

The Extracellular Matrix, Matrix Metalloproteinases and their roles in Disease.

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14 JUN 2013

Abstract

Biomedical research is often thought to primarily focus upon the trillions of cells that make up the body. However just as study of the immune system cannot ignore the lymph in favor of blood, the extracellular matrix (ECM) cannot be ignored in favor of the cells. The ECM is a dynamic structure that acts as scaffolding substrate for cell adhesion, as well as a reservoir of various proteins, growth factors, and signaling molecules. The matrix metalloproteinases (MMPs) are the principle proteins responsible for the degradation that occurs with normal remodeling of the ECM. However, increases in the presence and activity of MMPs are associated with diseases that are known to modify tissues such as rheumatoid arthritis, atherosclerotic plaque formation, aortic aneurysm, plaque instability, progressive heart failure, meningitis, multiple sclerosis, Alzheimer's and gliomas. The associations and contributions of MMPs in these diseases, and how to inhibit the activity of MMPs in order to hinder disease progression, have been areas of intensive research. Herein is a short description of some of the results of that research on the ECM and some important selected MMPs.

The Extracellular Matrix

The extracellular matrix (ECM) is composed of proteins and polysaccharide macromolecules such as collagen, elastin, fibronectin, and laminin. The ECM provides substrata for cell adhesion, growth, and differentiation [1]. The macromolecules that make up the ECM are produced locally by the cells attached and adjacent to the ECM [2]. The major macromolecules that comprise the ECM are glycoaminoglycans; polysaccharide chains often linked to protein forming proteoglycans, and fibrous proteins like collagen elastin, fibronectin, and laminin [2]. Glycosaminoglycans are classified into four main groups 1) hyaluran, 2) chondroitin and dermatan sulfate, 3) heparan sulfate, and 4) keratan sulfate [2]. The polysaccharide chains are less flexible than amino acid polypeptide chains and are very anionic and hydrophilic therefore, form gels that are high volume compared to their mass. These gels attract cations and create a swelling pressure that can withstand compressive forces such as those in joints [2]. Hyaluronan is thought to contribute to resisting compressive forces, serves to create cell-free spaces into which cells can migrate, acts as a lubricant in joints, and is produced in large quantities during wound healing [2]. Chondroitin and dermatin sulfate are strongly regulated by transforming growth factor (TGF)- β [3]. The production of chondroitin sulfate has been shown to increase following brain injury and may contribute to the inability of damaged neurons to regenerate [4] and limit the production of new neurites in development [5]. Dermatan sulfate has been shown to bind to over 17 different proteins including platelet factor-4, low density lipoprotein, interferon gamma, TGF- β , and RANTES [6]. There are also a growing number of viruses, parasites, and bacteria that have been found to use chondroitin, dermatan, and heparin sulfate for attachment to host tissues and cells [7]. Dermatan sulfate glycoaminoglycans have been shown to be involved in the coagulation cascade with binding to thrombin and enhancing the effect of activated protein C as proposed mechanisms [6]. Heparin sulfate glycosaminoglycans are increased during skeletal muscle regeneration [8] and have important roles in development influencing the hedgehog, wiggless, and other developmental pathways [9].

The ECM is not merely a scaffold but influences the development, proliferation, shape, form and migration of the cells attached to it. The glycoaminoglycans can act as cofactors, co-receptors and stabilizers for growth factors, cytokines, and chemokines. For example, proteoglycan binding to

chemokines can lead to their concentration and prolongation of action or more effective presentation to activating receptors [2]. Glycoaminoglycans can regulate enzyme activity and behave as signaling molecules in response to wound healing tumorigenesis and infections. They also act as targets for pathogens to bind to, use to invade cells and hide from the immune system [6]. The ECM helps regulate stem cells and prevent cancer cell invasion by maintaining cellular polarity and architecture [10]. The ECM is also constantly changing with degradation and remodeling constantly occurring as a normal part of tissue and organ function [10, 11]. Almost all cellular behavior is regulated directly or indirectly by the ECM [10].

ECM's Role in Disease

Given the importance of the ECM it is no surprise that its malformation and degradation are associated with a number of diseases. For example the profile of cell surface of heparin sulfate glycosaminoglycans has been shown to be modified in the cancer transformation process [12]. Increased ECM production or reduced ECM turnover are prominent in tissue fibrosis and abnormal alterations to the composition if the ECM potentiate growth factor signaling during transformation [10]. Alterations in the ECM contribute to angiogenesis, tumor associated inflammation, and recruitment of hematopoietic progenitor cells in tumor development [10].

Matrix Metalloproteinases

The modification and degradation of the ECM involves matrix metalloproteinases (MMP)'s which are zinc dependent endopeptidases. There are over 25 different MMPs [13] which are quite promiscuous in their substrates [14]. However, many MMPs are classified according to their substrates such as collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs [13]. MMPs control activation of signaling molecules such as death receptors and growth factors [15]. They are active at neutral pH and require calcium ions [16, 17] Most are exported from the cell and require proteolytic cleavage for activation, however, some are activated within the cell [17]. Most cells in the body express some type of MMP [17]. Thus far, eight different MMP genes have been found on a single chromosome. Chromosome 11[1].

MMP's Role in Disease

MMP activity has been associated with a myriad of diseases such as aneurysm, rupture of atherosclerotic plaques, myocardial infarction, and cerebral ischemia. MMPs are associated with multiple tumor types, matrix degradation in arthritis, idiopathic pulmonary fibrosis, asthma, and emphysema[18]. MMP activity has been implicated in chronic neurodegenerative disease [13] as well as rheumatoid arthritis, carcinogenesis, atherogenic plaque formation and rupture, and tumor metastasis and invasion [1]. MMPs increase the permeability of the blood brain barrier in hypoxia/ischemia, multiple sclerosis and infection [15].MMP activity is not always detrimental. For example MMP-3 and MMP-9 can be protective in that they limit plaque growth and enhance plaque stability[19]. MMP mediated degradation of the extracellular matrix may result in reduced fibrosis [20].MMP activity is important in normal remodeling processes such as development, post partum uterine involution, ovulation, and wound healing. [1]. In cancer models MMPs 3, 8, 9, and 12 have been seen as protective in knockout mice. While those that

lack MMP-9 have less tumors, those that develop are more aggressive [18]. MMPs are also important in bone remodeling [21]. As we age MMP activity increases and collagen deposition decreases [10].

Control and Regulation of MMP

MMPs are controlled on a number of levels. There is tight transcriptional control, via a number of transcription factors, control from proteolytic cleavage from a full length inactive zymogen to an active state. There is also regulation of MMP activity by two classes of proteins the α 2 macroglobulins and the tissue inhibitors of metalloproteinases (TIMPs)[19]. TIMPs are small proteins have been found to bind with the active site of MMPs. As there are 25 MMPs and four TIMPs, the TIMP-MMP binding is not highly specific. TIMPs inhibit MMPs stoichiometrically on a 1:1 ratio with a wedge shape that occupies the MMP active site [22]. All TIMPs inhibit MMPs with knockout mouse experiments showing that TIMP-1 null mice exhibited impaired learning and memory, TIMP-2 knockouts showing motor function and neurological abnormalities, and TIMP-3 knockout mice showing alveolar damage reminiscent of emphysema [19, 23]. TIMPs also inhibit a disintegrin and metalloproteinase (ADAMs) and ADAMs with thrombospondin motifs (ADAMTS). Like many housekeeping genes the TIMP-1,2,and 4 promoters lack TATