functions following G-protein coupled receptor binding, and the ligand activity and anti-migratory effects of metastin in HT1080 fibrosarcoma cells is abolished in an MMP dependent process [142]. In another example, the blockage of leukocyte chemoattracting CXC chemokine stromal cell-derived-factor 1 (CXCL12/SDF-1) binding to its receptor (CXCR4) in breast cancer cells strongly decreases metastasis to lung and lymph nodes in mice [143]. CXCL12/SDF-1 is proteolytically inactivated by MMPs-1, -3, -9 and -14 *in vitro* [14], and MMPs are proposed to display anti-metastatic properties through CXCL12/SDF-1 cleavage [7].

The discovery of the signaling molecule substrates described above indicates a different role for MMPs in cellular migration–either in homeostasis or during late cancer stages–one that is much more complex than previously thought, and demonstrates the need for improved understanding of MMP biological functions for the successful use of MMPIs in cancer therapy.

2.3.7. MMP substrates in cell proliferation, tumour growth and apoptosis

The main strategy for MMP control of cell division and proliferation is based on the regulation of growth factor availability and the activation or inactivation of growth factor receptors. We have already described some strategies for the proteolytic release of different ECM- or cell surface-trapped angiogenic factors, which can also show potent mitogenic properties, like FGFs or TGF-β. In addition, the physiological levels of insulin-like growth factors (IGFs) available for receptor binding and triggering of downstream signaling pathways depend on the breakdown of a latency complex formed by IGFs and IGF-binding proteins (IGFBP). MMPs are known to cleave IGFBPs to release active

IGFs e.g. MMPs-1, -3 and -2 cleavage of IGFBP-3 and -5, and MMP-11 cleavage of IGFBP-1 [28,144–146]. The proliferative actions of IGF-1 on smooth muscle cells can be blocked by the expression of an MMP-resistant IGFBP-5 [147], while degradation of IGFBP-1 by MMP-11 enables IGF-1 dependent growth of breast cancer cells [145]. Recently, IGFBP-6 has been identified as a novel MMP-2 substrate in culture by proteomics analysis [55].

MMP-2 also cleaves the hepatoma-derived growth factor (HDGF), which shows growth-stimulating activity in fibroblasts, hepatoma cells, vascular smooth muscle cells, and endothelial cells [148]. Mass-spectrometry-based peptide mapping analysis suggests that MMP-2 is a potential sheddase of HDGF, with part of the full-length molecule being potentially released from the cell-surface or from heparin-bound ECM stores [55].

There is evidence that the multiple protease-regulated functions of heparin-binding epidermal growth factor-like growth factor (HB-EGF) may be mediated by MMPs. The transmembrane form (mHB-EGF) of this molecule acts as a juxtacrine growth inhibitor for neighboring cells, but interestingly, it is converted into a potent paracrine mitogen after shedding of the soluble form (sHB-EGF) [149,150]. sHB-EGF is mitogenic in different cell types [149,151] and is potentially involved in several important physiological processes, including wound healing, neuroendocrine regulation and uterine implantation [149,151]. In cancer, sHB-EGF accelerates tumour growth rate and induces angiogenesis [151,152]. mHB-EGF is cleaved by MMP-3 in culture [153] and by MMPs-2, -9 and -7 *in vivo*; in the latter case there is an additional induction of vasoconstriction in mice [150,154].

The cleavage of HARP/pleiotrophin by MMP-2 induces angiogenesis through the release of VEGF (see above), but in addition HARP itself is a chemoattractant and mitogenic protein. The C-terminal fragment generated upon proteolytic processing of HARP by MMP-2 antagonizes the chemoattractanct and growth promoting activities of the full-length molecule [28]. Members of the follistatin protein family have been recently identified as novel MMP substrates in system-wide proteomics approaches. MMP-2 has been shown to cleave follistatin like-1 protein [28,55], a potent pro-inflammatory and pro-angiogenic molecule that also exerts important growth inhibitory activities in vascular smooth muscle cells and human lung cancer cell lines [155], thereby suggesting another pro-tumourigenic mechanism for MMP-2 [28]. The related follistatin-like 3 protein is cleaved by both MMP-2 and MMP-14 *in vitro* [27]. Hence, growth factor binding proteins that mask cytokine activity are proposed to constitute a major new class of MMP substrates [28].

MMPs-2, -3 and -7 cleave osteopontin [55,156], a multifunctional small integrin binding ligand N-linked glycoprotein (SIBLING) that is involved in cell adhesion and migration, wound healing, bone remodeling or apoptosis. Agnihotri and colleagues [156] have shown that MMP-3 and MMP-7 processing of osteopontin enhances its cell adhesive and migratory properties through integrin binding *in vitro*. Co-expression of MMPs with osteopontin and detection of MMP-specific cleavage products in tumour cell lines and remodeling tissues *in vivo* suggests MMP-dependent regulation of osteopontin activities in wound healing and in tumours. Another SIBLING, bone sialoprotein (BSP), was recently shown in a critical analysis of its binding and proMMP-2 activation properties neither to bind nor activate the enzyme [157], as originally proposed [158].

Tumours have developed many mechanisms, some of them mediated by MMPs, to block immune cell infiltration and evade the immune response. MMP-9 cleaved the interleukin-2 receptor-α (IL-2Rα) of T lymphocytes in culture, thus blocking their proliferative response to IL-2, and MMP degradation of IL-2Rα suppressed the infiltration of tumour-specific cytotoxic T lymphocytes *in vivo* [159]. Cleavage of α1-proteinase inhibitor (α1-PI) by MMPs-1, -3, -7, -8, and -11 releases a proteolytic fragment that reduces natural killer cytotoxic effects on tumour cells [160]. MMPs can target different cytokines and chemokines to modulate inflammatory and immunologic responses as well (see following section). These mechanisms are also relevant in the promotion or prevention of leukocyte infiltration into the tumour, but it must be stressed that anti-tumour immune activities can eventually enhance cancer progression through the negative effects of chronic inflammation, including MMP secretion by inflammatory cells [7].

Evasion of cell death and apoptosis is another successful strategy used by tumours to promote cell proliferation, division and growth. MMPs can either trigger or prevent apoptosis by proteolytic processing of certain signaling molecules. The Fas/Fas ligand (FasL) signal transduction axis is one of the most important pro-apoptotic systems affected by MMPs. MMP-3 and MMP-7 can shed the transmembrane FasL from the cell surface, rendering a soluble FasL (sFasL) that retains the ability to bind to its receptor, Fas, but that exhibits lower pro-apoptotic potency [161]. MMP-7-mediated release of sFasL has different cell-type-specific outcomes, as it induces apoptosis in epithelial cells whereas inhibits cell death in tumours [162,163]. However, MMP-7 also cleaves Fas to inhibit apoptosis in cancer cells [164].

MMP-14 is responsible for cleaving the ectodomain of death receptor-6 [53], a transmembrane member of the tumour necrosis factor (TNF) receptor family that induces cell death by signal transduction through its cytoplasmic domain [165]. Another mechanism of inhibition of apoptosis is related to the previously described activation of HB-EGF by MMP-7, which triggers an intracellular signaling pathway to promote cell survival following interaction of HB-EGF with the ERBB4 receptor tyrosine kinase [123]. On the contrary, MMPs can also enhance apoptotic processes through disruption of cell-cell interactions and subsequent cell rounding, mediated mainly through the cleavage of cadherins and other molecules like the platelet endothelial-cell adhesion molecule (PECAM-1). MMP-dependent proteolysis of the full-length membrane-bound PECAM-1 generates a truncated form that reduces cell-proliferation and increases apoptosis [166].

2.3.8. MMP substrates in inflammation, innate immunity and wound healing

Inflammation is a common process in many pathologies and during the healing response to injury and infection. Leukocyte recruitment, a hallmark of inflammation, is primarily triggered and regulated by chemoattractant cytokines known as chemokines. We have found that precise MMP processing can inactivate, enhance, antagonize or disperse the chemotactic properties of chemokines, therefore influencing the final outcome of the inflammatory response and regulating leukocyte homing in homeostasis.

Utilizing a yeast two-hybrid Exosite Scanning screen, McQuibban et al. [15] first identified the chemokine CCL7/MCP-3 as a novel MMP-2 substrate, so opening the door to a new field of investigation for MMP biology. MMP-2 cleavage of CCL7/MCP-3 generated an inactive antagonist molecule that retains the ability to interact with its CCR1, CCR2 and CCR3 receptors, thus dampening full-length CCL7/MCP-3-dependent responses *in vitro* and inflammation *in vivo* [15]. Follow up experiments showed that this functional shift upon MMP cleavage is conserved throughout the CC chemokine family, as CCL7/MCP-3, CCL2/MCP-1, CCL8/MCP-2 and CCL13/MCP4 are also cleaved by multiple MMPs [15,17,20] (Table 1). Another chemokine, CX_3CL1/fractalkine, which has been described as pro-inflammatory in rheumatoid arthritis [167], is also sequentially cleaved by MMP-2. After a shedding from the cell membrane, the molecule undergoes N-terminal processing by MMP-2 to convert a cell surface agonist to a soluble antagonist that could have protective effects for arthritis and other disease processes [55]. Hence, MMPs form a negative feedback loop for controlling macrophage recruitment so suppressing macrophage influx and activation *in vivo* [15].

In neutrophils, the story is reversed. The major polymorphonuclear leukocyte (PMN) chemoattractants are the ELR^+ CXC

Table 1
Cleavage of human chemokines by MMPs.

Function	Chemokine	MMP	Comments
Activation	CXCL8/IL-8	MMP1, -8, -9, -13, - 14	
	CXCL5/ENA-78	MMP-1, -8	
Inactivation	CXCL1/Gro-α	MMP-9, -12	Cleaved at E-LR by MMP-12
	CXCL2/Gro-β	MMP-12	Cleaved at E-LR
	CXCL3/Gro-γ	MMP-12	Cleaved at E-LR
	CXCL8/IL-8	MMP-12	Cleaved at E-LR
	CXCL4/PF-4	MMP-9	
	CXCL7/CTAP-III	MMP-9	
	CXCL11/ITAC	MMP-7, -9	MMP-7 degrades the chemokine
	CXCL12/SDF-1	MMP-1, -2, -3, -9, - 13, -14	MMP-2-cleaved form is neurotoxic
	CXCL5/ENA-78	MMP-9, -12	Cleaved at E-LR by MMP-12, MMP-9 could transiently activate before degradation *in vitro*.
	CXCL9/MIG	MMP-7, -8, -9, -12	Cox et al. [177] could not detect previously reported MMP-8, -9 cleavage [172].
	CXCL10/IP-10	MMP-7, -8, -9, -12	Cox et al. [177] could not detect previously reported MMP-8, -9 cleavage [172].
	CXCL6/GCP-2	MMP-1, -9, -12	MMP-12 cleaves C-terminal to ELR. MMP-1 and -9 degrade the chemokine.
Switch to antagonist	CCL2/MCP-1	MMP-1, -3, -8, -12	
	CCL8/MCP-2	MMP-1, -3, -12	
	CCL7/MCP-3	MMP-1, -2, -3, -12, - 13, -14	
	CCL13/MCP-4	MMP-1, -12	
	CX_3CL1/fractalkine	MMP-2	Two cleavages, First for shedding, second for conversion.
	CXCL11/ITAC	MMP-8, -12	Antagonist fragment (5-73) shows enhanced heparin binding. MMP-12 products are transient followed by degradation *in vitro*.
No change	CXCL6/GCP-2	MMP-8, -9	

chemokines, which signal through the receptor CXCL2 and that are exemplified by CXCL8/IL-8 in man and by GCP-2/LIX (LPS induced CXC chemokine) in the mouse. Interestingly, the neutrophil-specific MMP, MMP-8 (also known as collagenase-2), processes the N-terminus of both CXCL8/IL-8 and GCP-2/LIX upstream of the ELR motif to *enhance* chemotactic potency [18,19]. Indeed, MMP-8 null mice showed impaired recruitment of PMNs in an LPS-induced inflammation model, indicating the high specificity and relevance of MMP-8 in the immune response, in this instance by a feed-forward mechanism for cell recruitment [19]. Notably, MMPs-1, -2, -8, -9, -12, -13 and -14 also process and activate GCP-2/LIX and/or CXCL8/IL-8 at the same or nearby sites [18,19] (Table 1). What is critically important is that *in vivo* GCP-2/LIX cleavage in the *Mmp8*$^{-/-}$ mice is not compensated by the other MMPs that were shown to cleave and activate GCP-2/LIX in biochemical assays. For instance, MMP-9 cleaves GCP-2/LIX (and CXCL8/IL-8) at the exact same cleavage site that MMP-8 does with equally good kinetic parameters; in addition, it is also present in the same PMN secretory granules as MMP-8, and is even expressed at higher levels than in wild-type mice. Yet, MMP-9 does not compensate for the lack of MMP-8 cleavage and activation of GCP-2/LIX *in vivo* [19]. This is not to say that MMP-9 is not involved in the response, but that it is biologically a minor contribution compared with the MMP-8 response, despite the similar biochemical parameters. Indeed, similar perturbations in PMN chemotaxis have not been reported for the *Mmp9*$^{-/-}$ mouse. As found with CXCL8/IL-8, similar effects were found for another human orthologue of GCP-2/LIX, CXCL5/ENA-78. The induction of an intracellular calcium influx and chemotaxis by CXCL8/IL-8 were strongly increased after MMP-8, -9, -13 and -14 cleavage, akin to the MMP-1 and MMP-8 cleaved forms of CXCL5/ENA-78 [18]. In addition, CXCL5/ENA-78 is sequentially cleaved by MMP-9 (Table 1). Initial cleavage by MMP-9 resulted in N-terminally truncated forms of the chemokine that were 3-8-fold more active than the full-length molecule, however after prolonged incubation complete degradation of CXCL5/ENA-78 occurred [18].

Most members of the CXCL chemokine family are inactivated by proteolysis of their specific ELR receptor-binding motif by the macrophage-specific MMP-12. Such disruption of the interaction of CXCL1/Gro-α, CXCL2/Gro-β, CXCL3/Gro-γ, CXCL5/ENA-78, CXCL6/GCP-2 and CXCL8/IL-8 with their receptor has been proposed to participate in the resolution of LPS-induced influx of PMN leukocytes by macrophages by taking an active role in this shutting down of the neutrophil response [20]. CXCL1/Gro-α is also inactivated by MMP-9, like CXCL4/PF-4 and CXCL7/CTAP-III [16]. CXCL9/MIG and CXCL10/IP-10 are rendered functionally inactive after C-terminal cleavage by MMP-8 and MMP-9 [168]. During HIV infection, astrocytes over-express the chemokine CXCL12/SDF-1, which is cleaved at position 4-5 by CNS macrophage MMP-2, itself activated by neuron derived MMP-14. The cleaved form of CXCL12/SDF-1 is chemotactically inactive but highly neurotoxic, and induces neuronal apoptosis and inflammation in mice [169]. This effect was later found to be exerted through switching receptor specificity to CXCL3 [170].

In vivo, chemokines are generally immobilized on the ECM or cell membrane through interactions with glycosaminoglycans (GAGs) to form temporally and spatially stable haptotactic gradients. MMPs have the capability to modulate these gradients, either by cleavage of the proteoglycan core protein [171,172] or by cleaving off the C-terminal GAG binding motifs from the chemokines themselves so removing the chemokine-ECM binding properties [173]. For instance, it has been shown that after bleomycin lung injury, neutrophils of *Mmp7*-null mice accumulate in the perivascular region and are unable to infiltrate into the alveolar lumen. It is also known that, after damage, endothelial cells secrete the neutrophil chemokine CXCL1/ Gro-α, which is tethered to syndecan-1 after secretion. MMP-7, secreted by the same cells, cleaves syndecan to create a syndecan-1-CXCL1/ Gro-α neutrophil chemokine gradient across the alveolar wall that seems to be essential for neutrophil invasion of the alveolar space. In *Mmp7*$^{-/-}$ mice, this gradient formation is reduced since MMP-7 cleavage of syndecan is lost [171,172]. Other MMPs shed syndecan-1, like MMPs-14, -16 and -9, with the latter also cleaving syndecan-4 [128,174]. In contrast, the human T lymphocyte chemokine CXCL11/ITAC, which signals via CXCR3, is cleaved at its C-terminal α-helix. This results in loss of GAG binding and hence causes dispersion of the haptotactic gradient with concomitant loss of directed T lymphocyte migration [173]. Collectively, these types of activities appear pivotal for the establishment, maintenance and resolution of chemokine gradients during inflammatory responses.

Many studies also show the involvement of MMPs *in vivo* in the generation of chemotactic gradients. Leukocyte accumulation in the lung parenchyma of allergen-induced asthmatic *Mmp2*-null mice is correlated with a decrease of free CCL11/eotaxin levels [175]. *Mmp8*-knockout mice showed defective neutrophil recruitment due to

impaired release of GCP-2/LIX [176]. Reduction of eosinophils and neutrophils in brochoalveolar lavages of allergen-challenged lungs in *Mmp2* and *Mmp2/9*-deficient mice correlates with the significant decrease of CCL11/eotaxin, CCL7/MCP-3 and CCL17/TARC levels [177]. Nevertheless, similar experiments performed with *Mmp9*-null mice reported different outcomes, with different cell migration effects and chemokine levels in both BAL fluids and parenchyma tissues [178–180], suggesting that regulation of the inflammatory response by MMPs may depend on the nature of the stimulus, and hence the MMPs expressed. The MMP substrates that mediate these altered functions in the resolution of inflammation remain unknown, but they are likely pericellular proteoglycans or other ECM-components involved in chemokine interactions.

The involvement of MMP activities in inflammatory responses and wound healing could explain the beneficial roles played by certain MMPs. Balbín and colleagues [45] reported the first protective function for an MMP after the observation of increased chemical-induced skin tumourigenesis in MMP-8-deficient mice. These mice also showed delayed wound healing, associated with impaired neutrophil infiltration to the injured area [181] and worsened outcome in arthritis [182]. Interestingly, enhanced inflammation due to neutrophil retention is observed during the last stages of the healing process at a stage normally characterized by massive apoptosis of the inflammatory cells. Altered SMAD2 activation and SMAD3 phosphorylation were observed in these mice due to low levels of circulating TGF-β1, which is also an important pro-inflammatory cytokine with relevant implications in wound healing [181]. In addition, perturbed generation and dispersion of chemotactic gradients involved in the resolution of inflammation could lead to the development of chronic inflammatory response in arthritis and other inflammatory diseases, and so also to the increased tumourigenesis of the chemical-induced skin cancer model.

The interaction of MMP activities with chemotactic and inflammatory processes is not limited to chemokine processing. Several proinflammatory cytokines are also affected by MMP proteolysis. TNF-α, a potent pro-inflammatory cytokine, can be proteolytically activated by many MMPs *in vitro* and by MMPs-7, -12, -14 and -17 in cultured cells [53,183,184]. MMP-7 has been shown to activate TNF-α signaling in a herniated disc culture model to eventually induce macrophage infiltration leading to disc resorption [185]. Another pro-inflammatory cytokine, interleukin-1β (IL-1β) is activated by MMPs-2, -3 and -9, with further incubation driving to degradation of the mature cytokine, suggesting a tight regulation of IL-1β activity by MMPs [186,187]. However, these analyses were performed at very high enzyme to substrate ratios and so it cannot be assumed that this is a biologically relevant pathway *in vivo*. Some reports also suggest alternative regulation of IL-1β functions either through shedding of its type II IL-1 decoy receptor to generate a soluble form that interacts and inhibits IL-1β [188], or through degradation of the receptor [189]. Another example of the relevance of MMP actions in inflammation is the ability of MMP-9 to inactivate interferon-β [190].

MMP processing of ECM components can also release cleavage fragments with chemotactic properties. The tripeptide Pro–Gly–Pro (PGP) is obtained by MMP-9-driven proteolysis of collagen. This product mimics binding of Glu–Leu–Arg motif-containing ELR^+ CXC chemokines to CXCR1 and 2 and stimulates neutrophil migration [191]. On the other hand, elastin fragments obtained through MMP-12 cleavage can drive the influx of macrophages, as observed in a murine model of emphysema [192]. Even non-ECM proteins like the MMP-12-cleaved serine protease inhibitor α1-PI can generate proteolytic products that induce chemoattraction of neutrophils [193].

MMPs also influence chemoattraction by non-chemokine substrates by creating gradients with different classes of chemotactic proteins. In the search for MMP-substrates linked to allergic inflammation, *Mmp2* and *Mmp9*-defective mice were compared with their wild type counterparts after being challenged with an allergen. Three candidate pro-inflammatory proteins identified proteomically, Ym1, S100A8 and S100A9 showed further altered chemoattraction *in vitro* after being cleaved by MMP-2 and -9. The reduction of inflammatory cell migration into the alveolar space after blockage of the S100 proteins with specific antibodies indicates that MMPs might also influence the resolution of inflammation by cleavage of S100 proteins [60]. This is also true for the cyclophilin B or peptidyl-prolyl isomerase, an intracellular protein that is exported to the cell surface and mobilized by MMPs via heparan sulfate proteglycan cleavage. Release of cyclophilin B from chondrocytes by MMPs-1, -2, -3, -9 and -13, induces chemotaxis in human neutrophils and T cells [194]. In addition, cyclophilin A was recently found to be cleaved by MMP-14 in cultured MDA-MB-213 breast cancer cells, likely resulting in release from its tethering interaction with heparan sulphate glycosaminoglycans, but through a different MMP-dependent mechanism than cyclophilin B [27]. MMPs can participate in inflammatory cell trafficking into damaged tissues through non-chemotactic signaling pathways. MMP-2 cleaves big endothelin-1 (ET-1) to produce an active peptide that binds to the neutrophil receptor endothelin A and triggers the MAP kinases (MAPK) pathway to participate in the transition from adhesive to migratory stage in endothelial cells [195].

MMP release of signaling molecules can also impact the immune response via the mobilization of hematopoietic progenitor cells from the bone marrow as first proposed for MMP-9 cleavage of CXCL12/SDF [14]. Several MMPs including MMP-9, which is stimulated by macrophage colony-stimulating factor (GMCSF) and granulocyte colony-stimulating factor (GCSF) in bone marrow stromal cells, cleave and inactivate CXCL12/SDF-1 at position 4–5. So, by transiently reducing the bone marrow hematopoietic cell retention signal, McQuibban and colleagues [14] proposed that this is the mechanism for mobilization of stem cells, rather than by MMP-9 cleavage of basement membrane components. MMP-9 also promoted shedding of the membrane-associated kit-ligand (also termed stem cell factor) following the activation of a VEGF receptor-1 (VEGFR1)-dependent signaling pathway [196]. Proteolytic processing by MMPs is also involved in pathogen-induced innate immunologic responses. For example MMP-7 activates the bactericidal α-defensins cryptdin-1 and -4 in the murine intestinal Paneth cells [197,198]. In contrast, MMP-1 and MMP-14 cleave the multifunctional protein pentraxin-3 [27], which is involved in pro-inflammatory response and protection against bacterial infections [199].

MMPs have developed many precise and non-degradative ways to modulate the inflammatory and immune responses via tight proteolytic modification of bioactive mediators that participate in highly regulated cellular functions. The complete understanding of the MMP-mediated processes described above–and many others yet to be discovered–is pivotal for the development of efficient MMPI strategies, thus representing a degradomics challenge for the following years.

2.3.9. Interactive proteolytic systems. The protease web

MMP biological functions reach another level of complexity when their interconnections with other proteases are taken into account. There are more than 560 putative proteases in the human degradome [48,200] that are responsible for the generation of irreversible (with the exception of deubiquitination) post-translational modifications of every protein [49]. Tight regulation and control of these activities is achieved through interconnected pathways, amplification cascades and self-regulatory feedback loops that occur within and between families of proteases. Degradomics data generated by recent high-throughput proteomics screens has further revealed the depth of the roles that MMPs play in regulating other classes of proteases and their inhibitors [27,53,55]. In addition to cleaving other members of their

own family, MMPs can process other proteases, such as ADAMTS-4 [201] or uPA [202], and also many different protease inhibitors like cystatin C [55], plasma serine protease inhibitor (SLPI) [53], tissue factor pathway inhibitor [203,204] and several serpins [205–208]. Hence, MMPs can also modulate the net proteolytic potential through modulation of the activation states of different protease classes. From a system biology perspective, different protease classes and families are interconnected in an interactive functional network, termed the protease web [26,49], with MMPs acting as key nodal proteases. As a consequence, deregulation or inhibition of an MMP can cause unexpected pleiotropic effects derived from alteration of other proteolytic pathways. In the previously described pharmacoproteomics experiment carried out in MDA-MB-231 MMP-14-transfected cells, quantitative proteomics analyses reported global changes in protease levels upon inhibition with Prinomastat. Many of the 37 proteases and 10 protease inhibitors identified in the experiment showed significant changes in level, reflecting strong disturbances along the protease web when MMP activities are blocked [27]. Other proteases, and mainly the closely related ADAM metalloproteinases, may also perform some of the signaling functions ascribed to MMPs. For instance, functional redundancy could exist for the MMP- and ADAM-mediated ectodomain shedding of molecules like N-, E- and VE-cadherins [209–211], TNF-α [212–214], CD44 [215], FasL [216] or EGFR ligands like HB-EGFR [217–222]. These findings stress the need for a comprehensive understanding of the underlying mechanisms of the protease web, in order to predict the consequences of MMP inhibition for optimal drug development. Indeed, the Kruger lab has shown that MMPIs can lead to altered protease expression in murine models of cancer [223–225]. Host overexpression of TIMP-1 induces an important increase in liver metastasis, partially supported by upregulation of metastasis-related genes, including several cystein proteases that enhanced net proteolytic activity in the liver microenvironment [223]. Interestingly, overexpression of cystatin C did not reduce TIMP-1-induced metastasis burden and, moreover, induced expression of plasminogen activators. Further overexpression of plasminogen activator inhibitor-2 reverted the metastatic phenotype [224]. The authors propose that excess of circulating TIMP-1 could be priming liver cells by promoting the formation of a *premetastatic niche*. Thus, microenvironmental disturbances of the protease web that alter gene expression and consequently organ homeostasis can be responsible for the increase in liver subsceptibility to metastasis [224].

3. Conclusions and future perspectives

The great number of new substrates discovered for MMPs in the last few years raises the significance of MMPs in regulating many signaling pathways and hence cell behavior. However, in simple biochemical experiments where substrates are cleaved by MMPs in the test tube, careful interpretation is needed. Many substrates discovered are not cleaved in cell culture or *in vivo* using mouse models of disease. This reiterates an important axiom in substrate discovery and the search for biological roles for proteases: "Just because it can, does not mean it does" [51]—biochemical evidence cannot necessarily be translated to cell culture nor to animal models and certainly not necessarily to man. Nonetheless, *Mmp* gene knockout mice show the response of a complex system the loss of one of its components, and not just the effects of the loss of the enzyme alone. Hence, interpretation of knockout mice is difficult. However, what is now clear is that the majority of MMP substrates are non-matrix molecules and indeed we propose that a major function of MMPs is to mobilize growth factors and cytokines—not only by cleaving the ECM molecules they might be bound to as originally proposed, but by cleavage and dissociation of cytokine and growth factor binding proteins such as CTGF, pleotrophin, follistatin and IGFBPs from their cognate cytokines and growth factors. This unmasking of cytokines is likely to be a key new biological role for MMPs in the control of cell function and homeostasis in many tissues.

The strong association between deregulated MMP activities and disease development raised great expectations for MMPs as promising drug targets. The unexpected failure of early clinical trials was discouraging but this does not justify the total resignation of MMPI programs, as these trials were designed using incorrect or at least incomplete assumptions about the actual *in vivo* functional roles of MMPs [26,33]. Recent efforts in the degradomics field are helping to understand that MMPs orchestrate highly complex signaling pathways through sophisticated proteolytic processing of messenger molecules and receptors, leaving ECM degradation and remodeling as a secondary role in homeostasis and disease. Hence, the positive association between MMP activities and disease development is not as straightforward as it was assumed before. Now we know that MMPs can regulate cell proliferation and migration, programmed death, angiogenesis, immunogenic response and inflammation, tumour growth and several other processes. Further, MMP regulation of other proteases, both directly or indirectly through cleavage and inactivation of protease inhibitors, renders interpretation of data from MMPI experiments and $Mmp^{-/-}$ mouse models of disease very difficult.

From this new perspective, many MMP substrates and functions remain unknown, particularly for the more recently described MMPs, necessitating the development of high-throughput, high-content genomics and proteomics discovery techniques. Definitive validation of the new functional activities must then be performed at the organism level using specifically designed *in vivo* animal models. The achievement of an integrative, system-wide functional annotation of MMPs and their interactions with the protease web during development, homeostasis and disease should lay the foundations for a new wave of clinical trials, with MMPIs blocking disease related-MMP targets and/or their downstream effectors, while leaving anti-targets unaffected for the sake of high efficiency and reduced secondary effects. At least initially, however, these clinical indications will be limited to short term clinical dosing in order to reduce side effects, but more particularly, those derived from blockage of MMP anti-targets. Nonetheless, the importance and widespread biological roles of MMPs strongly suggest that MMP deregulation is an important pathogenic factor in many diseases and so too remains a promising vista for drug targeting.

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References

[1] J. Gross, C.M. Lapiere, Collagenolytic activity in amphibian tissues: a tissue culture assay, Proc. Natl. Acad. Sci. U. S. A. 48 (1962) 1014–1022.
[2] H. Nagase, J.F.J. Woessner, Matrix metalloproteinases, J. Biol. Chem. 274 (1999) 21491–21494.
[3] C.M. Overall, Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites, Mol. Biotechnol. 22 (2002) 51–86.
[4] J.D. Mott, Z. Werb, Regulation of matrix biology by matrix metalloproteinases, Curr. Opin. Cell Biol. 16 (2004) 558–564.

[5] A. Page-McCaw, A.J. Ewald, Z. Werb, Matrix metalloproteinases and the regulation of tissue remodelling, Nat. Rev., Mol. Cell Biol. 8 (2007) 221–233.
[6] C.M. Alexander, E.J. Hansell, O. Behrendtsen, M.L. Flannery, N.S. Kishnani, S.P. Hawkes, Z. Werb, Expression and function of matrix metalloproteinases and their inhibitors at the maternal-embryonic boundary during mouse embryo implantation, Development 122 (1996) 1723–1736.
[7] M. Egeblad, Z. Werb, New functions for the matrix metalloproteinases in cancer progression, Nat. Rev. Cancer 2 (2002) 161–174.
[8] J.C. Currie, S. Fortier, A. Sina, J. Galipeau, J. Cao, B. Annabi, MT1-MMP down-regulates the glucose 6-phosphate transporter expression in marrow stromal cells: a molecular link between pro-MMP-2 activation, chemotaxis, and cell survival, J. Biol. Chem. 282 (2007) 8142–8149.
[9] T.H. Vu, Z. Werb, Matrix metalloproteinases: effectors of development and normal physiology, Genes Dev. 14 (2000) 2123–2133.
[10] W.C. Parks, C.L. Wilson, Y.S. Lopez-Boado, Matrix metalloproteinases as modulators of inflammation and innate immunity, Nat. Rev. Immunol. 4 (2004) 617–629.
[11] T.E.J. Curry, K.G. Osteen, The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle, Endocr. Rev. 24 (2003) 428–465.
[12] D.L. Hulboy, L.A. Rudolph, L.M. Matrisian, Matrix metalloproteinases as mediators of reproductive function, Mol. Hum. Reprod. 3 (1997) 27–45.
[13] Y.S. Lopez-Boado, C.L. Wilson, L.V. Hooper, J.I. Gordon, S.J. Hultgren, W.C. Parks, Bacterial exposure induces and activates matrilysin in mucosal epithelial cells, J. Cell Biol. 148 (2000) 1305–1315.
[14] G.A. McQuibban, G.S. Butler, J.H. Gong, L. Bendall, C. Power, I. Clark-Lewis, C.M. Overall, Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1, J. Biol. Chem. 276 (2001) 43503–43508.
[15] G.A. McQuibban, J.H. Gong, E.M. Tam, C.A. McCulloch, I. Clark-Lewis, C.M. Overall, Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3, Science 289 (2000) 1202–1206.
[16] P.E. Van den Steen, P. Proost, A. Wuyts, J. Van Damme, G. Opdenakker, Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-alpha and leaves RANTES and MCP-2 intact, Blood 96 (2000) 2673–2681.
[17] G.A. McQuibban, J.H. Gong, J.P. Wong, J.L. Wallace, I. Clark-Lewis, C.M. Overall, Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties *in vivo*, Blood 100 (2002) 1160–1167.
[18] P.E. Van Den Steen, A. Wuyts, S.J. Husson, P. Proost, J. Van Damme, G. Opdenakker, Gelatinase B/MMP-9 and neutrophil collagenase/MMP-8 process the chemokines human GCP-2/CXCL6, ENA-78/CXCL5 and mouse GCP-2/LIX and modulate their physiological activities, Eur. J. Biochem. 270 (2003) 3739–3749.
[19] A.M. Tester, J.H. Cox, A.R. Connor, A.E. Starr, R.A. Dean, X.S. Puente, C. Lopez-Otin, C.M. Overall, LPS responsiveness and neutrophil chemotaxis *in vivo* require PMN MMP-8 activity, PLoS ONE 2 (2007) e312.
[20] R.A. Dean, J.H. Cox, C.L. Bellac, A. Doucet, A.E. Starr, C.M. Overall, Macrophage-specific metalloelastase (MMP-12) truncates and inactivates ELR+ CXC chemokines and generates CCL2, -7, -8, and -13 antagonists: potential role of the macrophage in terminating polymorphonuclear leukocyte influx, Blood 112 (2008) 3455–3464.
[21] M. Ferreras, U. Felbor, T. Lenhard, B.R. Olsen, J. Delaisse, Generation and degradation of human endostatin proteins by various proteinases, FEBS Lett. 486 (2000) 247–251.
[22] Z. Dong, R. Kumar, X. Yang, I.J. Fidler, Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma, Cell 88 (1997) 801–810.
[23] M.S. O'Reilly, D. Wiederschain, W.G. Stetler-Stevenson, J. Folkman, M.A. Moses, Regulation of angiostatin production by matrix metalloproteinase-2 in a model of concomitant resistance, J. Biol. Chem. 274 (1999) 29568–29571.
[24] B.C. Patterson, Q.A. Sang, Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9), J. Biol. Chem. 272 (1997) 28823–28825.
[25] C.M. Overall, Dilating the degradome: matrix metalloproteinase 2 (MMP-2) cuts to the heart of the matter, Biochem. J. 383 (2004) e5–e7.
[26] C.M. Overall, O. Kleifeld, Tumour microenvironment-opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy, Nat. Rev. Cancer 6 (2006) 227–239.
[27] G.S. Butler, R.A. Dean, E.M. Tam, C.M. Overall, Pharmacoproteomics of a metalloproteinase hydroxamate inhibitor in breast cancer cells: dynamics of membrane type 1 matrix metalloproteinase-mediated membrane protein shedding, Mol. Cell. Biol. 28 (2008) 4896–4914.
[28] R.A. Dean, G.S. Butler, Y. Hamma-Kourbali, J. Delbe, D.R. Brigstock, J. Courty, C.M. Overall, Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affin regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis, Mol. Cell. Biol. 27 (2007) 8454–8465.
[29] M.H. Tayebjee, G.Y. Lip, R.J. MacFadyen, Matrix metalloproteinases in coronary artery disease: clinical and therapeutic implications and pathological significance, Curr. Med. Chem. 12 (2005) 917–925.
[30] J. Hu, P.E. Van den Steen, Q.X. Sang, G. Opdenakker, Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases, Nat. Rev. Drug Discov. 6 (2007) 480–498.
[31] G. Murphy, H. Nagase, Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat. Clin. Pract. Rheumatol. 4 (2008) 128–135.
[32] V.W. Yong, Metalloproteinases: mediators of pathology and regeneration in the CNS, Nat. Rev. Neurosci. 6 (2005) 931–944.
[33] C.M. Overall, C. Lopez-Otin, Strategies for MMP inhibition in cancer: innovations for the post-trial era, Nat. Rev. Cancer 2 (2002) 657–672.
[34] S.R. Bramhall, A. Rosemurgy, P.D. Brown, C. Bowry, J.A. Buckels, Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial, J. Clin. Oncol. 19 (2001) 3447–3455.
[35] L.M. Coussens, B. Fingleton, L.M. Matrisian, Matrix metalloproteinase inhibitors and cancer: trials and tribulations, Science 295 (2002) 2387–2392.
[36] S. Filippov, G.C. Koenig, T.H. Chun, K.B. Hotary, I. Ota, T.H. Bugge, J.D. Roberts, W.P. Fay, H. Birkedal-Hansen, K. Holmbeck, F. Sabeh, E.D. Allen, S.J. Weiss, MT1-matrix metalloproteinase directs arterial wall invasion and neointima formation by vascular smooth muscle cells, J. Exp. Med. 202 (2005) 663–671.
[37] F. Sabeh, I. Ota, K. Holmbeck, H. Birkedal-Hansen, P. Soloway, M. Balbin, C. Lopez-Otin, S. Shapiro, M. Inada, S. Krane, E. Allen, D. Chung, S.J. Weiss, Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP, J. Cell Biol. 167 (2004) 769–781.
[38] H. Lee, C.M. Overall, C.A. McCulloch, J. Sodek, A critical role for the membrane-type 1 matrix metalloproteinase in collagen phagocytosis, Mol. Biol. Cell 17 (2006) 4812–4826.
[39] V. Everts, E. van der Zee, L. Creemers, W. Beertsen, Phagocytosis and intracellular digestion of collagen, its role in turnover and remodelling, Histochem. J. 28 (1996) 229–245.
[40] L.H. Engelholm, K. List, S. Netzel-Arnett, E. Cukierman, D.J. Mitola, H. Aaronson, L. Kjoller, J.K. Larsen, K.M. Yamada, D.K. Strickland, K. Holmbeck, K. Dano, H. Birkedal-Hansen, N. Behrendt, T.H. Bugge, uPARAP/Endo180 is essential for cellular uptake of collagen and promotes fibroblast collagen adhesion, J. Cell Biol. 160 (2003) 1009–1015.
[41] J. Sodek, C.M. Overall, in: Z. Davidovitch (Ed.), Biological Mechanisms of Tooth Eruption and Root Resorption, EBSCO Media, Birmingham, AL, 1988, pp. 303–312.
[42] A.R. Folgueras, A.M. Pendas, L.M. Sanchez, C. Lopez-Otin, Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies, Int. J. Dev. Biol. 48 (2004) 411–424.
[43] C.P. Blobel, Functional and biochemical characterization of ADAMs and their predicted role in protein ectodomain shedding, Inflamm. Res. 51 (2002) 83–84.
[44] S. Cal, A.J. Obaya, M. Llamazares, C. Garabaya, V. Quesada, C. Lopez-Otin, Cloning, expression analysis, and structural characterization of seven novel human ADAMTSs, a family of metalloproteinases with disintegrin and thrombospondin-1 domains, Gene 283 (2002) 49–62.
[45] M. Balbin, A. Fueyo, A.M. Tester, A.M. Pendas, A.S. Pitiot, A. Astudillo, C.M. Overall, S.D. Shapiro, C. Lopez-Otin, Loss of collagenase-2 confers increased skin tumor susceptibility to male mice, Nat. Genet. 35 (2003) 252–257.
[46] C. Lopez-Otin, L.M. Matrisian, Emerging roles of proteases in tumour suppression, Nat. Rev. Cancer 7 (2007) 800–808.
[47] M.D. Martin, L.M. Matrisian, The other side of MMPs: protective roles in tumor progression, Cancer Metastasis Rev. 26 (2007) 717–724.
[48] C. Lopez-Otin, C.M. Overall, Protease degradomics: a new challenge for proteomics, Nat. Rev., Mol. Cell Biol. 3 (2002) 509–519.
[49] A. Doucet, G.S. Butler, D. Rodriguez, A. Prudova, C.M. Overall, Metadegradomics: toward *in vivo* quantitative degradomics of proteolytic post-translational modifications of the cancer proteome, Mol. Cell Proteomics 7 (2008) 1925–1951.
[50] B.E. Turk, L.L. Huang, E.T. Piro, L.C. Cantley, Determination of protease cleavage site motifs using mixture-based oriented peptide libraries, Nat. Biotechnol. 19 (2001) 661–667.
[51] C.M. Overall, C.P. Blobel, In search of partners: linking extracellular proteases to substrates, Nat. Rev., Mol. Cell Biol. 8 (2007) 245–257.
[52] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, R. Aebersold, Quantitative analysis of complex protein mixtures using isotope-coded affinity tags, Nat. Biotechnol. 17 (1999) 994–999.
[53] E.M. Tam, C.J. Morrison, Y.I. Wu, M.S. Stack, C.M. Overall, Membrane protease proteomics: Isotope-coded affinity tag MS identification of undescribed MT1-matrix metalloproteinase substrates, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6917–6922.
[54] P.L. Ross, Y.N. Huang, J.N. Marchese, B. Williamson, K. Parker, S. Hattan, N. Khainovski, S. Pillai, S. Dey, S. Daniels, S. Purkayastha, P. Juhasz, S. Martin, M. Bartlet-Jones, F. He, A. Jacobson, D.J. Pappin, Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents, Mol. Cell Proteomics 3 (2004) 1154–1169.
[55] R.A. Dean, C.M. Overall, Proteomics discovery of metalloproteinase substrates in the cellular context by iTRAQ labeling reveals a diverse MMP-2 substrate degradome, Mol. Cell Proteomics 6 (2007) 611–623.
[56] D. Xu, N. Suenaga, M.J. Edelmann, R. Fridman, R.J. Muschel, B.M. Kessler, Novel MMP-9 substrates in cancer cells revealed by a label-free quantitative proteomics approach, Mol. Cell Proteomics 7 (2008) 2215–2228.
[57] T. Vaisar, S.Y. Kassim, I.G. Gomez, P.S. Green, S.A. Hargarten, P.J. Gough, W.C. Parks, C.L. Wilson, E.W. Raines, J.W. Heinecke, MMP-9 sheds the beta 2 integrin subunit (CD18) from macrophages, Mol. Cell Proteomics 8 (2009) 1044–1060.
[58] I.K. Hwang, S.M. Park, S.Y. Kim, S.T. Lee, A proteomic approach to identify substrates of matrix metalloproteinase-14 in human plasma, Biochim. Biophys. Acta 1702 (2004) 79–87.
[59] S.Y. Kim, S.M. Park, S.T. Lee, ApolipoproteinC-II , is a novel substrate for matrix metalloproteinases, Biochem. Biophys. Res. Commun. 339 (2006) 47–54.
[60] K.J. Greenlee, D.B. Corry, D.A. Engler, R.K. Matsunami, P. Tessier, R.G. Cook, Z. Werb, F. Kheradmand, Proteomic identification of *in vivo* substrates for matrix metalloproteinases 2 and 9 reveals a mechanism for resolution of inflammation, J. Immunol. 177 (2006) 7312–7321.

[61] K. Holmbeck, P. Bianco, J. Caterina, S. Yamada, M. Kromer, S.A. Kuznetsov, M. Mankani, P.G. Robey, A.R. Poole, I. Pidoux, J.M. Ward, H. Birkedal-Hansen, MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover, Cell 99 (1999) 81–92.
[62] Z. Zhou, S.S. Apte, R. Soininen, R. Cao, G.Y. Baaklini, R.W. Rauser, J. Wang, Y. Cao, K. Tryggvason, Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4052–4057.
[63] E. Ohuchi, K. Imai, Y. Fujii, H. Sato, M. Seiki, Y. Okada, Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules, J. Biol. Chem. 272 (1997) 2446–2451.
[64] T.H. Vu, J.M. Shipley, G. Bergers, J.E. Berger, J.A. Helms, D. Hanahan, S.D. Shapiro, R.M. Senior, Z. Werb, MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes, Cell 93 (1998) 411–422.
[65] J. Ochieng, B. Green, S. Evans, O. James, P. Warfield, Modulation of the biological functions of galectin-3 by matrix metalloproteinases, Biochim. Biophys. Acta 1379 (1998) 97–106.
[66] C. Colnot, S.S. Sidhu, N. Balmain, F. Poirier, Uncoupling of chondrocyte death and vascular invasion in mouse galectin 3 null mutant bones, Dev. Biol. 229 (2001) 203–214.
[67] N. Ortega, D.J. Behonick, C. Colnot, D.N. Cooper, Z. Werb, Galectin-3 is a downstream regulator of matrix metalloproteinase-9 function during endochondral bone formation, Mol. Biol. Cell 16 (2005) 3028–3039.
[68] C. Colnot, Z. Thompson, T. Miclau, Z. Werb, J.A. Helms, Altered fracture repair in the absence of MMP9, Development 130 (2003) 4123–4133.
[69] M. Heroult, I. Bernard-Pierrot, J. Delbe, Y. Hamma-Kourbali, P. Katsoris, D. Barritault, E. Papadimitriou, J. Plouet, J. Courty, Heparin affin regulatory peptide binds to vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis, Oncogene 23 (2004) 1745–1753.
[70] D. Stickens, D.J. Behonick, N. Ortega, B. Heyer, B. Hartenstein, Y. Yu, A.J. Fosang, M. Schorpp-Kistner, P. Angel, Z. Werb, Altered endochondral bone development in matrix metalloproteinase 13-deficient mice, Development 131 (2004) 5883–5895.
[71] M. Inada, Y. Wang, M.H. Byrne, M.U. Rahman, C. Miyaura, C. Lopez-Otin, S.M. Krane, Critical roles for collagenase-3 (Mmp13) in development of growth plate cartilage and in endochondral ossification, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17192–17197.
[72] J.J. Pinzone, B.M. Hall, N.K. Thudi, M. Vonau, Y.W. Qiang, T.J. Rosol, J.D.J. Shaughnessy, The role of Dickkopf-1 in bone development, homeostasis, and disease, Blood 113 (2009) 517–525.
[73] L. Wilkinson, G. Kolle, D. Wen, M. Piper, J. Scott, M. Little, CRIM1 regulates the rate of processing and delivery of bone morphogenetic proteins to the cell surface, J. Biol. Chem. 278 (2003) 34181–34188.
[74] G. Bergers, R. Brekken, G. McMahon, T.H. Vu, T. Itoh, K. Tamaki, K. Tanzawa, P. Thorpe, S. Itohara, Z. Werb, D. Hanahan, Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis, Nat. Cell. Biol. 2 (2000) 737–744.
[75] S. Lee, S.M. Jilani, G.V. Nikolova, D. Carpizo, M.L. Iruela-Arispe, Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors, J. Cell Biol. 169 (2005) 681–691.
[76] G. Hashimoto, I. Inoki, Y. Fujii, T. Aoki, E. Ikeda, Y. Okada, Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor 165, J. Biol. Chem. 277 (2002) 36288–36295.
[77] N.E. Sounni, C. Roghi, V. Chabottaux, M. Janssen, C. Munaut, E. Maquoi, B.G. Galvez, C. Gilles, F. Frankenne, G. Murphy, J.M. Foidart, A. Noel, Up-regulation of vascular endothelial growth factor-A by active membrane-type 1 matrix metalloproteinase through activation of Src-tyrosine kinases, J. Biol. Chem. 279 (2004) 13564–13574.
[78] J.M. Whitelock, A.D. Murdoch, R.V. Iozzo, P.A. Underwood, The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases, J. Biol. Chem. 271 (1996) 10079–10086.
[79] G. Opdenakker, P.E. Van den Steen, J. Van Damme, Gelatinase B: a tuner and amplifier of immune functions, Trends Immunol. 22 (2001) 571–579.
[80] K. Lehti, E. Allen, H. Birkedal-Hansen, K. Holmbeck, Y. Miyake, T.H. Chun, S.J. Weiss, An MT1-MMP-PDGF receptor-beta axis regulates mural cell investment of the microvasculature, Genes Dev. 19 (2005) 979–991.
[81] K. Lehti, N.F. Rose, S. Valavaara, S.J. Weiss, J. Keski-Oja, MT1-MMP promotes vascular smooth muscle dedifferentiation through LRP1 processing, J. Cell. Sci. 122 (2009) 126–135.
[82] M. Hangai, N. Kitaya, J. Xu, C.K. Chan, J.J. Kim, Z. Werb, S.J. Ryan, P.C. Brooks, Matrix metalloproteinase-9-dependent exposure of a cryptic migratory control site in collagen is required before retinal angiogenesis, Am. J. Pathol. 161 (2002) 1429–1437.
[83] J. Xu, D. Rodriguez, E. Petitclerc, J.J. Kim, M. Hangai, Y.S. Moon, G.E. Davis, P.C. Brooks, Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth *in vivo*, J. Cell Biol. 154 (2001) 1069–1079.
[84] Y. Hamano, M. Zeisberg, H. Sugimoto, J.C. Lively, Y. Maeshima, C. Yang, R.O. Hynes, Z. Werb, A. Sudhakar, R. Kalluri, Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin, Cancer Cell 3 (2003) 589–601.
[85] P. Koolwijk, N. Sidenius, E. Peters, C.F. Sier, R. Hanemaaijer, F. Blasi, V.W. van Hinsbergh, Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices, Blood 97 (2001) 3123–3131.
[86] A. Andolfo, W.R. English, M. Resnati, G. Murphy, F. Blasi, N. Sidenius, Metalloproteases cleave the urokinase-type plasminogen activator receptor in the D1-D2 linker region and expose epitopes not present in the intact soluble receptor, Thromb. Haemost. 88 (2002) 298–306.
[87] B. Cauwe, P.E. Van den Steen, G. Opdenakker, The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases, Crit. Rev. Biochem. Mol. Biol. 42 (2007) 113–185.
[88] E. Levi, R. Fridman, H.Q. Miao, Y.S. Ma, A. Yayon, I. Vlodavsky, Matrix metalloproteinase 2 releases active soluble ectodomain of fibroblast growth factor receptor 1, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 7069–7074.
[89] J.R. Basile, K. Holmbeck, T.H. Bugge, J.S. Gutkind, MT1-MMP controls tumor-induced angiogenesis through the release of semaphorin 4D, J. Biol. Chem. 282 (2007) 6899–6905.
[90] Y. Macotela, M.B. Aguilar, J. Guzman-Morales, J.C. Rivera, C. Zermeno, F. Lopez-Barrera, G. Nava, C. Lavalle, G. Martinez de la Escalera, C. Clapp, Matrix metalloproteases from chondrocytes generate an antiangiogenic 16 kDa prolactin, J. Cell. Sci. 119 (2006) 1790–1800.
[91] K. Frazier, S. Williams, D. Kothapalli, H. Klapper, G.R. Grotendorst, Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor, J. Invest. Dermatol. 107 (1996) 404–411.
[92] Y. Chen, X.Y. Du, Functional properties and intracellular signaling of CCN1/Cyr61, J. Cell. Biochem. 100 (2007) 1337–1345.
[93] Q. Yu, I. Stamenkovic, Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis, Genes Dev. 14 (2000) 163–176.
[94] M. Dangelo, D.P. Sarment, P.C. Billings, M. Pacifici, Activation of transforming growth factor beta in chondrocytes undergoing endochondral ossification, J. Bone Miner. Res. 16 (2001) 2339–2347.
[95] D. Mu, S. Cambier, L. Fjellbirkeland, J.L. Baron, J.S. Munger, H. Kawakatsu, D. Sheppard, V.C. Broaddus, S.L. Nishimura, The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1, J. Cell Biol. 157 (2002) 493–507.
[96] S.L. Dallas, J.L. Rosser, G.R. Mundy, L.F. Bonewald, Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix, J. Biol. Chem. 277 (2002) 21352–21360.
[97] S. Maeda, D.D. Dean, R. Gomez, Z. Schwartz, B.D. Boyan, The first stage of transforming growth factor beta1 activation is release of the large latent complex from the extracellular matrix of growth plate chondrocytes by matrix vesicle stromelysin-1 (MMP-3), Calcif. Tissue Int. 70 (2002) 54–65.
[98] K. Imai, A. Hiramatsu, D. Fukushima, M.D. Pierschbacher, Y. Okada, Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release, Biochem. J. 322 (1997) 809–814.
[99] G. Velasco-Loyden, J. Arribas, F. Lopez-Casillas, The shedding of betaglycan is regulated by pervanadate and mediated by membrane type matrix metalloprotease-1, J. Biol. Chem. 279 (2004) 7721–7733.
[100] S. Kojima, K. Nara, D.B. Rifkin, Requirement for transglutaminase in the activation of latent transforming growth factor-beta in bovine endothelial cells, J. Cell Biol. 121 (1993) 439–448.
[101] I. Nunes, P.E. Gleizes, C.N. Metz, D.B. Rifkin, Latent transforming growth factor-beta binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-beta, J. Cell Biol. 136 (1997) 1151–1163.
[102] S.M. Ellerbroek, Y.I. Wu, C.M. Overall, M.S. Stack, Functional interplay between type I collagen and cell surface matrix metalloproteinase activity, J. Biol. Chem. 276 (2001) 24833–24842.
[103] C. Gilles, M. Polette, M. Seiki, P. Birembaut, E.W. Thompson, Implication of collagen type I-induced membrane-type 1-matrix metalloproteinase expression and matrix metalloproteinase-2 activation in the metastatic progression of breast carcinoma, Lab. Invest. 76 (1997) 651–660.
[104] B. Steffensen, H.F. Bigg, C.M. Overall, The involvement of the fibronectin type II-like modules of human gelatinase A in cell surface localization and activation, J. Biol. Chem. 273 (1998) 20622–20628.
[105] C. Gilles, M. Polette, C. Coraux, J.M. Tournier, G. Meneguzzi, C. Munaut, L. Volders, P. Rousselle, P. Birembaut, J.M. Foidart, Contribution of MT1-MMP and of human laminin-5 gamma2 chain degradation to mammary epithelial cell migration, J. Cell. Sci. 114 (2001) 2967–2976.
[106] N. Koshikawa, G. Giannelli, V. Cirulli, K. Miyazaki, V. Quaranta, Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5, J. Cell Biol. 148 (2000) 615–624.
[107] N. Koshikawa, S. Schenk, G. Moeckel, A. Sharabi, K. Miyazaki, H. Gardner, R. Zent, V. Quaranta, Proteolytic processing of laminin-5 by MT1-MMP in tissues and its effects on epithelial cell morphology, FASEB J. 18 (2004) 364–366.
[108] E. Pirila, A. Sharabi, T. Salo, V. Quaranta, H. Tu, R. Heljasvaara, N. Koshikawa, T. Sorsa, P. Maisi, Matrix metalloproteinases process the laminin-5 gamma 2-chain and regulate epithelial cell migration, Biochem. Biophys. Res. Commun. 303 (2003) 1012–1017.
[109] J.K. McGuire, Q. Li, W.C. Parks, Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium, Am. J. Pathol. 162 (2003) 1831–1843.
[110] A. Lochter, S. Galosy, J. Muschler, N. Freedman, Z. Werb, M.J. Bissell, Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells, J. Cell Biol. 139 (1997) 1861–1872.
[111] V. Noe, B. Fingleton, K. Jacobs, H.C. Crawford, S. Vermeulen, W. Steelant, E. Bruyneel, L.M. Matrisian, M. Mareel, Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1, J. Cell. Sci. 114 (2001) 111–118.

[112] A. Hecht, R. Kemler, Curbing the nuclear activities of beta-catenin. Control over Wnt target gene expression, EMBO Rep. 1 (2000) 24–28.
[113] B. Nawrocki-Raby, C. Gilles, M. Polette, E. Bruyneel, J.Y. Laronze, N. Bonnet, J.M. Foidart, M. Mareel, P. Birembaut, Upregulation of MMPs by soluble E-cadherin in human lung tumor cells, Int. J. Cancer 105 (2003) 790–795.
[114] U. Cavallaro, G. Christofori, Cell adhesion and signalling by cadherins and Ig-CAMs in cancer, Nat. Rev. Cancer 4 (2004) 118–132.
[115] Y. Ichikawa, T. Ishikawa, N. Momiyama, M. Kamiyama, H. Sakurada, R. Matsuyama, S. Hasegawa, T. Chishima, Y. Hamaguchi, S. Fujii, S. Saito, K. Kubota, S. Hasegawa, H. Ike, S. Oki, H. Shimada, Matrilysin (MMP-7) degrades VE-cadherin and accelerates accumulation of beta-catenin in the nucleus of human umbilical vein endothelial cells, Oncol. Rep. 15 (2006) 311–315.
[116] J.D. Hood, D.A. Cheresh, Role of integrins in cell invasion and migration, Nat. Rev. Cancer 2 (2002) 91–100.
[117] P.C. Brooks, S. Silletti, T.L. von Schalscha, M. Friedlander, D.A. Cheresh, Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity, Cell 92 (1998) 391–400.
[118] R.E. Nisato, G. Hosseini, C. Sirrenberg, G.S. Butler, T. Crabbe, A.J. Docherty, M. Wiesner, G. Murphy, C.M. Overall, S.L. Goodman, M.S. Pepper, Dissecting the role of matrix metalloproteinases (MMP) and integrin alpha(v)beta3 in angiogenesis *in vitro*: absence of hemopexin C domain bioactivity, but membrane-Type 1-MMP and alpha(v)beta3 are critical, Cancer Res. 65 (2005) 9377–9387.
[119] E.I. Deryugina, M.A. Bourdon, K. Jungwirth, J.W. Smith, A.Y. Strongin, Functional activation of integrin alpha V beta 3 in tumor cells expressing membrane-type 1 matrix metalloproteinase, Int. J. Cancer 86 (2000) 15–23.
[120] E.I. Deryugina, B.I. Ratnikov, T.I. Postnova, D.V. Rozanov, A.Y. Strongin, Processing of integrin alpha(v) subunit by membrane type 1 matrix metalloproteinase stimulates migration of breast carcinoma cells on vitronectin and enhances tyrosine phosphorylation of focal adhesion kinase, J. Biol. Chem. 277 (2002) 9749–9756.
[121] M. Abdel-Ghany, H.C. Cheng, R.C. Elble, B.U. Pauli, The breast cancer beta 4 integrin and endothelial human CLCA2 mediate lung metastasis, J. Biol. Chem. 276 (2001) 25438–25446.
[122] H. Mori, T. Tomari, N. Koshikawa, M. Kajita, Y. Itoh, H. Sato, H. Tojo, I. Yana, M. Seiki, CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain, EMBO J. 21 (2002) 3949–3959.
[123] W.H. Yu, J.F.J. Woessner, J.D. McNeish, I. Stamenkovic, CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling, Genes Dev. 16 (2002) 307–323.
[124] M. Seiki, The cell surface: the stage for matrix metalloproteinase regulation of migration, Curr. Opin. Cell Biol. 14 (2002) 624–632.
[125] N. Suenaga, H. Mori, Y. Itoh, M. Seiki, CD44 binding through the hemopexin-like domain is critical for its shedding by membrane-type 1 matrix metalloproteinase, Oncogene 24 (2005) 859–868.
[126] M. Kajita, Y. Itoh, T. Chiba, H. Mori, A. Okada, H. Kinoh, M. Seiki, Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration, J. Cell Biol. 153 (2001) 893–904.
[127] Q. Yu, I. Stamenkovic, Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion, Genes Dev. 13 (1999) 35–48.
[128] K. Endo, T. Takino, H. Miyamori, H. Kinsen, T. Yoshizaki, M. Furukawa, H. Sato, Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration, J. Biol. Chem. 278 (2003) 40764–40770.
[129] V.K. Asundi, R. Erdman, R.C. Stahl, D.J. Carey, Matrix metalloproteinase-dependent shedding of syndecan-3, a transmembrane heparan sulfate proteoglycan, in Schwann cells, J. Neurosci. Res. 73 (2003) 593–602.
[130] A.M. Belkin, E.A. Zemskov, J. Hang, S.S. Akimov, S. Sikora, A.Y. Strongin, Cell-surface-associated tissue transglutaminase is a target of MMP-2 proteolysis, Biochemistry 43 (2004) 11760–11769.
[131] A.M. Belkin, S.S. Akimov, L.S. Zaritskaya, B.I. Ratnikov, E.I. Deryugina, A.Y. Strongin, Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion, J. Biol. Chem. 276 (2001) 18415–18422.
[132] E.A. Zemskov, A. Janiak, J. Hang, A. Waghray, A.M. Belkin, The role of tissue transglutaminase in cell-matrix interactions, Front. Biosci. 11 (2006) 1057–1076.
[133] D.K. Strickland, S.L. Gonias, W.S. Argraves, Diverse roles for the LDL receptor family, Trends Endocrinol. Metab. 13 (2002) 66–74.
[134] D.V. Rozanov, E. Hahn-Dantona, D.K. Strickland, A.Y. Strongin, The low density lipoprotein receptor-related protein LRP is regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) proteolysis in malignant cells, J. Biol. Chem. 279 (2004) 4260–4268.
[135] H. Emonard, G. Bellon, P. de Diesbach, M. Mettlen, W. Hornebeck, P.J. Courtoy, Regulation of matrix metalloproteinase (MMP) activity by the low-density lipoprotein receptor-related protein (LRP). A new function for an "old friend", Biochimie 87 (2005) 369–376.
[136] P.E. Van den Steen, I. Van Aelst, V. Hvidberg, H. Piccard, P. Fiten, C. Jacobsen, S.K. Moestrup, S. Fry, L. Royle, M.R. Wormald, R. Wallis, P.M. Rudd, R.A. Dwek, G. Opdenakker, The hemopexin and O-glycosylated domains tune gelatinase B/MMP-9 bioavailability via inhibition and binding to cargo receptors, J. Biol. Chem. 281 (2006) 18626–18637.
[137] A. Boire, L. Covic, A. Agarwal, S. Jacques, S. Sherifi, A. Kuliopulos, PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells, Cell 120 (2005) 303–313.
[138] D. Pei, Matrix metalloproteinases target protease-activated receptors on the tumor cell surface, Cancer Cell 7 (2005) 207–208.
[139] L. Blavier, Y.A. Declerck, Considering the critical interface between tumor cells and stromal cells in the search for targets for anticancer therapy, Cancer Cell 7 (2005) 408–409.
[140] C.C. Lynch, A. Hikosaka, H.B. Acuff, M.D. Martin, N. Kawai, R.K. Singh, T.C. Vargo-Gogola, J.L. Begtrup, T.E. Peterson, B. Fingleton, T. Shirai, L.M. Matrisian, M. Futakuchi, MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL, Cancer Cell 7 (2005) 485–496.
[141] J. Schlondorff, L. Lum, C.P. Blobel, Biochemical and pharmacological criteria define two shedding activities for TRANCE/OPGL that are distinct from the tumor necrosis factor alpha convertase, J. Biol. Chem. 276 (2001) 14665–14674.
[142] T. Takino, N. Koshikawa, H. Miyamori, M. Tanaka, T. Sasaki, Y. Okada, M. Seiki, H. Sato, Cleavage of metastasis suppressor gene product KiSS-1 protein/metastin by matrix metalloproteinases, Oncogene 22 (2003) 4617–4626.
[143] A. Muller, B. Homey, H. Soto, N. Ge, D. Catron, M.E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S.N. Wagner, J.L. Barrera, A. Mohar, E. Verastegui, A. Zlotnik, Involvement of chemokine receptors in breast cancer metastasis, Nature 410 (2001) 50–56.
[144] J.L. Fowlkes, D.M. Serra, H. Nagase, K.M. Thrailkill, MMPs are IGFBP-degrading proteinases: implications for cell proliferation and tissue growth, Ann. N.Y. Acad. Sci. 878 (1999) 696–699.
[145] S. Manes, E. Mira, M.M. Barbacid, A. Cipres, P. Fernandez-Resa, J.M. Buesa, I. Merida, M. Aracil, G. Marquez, C. Martinez-A, Identification of insulin-like growth factor-binding protein-1 as a potential physiological substrate for human stromelysin-3, J. Biol. Chem. 272 (1997) 25706–25712.
[146] J.L. Fowlkes, D.M. Serra, R.C. Bunn, K.M. Thrailkill, J.J. Enghild, H. Nagase, Regulation of insulin-like growth factor (IGF)-I action by matrix metalloproteinase-3 involves selective disruption of IGF-I/IGF-binding protein-3 complexes, Endocrinology 145 (2004) 620–626.
[147] Y. Imai, W.H.J. Busby, C.E. Smith, J.B. Clarke, A.J. Garmong, G.D. Horwitz, C. Rees, D.R. Clemmons, Protease-resistant form of insulin-like growth factor-binding protein 5 is an inhibitor of insulin-like growth factor-I actions on porcine smooth muscle cells in culture, J. Clin. Invest. 100 (1997) 2596–2605.
[148] Y. Kishima, H. Yamamoto, Y. Izumoto, K. Yoshida, H. Enomoto, M. Yamamoto, T. Kuroda, H. Ito, K. Yoshizaki, H. Nakamura, Hepatoma-derived growth factor stimulates cell growth after translocation to the nucleus by nuclear localization signals, J. Biol. Chem. 277 (2002) 10315–10322.
[149] R. Iwamoto, E. Mekada, Heparin-binding EGF-like growth factor: a juxtacrine growth factor, Cytokine Growth Factor Rev. 11 (2000) 335–344.
[150] S. Higashiyama, Metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor and its pathophysiological roles, Protein Pept. Lett. 11 (2004) 443–450.
[151] G. Raab, M. Klagsbrun, Heparin-binding EGF-like growth factor, Biochim. Biophys. Acta 1333 (1997) F179–F199.
[152] P.P. Ongusaha, J.C. Kwak, A.J. Zwible, S. Macip, S. Higashiyama, N. Taniguchi, L. Fang, S.W. Lee, HB-EGF is a potent inducer of tumor growth and angiogenesis, Cancer Res. 64 (2004) 5283–5290.
[153] M. Suzuki, G. Raab, M.A. Moses, C.A. Fernandez, M. Klagsbrun, Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site, J. Biol. Chem. 272 (1997) 31730–31737.
[154] L. Hao, M. Du, A. Lopez-Campistrous, C. Fernandez-Patron, Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway, Circ. Res. 94 (2004) 68–76.
[155] K. Sumitomo, A. Kurisaki, N. Yamakawa, K. Tsuchida, E. Shimizu, S. Sone, H. Sugino, Expression of a TGF-beta1 inducible gene, TSC-36, causes growth inhibition in human lung cancer cell lines, Cancer Lett. 155 (2000) 37–46.
[156] R. Agnihotri, H.C. Crawford, H. Haro, L.M. Matrisian, M.C. Havrda, L. Liaw, Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin), J. Biol. Chem. 276 (2001) 28261–28267.
[157] Q. Hwang, S. Cheifetz, C.M. Overall, C.A. McCulloch, J. Sodek, Bone sialoprotein does not interact with pro-gelatinase A (MMP-2) or mediate MMP-2 activation, BMC Cancer 9 (2009) 121.
[158] N.S. Fedarko, A. Jain, A. Karadag, L.W. Fisher, Three small integrin binding ligand N-linked glycoproteins (SIBLINGs) bind and activate specific matrix metalloproteinases, FASEB J. 18 (2004) 734–736.
[159] B.C. Sheu, S.M. Hsu, H.N. Ho, H.C. Lien, S.C. Huang, R.H. Lin, A novel role of metalloproteinase in cancer-mediated immunosuppression, Cancer Res. 61 (2001) 237–242.
[160] H. Kataoka, H. Uchino, T. Iwamura, M. Seiki, K. Nabeshima, M. Koono, Enhanced tumor growth and invasiveness *in vivo* by a carboxyl-terminal fragment of alpha1-proteinase inhibitor generated by matrix metalloproteinases: a possible modulatory role in natural killer cytotoxicity, Am. J. Pathol. 154 (1999) 457–468.
[161] M. Tanaka, T. Itai, M. Adachi, S. Nagata, Downregulation of Fas ligand by shedding, Nat. Med. 4 (1998) 31–36.
[162] W.C. Powell, B. Fingleton, C.L. Wilson, M. Boothby, L.M. Matrisian, The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis, Curr. Biol. 9 (1999) 1441–1447.
[163] N. Mitsiades, W.H. Yu, V. Poulaki, M. Tsokos, I. Stamenkovic, Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity, Cancer Res. 61 (2001) 577–581.
[164] S. Strand, P. Vollmer, L. van den Abeelen, D. Gottfried, V. Alla, H. Heid, J. Kuball, M. Theobald, P.R. Galle, D. Strand, Cleavage of CD95 by matrix metalloproteinase-7 induces apoptosis resistance in tumour cells, Oncogene 23 (2004) 3732–3736.

[165] G. Pan, J.H. Bauer, V. Haridas, S. Wang, D. Liu, G. Yu, C. Vincenz, B.B. Aggarwal, J. Ni, V.M. Dixit, Identification and functional characterization of DR6, a novel death domain-containing TNF receptor, FEBS Lett. 431 (1998) 351–356.
[166] N. Ilan, A. Mohsenin, L. Cheung, J.A. Madri, PECAM-1 shedding during apoptosis generates a membrane-anchored truncated molecule with unique signaling characteristics, FASEB J. 15 (2001) 362–372.
[167] S. Blaschke, M. Koziolek, A. Schwarz, P. Benohr, P. Middel, G. Schwarz, K.M. Hummel, G.A. Muller, Proinflammatory role of fractalkine (CX3CL1) in rheumatoid arthritis, J. Rheumatol. 30 (2003) 1918–1927.
[168] P.E. Van den Steen, S.J. Husson, P. Proost, J. Van Damme, G. Opdenakker, Carboxyterminal cleavage of the chemokines MIG and IP-10 by gelatinase B and neutrophil collagenase, Biochem. Biophys. Res. Commun. 310 (2003) 889–896.
[169] K. Zhang, G.A. McQuibban, C. Silva, G.S. Butler, J.B. Johnston, J. Holden, I. Clark-Lewis, C.M. Overall, C. Power, HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration, Nat. Neurosci. 6 (2003) 1064–1071.
[170] D. Vergote, G.S. Butler, M. Ooms, J.H. Cox, C. Silva, M.D. Hollenberg, J.H. Jhamandas, C.M. Overall, C. Power, Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 19182–19187.
[171] W.H. Yu, J.F.J. Woessner, Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7), J. Biol. Chem. 275 (2000) 4183–4191.
[172] Q. Li, P.W. Park, C.L. Wilson, W.C. Parks, Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury, Cell 111 (2002) 635–646.
[173] J.H. Cox, R.A. Dean, C.R. Roberts, C.M. Overall, Matrix metalloproteinase processing of CXCL11/I-TAC results in loss of chemoattractant activity and altered glycosaminoglycan binding, J. Biol. Chem. 283 (2008) 19389–19399.
[174] S. Brule, N. Charnaux, A. Sutton, D. Ledoux, T. Chaigneau, L. Saffar, L. Gattegno, The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9, Glycobiology 16 (2006) 488–501.
[175] D.B. Corry, K. Rishi, J. Kanellis, A. Kiss, L.Z. Song Lz, J. Xu, L. Feng, Z. Werb, F. Kheradmand, Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency, Nat. Immunol. 3 (2002) 347–353.
[176] P. Van Lint, B. Wielockx, L. Puimege, A. Noel, C. Lopez-Otin, C. Libert, Resistance of collagenase-2 (matrix metalloproteinase-8)-deficient mice to TNF-induced lethal hepatitis, J. Immunol. 175 (2005) 7642–7649.
[177] D.B. Corry, A. Kiss, L.Z. Song, L. Song, J. Xu, S.H. Lee, Z. Werb, F. Kheradmand, Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines, FASEB J. 18 (2004) 995–997.
[178] K.Y. Vermaelen, D. Cataldo, K. Tournoy, T. Maes, A. Dhulst, R. Louis, J.M. Foidart, A. Noel, R. Pauwels, Matrix metalloproteinase-9-mediated dendritic cell recruitment into the airways is a critical step in a mouse model of asthma, J. Immunol. 171 (2003) 1016–1022.
[179] D.D. Cataldo, K.G. Tournoy, K. Vermaelen, C. Munaut, J.M. Foidart, R. Louis, A. Noel, R.A. Pauwels, Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation, Am. J. Pathol. 161 (2002) 491–498.
[180] S.J. McMillan, J. Kearley, J.D. Campbell, X.W. Zhu, K.Y. Larbi, J.M. Shipley, R.M. Senior, S. Nourshargh, C.M. Lloyd, Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation, J. Immunol. 172 (2004) 2586–2594.
[181] A. Gutierrez-Fernandez, M. Inada, M. Balbin, A. Fueyo, A.S. Pitiot, A. Astudillo, K. Hirose, M. Hirata, S.D. Shapiro, A. Noel, Z. Werb, S.M. Krane, C. Lopez-Otin, X.S. Puente, Increased inflammation delays wound healing in mice deficient in collagenase-2 (MMP-8), FASEB J. 21 (2007) 2580–2591.
[182] J.H. Cox, A.E. Starr, R. Kappelhoff, R. Yan, C.R. Roberts, C.M. Overall, Matrix metalloproteinase-8 deficiency causes more severe arthritis and delays neutrophil apoptosis through reduced caspase-11 expression, J. Immunol. (submitted for publication).
[183] A.J. Gearing, P. Beckett, M. Christodoulou, M. Churchill, J. Clements, A.H. Davidson, A.H. Drummond, W.A. Galloway, R. Gilbert, J.L. Gordon, et al., Processing of tumour necrosis factor-alpha precursor by metalloproteinases, Nature 370 (1994) 555–557.
[184] W.R. English, X.S. Puente, J.M. Freije, V. Knauper, A. Amour, A. Merryweather, C. Lopez-Otin, G. Murphy, Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-alpha convertase activity but does not activate pro-MMP2, J. Biol. Chem. 275 (2000) 14046–14055.
[185] H. Haro, H.C. Crawford, B. Fingleton, K. Shinomiya, D.M. Spengler, L.M. Matrisian, Matrix metalloproteinase-7-dependent release of tumor necrosis factor-alpha in a model of herniated disc resorption, J. Clin. Invest. 105 (2000) 143–150.
[186] U. Schonbeck, F. Mach, P. Libby, Generation of biologically active IL-1 beta by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 beta processing, J. Immunol. 161 (1998) 3340–3346.
[187] A. Ito, A. Mukaiyama, Y. Itoh, H. Nagase, I.B. Thogersen, J.J. Enghild, Y. Sasaguri, Y. Mori, Degradation of interleukin 1beta by matrix metalloproteinases, J. Biol. Chem. 271 (1996) 14657–14660.
[188] S. Orlando, M. Sironi, G. Bianchi, A.H. Drummond, D. Boraschi, D. Yabes, A. Mantovani, Role of metalloproteases in the release of the IL-1 type II decoy receptor, J. Biol. Chem. 272 (1997) 31764–31769.
[189] C. Bellehumeur, T. Collette, R. Maheux, J. Mailloux, M. Villeneuve, A. Akoum, Increased soluble interleukin-1 receptor type II proteolysis in the endometrium of women with endometriosis, Hum. Reprod. 20 (2005) 1177–1184.
[190] I. Nelissen, E. Martens, P.E. Van den Steen, P. Proost, I. Ronsse, G. Opdenakker, Gelatinase B/matrix metalloproteinase-9 cleaves interferon-beta and is a target for immunotherapy, Brain 126 (2003) 1371–1381.
[191] N.M. Weathington, A.H. van Houwelingen, B.D. Noerager, P.L. Jackson, A.D. Kraneveld, F.S. Galin, G. Folkerts, F.P. Nijkamp, J.E. Blalock, A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation, Nat. Med. 12 (2006) 317–323.
[192] A.M. Houghton, P.A. Quintero, D.L. Perkins, D.K. Kobayashi, D.G. Kelley, L.A. Marconcini, R.P. Mecham, R.M. Senior, S.D. Shapiro, Elastin fragments drive disease progression in a murine model of emphysema, J. Clin. Invest. 116 (2006) 753–759.
[193] M.J. Banda, A.G. Rice, G.L. Griffin, R.M. Senior, Alpha 1-proteinase inhibitor is a neutrophil chemoattractant after proteolytic inactivation by macrophage elastase, J. Biol. Chem. 263 (1988) 4481–4484.
[194] F. De Ceuninck, F. Allain, A. Caliez, G. Spik, P.M. Vanhoutte, High binding capacity of cyclophilin B to chondrocyte heparan sulfate proteoglycans and its release from the cell surface by matrix metalloproteinases: possible role as a proinflammatory mediator in arthritis, Arthritis Rheum. 48 (2003) 2197–2206.
[195] C. Fernandez-Patron, C. Zouki, R. Whittal, J.S. Chan, S.T. Davidge, J.G. Filep, Matrix metalloproteinases regulate neutrophil–endothelial cell adhesion through generation of endothelin-1[1-32], FASEB J. 15 (2001) 2230–2240.
[196] B. Heissig, K. Hattori, S. Dias, M. Friedrich, B. Ferris, N.R. Hackett, R.G. Crystal, P. Besmer, D. Lyden, M.A. Moore, Z. Werb, S. Rafii, Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand, Cell 109 (2002) 625–637.
[197] Y. Shirafuji, H. Tanabe, D.P. Satchell, A. Henschen-Edman, C.L. Wilson, A.J. Ouellette, Structural determinants of procryptdin recognition and cleavage by matrix metalloproteinase-7, J. Biol. Chem. 278 (2003) 7910–7919.
[198] C.L. Wilson, A.J. Ouellette, D.P. Satchell, T. Ayabe, Y.S. Lopez-Boado, J.L. Stratman, S.J. Hultgren, L.M. Matrisian, W.C. Parks, Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense, Science 286 (1999) 113–117.
[199] B. Bottazzi, A. Bastone, A. Doni, C. Garlanda, S. Valentino, L. Deban, V. Maina, A. Cotena, F. Moalli, L. Vago, A. Salustri, L. Romani, A. Mantovani, The long pentraxin PTX3 as a link among innate immunity, inflammation, and female fertility, J. Leukoc. Biol. 79 (2006) 909–912.
[200] X.S. Puente, L.M. Sanchez, C.M. Overall, C. Lopez-Otin, Human and mouse proteases: a comparative genomic approach, Nat. Rev. Genet. 4 (2003) 544–558.
[201] G. Gao, A. Plaas, V.P. Thompson, S. Jin, F. Zuo, J.D. Sandy, ADAMTS4 (aggrecanase-1) activation on the cell surface involves C-terminal cleavage by glycosylphosphatidyl inositol-anchored membrane type 4-matrix metalloproteinase and binding of the activated proteinase to chondroitin sulfate and heparan sulfate on syndecan-1, J. Biol. Chem. 279 (2004) 10042–10051.
[202] P.A. Marcotte, I.M. Kozan, S.A. Dorwin, J.M. Ryan, The matrix metalloproteinase pump-1 catalyzes formation of low molecular weight (pro)urokinase in cultures of normal human kidney cells, J. Biol. Chem. 267 (1992) 13803–13806.
[203] A.A. Belaaouaj, A. Li, T.C. Wun, H.G. Welgus, S.D. Shapiro, Matrix metalloproteinases cleave tissue factor pathway inhibitor. Effects on coagulation, J. Biol. Chem. 275 (2000) 27123–27128.
[204] A.C. Cunningham, K.A. Hasty, J.J. Enghild, A.E. Mast, Structural and functional characterization of tissue factor pathway inhibitor following degradation by matrix metalloproteinase-8, Biochem. J. 367 (2002) 451–458.
[205] A.E. Mast, J.J. Enghild, H. Nagase, K. Suzuki, S.V. Pizzo, G. Salvesen, Kinetics and physiologic relevance of the inactivation of alpha 1-proteinase inhibitor, alpha 1-antichymotrypsin, and antithrombin III by matrix metalloproteinases-1 (tissue collagenase), -2 (72-kDa gelatinase/type IV collagenase), and -3 (stromelysin), J. Biol. Chem. 266 (1991) 15810–15816.
[206] Z. Liu, X. Zhou, S.D. Shapiro, J.M. Shipley, S.S. Twining, L.A. Diaz, R.M. Senior, Z. Werb, The serpin alpha1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 *in vivo*, Cell 102 (2000) 647–655.
[207] H.R. Lijnen, B. Arza, B. Van Hoef, D. Collen, P.J. Declerck, Inactivation of plasminogen activator inhibitor-1 by specific proteolysis with stromelysin-1 (MMP-3), J. Biol. Chem. 275 (2000) 37645–37650.
[208] H.R. Lijnen, B. Van Hoef, D. Collen, Inactivation of the serpin alpha(2)-antiplasmin by stromelysin-1, Biochim. Biophys. Acta 1547 (2001) 206–213.
[209] T. Maretzky, K. Reiss, A. Ludwig, J. Buchholz, F. Scholz, E. Proksch, B. de Strooper, D. Hartmann, P. Saftig, ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and beta-catenin translocation, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9182–9187.
[210] K. Reiss, T. Maretzky, A. Ludwig, T. Tousseyn, B. de Strooper, D. Hartmann, P. Saftig, ADAM10 cleavage of N-cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signalling, EMBO J. 24 (2005) 742–752.
[211] B. Schulz, J. Pruessmeyer, T. Maretzky, A. Ludwig, C.P. Blobel, P. Saftig, K. Reiss, ADAM10 regulates endothelial permeability and T-Cell transmigration by proteolysis of vascular endothelial cadherin, Circ. Res. 102 (2008) 1192–1201.
[212] M.L. Moss, S.L. Jin, M.E. Milla, D.M. Bickett, W. Burkhart, H.L. Carter, W.J. Chen, W.C. Clay, J.R. Didsbury, D. Hassler, C.R. Hoffman, T.A. Kost, M.H. Lambert, M.A. Leesnitzer, P. McCauley, G. McGeehan, J. Mitchell, M. Moyer, G. Pahel, W. Rocque, L.K. Overton, F. Schoenen, T. Seaton, J.L. Su, J. Warner, D. Willard, J.D. Becherer, Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha, Nature 385 (1997) 733–736.
[213] R.A. Black, C.T. Rauch, C.J. Kozlosky, J.J. Peschon, J.L. Slack, M.F. Wolfson, B.J. Castner, K.L. Stocking, P. Reddy, S. Srinivasan, N. Nelson, N. Boiani, K.A. Schooley, M. Gerhart, R. Davis, J.N. Fitzner, R.S. Johnson, R.J. Paxton, C.J. March, D.P. Cerretti,

A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells, Nature 385 (1997) 729–733.
[214] Y. Zheng, P. Saftig, D. Hartmann, C. Blobel, Evaluation of the contribution of different ADAMs to tumor necrosis factor alpha (TNFalpha) shedding and of the function of the TNFalpha ectodomain in ensuring selective stimulated shedding by the TNFalpha convertase (TACE/ADAM17), J. Biol. Chem. 279 (2004) 42898–42906.
[215] U. Anderegg, T. Eichenberg, T. Parthaune, C. Haiduk, A. Saalbach, L. Milkova, A. Ludwig, J. Grosche, M. Averbeck, C. Gebhardt, V. Voelcker, J.P. Sleeman, J.C. Simon, ADAM10 is the constitutive functional sheddase of CD44 in human melanoma cells, J. Invest. Dermatol. 129 (2009) 1471–1482.
[216] M. Schulte, K. Reiss, M. Lettau, T. Maretzky, A. Ludwig, D. Hartmann, B. de Strooper, O. Janssen, P. Saftig, ADAM10 regulates FasL cell surface expression and modulates FasL-induced cytotoxicity and activation-induced cell death, Cell Death Differ. 14 (2007) 1040–1049.
[217] S.W. Sunnarborg, C.L. Hinkle, M. Stevenson, W.E. Russell, C.S. Raska, J.J. Peschon, B.J. Castner, M.J. Gerhart, R.J. Paxton, R.A. Black, D.C. Lee, Tumor necrosis factor-alpha converting enzyme (TACE) regulates epidermal growth factor receptor ligand availability, J. Biol. Chem. 277 (2002) 12838–12845.
[218] Y. Izumi, M. Hirata, H. Hasuwa, R. Iwamoto, T. Umata, K. Miyado, Y. Tamai, T. Kurisaki, A. Sehara-Fujisawa, S. Ohno, E. Mekada, A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor, EMBO J. 17 (1998) 7260–7272.
[219] M. Asakura, M. Kitakaze, S. Takashima, Y. Liao, F. Ishikura, T. Yoshinaka, H. Ohmoto, K. Node, K. Yoshino, H. Ishiguro, H. Asanuma, S. Sanada, Y. Matsumura, H. Takeda, S. Beppu, M. Tada, M. Hori, S. Higashiyama, Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy, Nat. Med. 8 (2002) 35–40.
[220] H. Lemjabbar, C. Basbaum, Platelet-activating factor receptor and ADAM10 mediate responses to Staphylococcus aureus in epithelial cells, Nat. Med. 8 (2002) 41–46.
[221] Y. Yan, K. Shirakabe, Z. Werb, The metalloprotease Kuzbanian (ADAM10) mediates the transactivation of EGF receptor by G protein-coupled receptors, J. Cell Biol. 158 (2002) 221–226.
[222] U. Sahin, G. Weskamp, K. Kelly, H.M. Zhou, S. Higashiyama, J. Peschon, D. Hartmann, P. Saftig, C.P. Blobel, Distinct roles for ADAM10 and ADAM17 in ectodomain shedding of six EGFR ligands, J. Cell Biol. 164 (2004) 769–779.
[223] C. Kopitz, M. Gerg, O.R. Bandapalli, D. Ister, C.J. Pennington, S. Hauser, C. Flechsig, H.W. Krell, D. Antolovic, K. Brew, H. Nagase, M. Stangl, C.W. von Weyhern, B.L. Brucher, K. Brand, L.M. Coussens, D.R. Edwards, A. Kruger, Tissue inhibitor of metalloproteinases-1 promotes liver metastasis by induction of hepatocyte growth factor signaling, Cancer Res. 67 (2007) 8615–8623.
[224] C. Kopitz, M. Gerg, B. Gansbacher, A. Kruger, Plasminogen activator inhibitor-2, but not cystatin C, inhibits the prometastatic activity of tissue inhibitor of metalloproteinases-1 in the liver, Hum. Gene Ther. 19 (2008) 1039–1049.
[225] A. Kruger, Functional genetic mouse models: promising tools for investigation of the proteolytic internet, Biol. Chem. 390 (2009) 91–97.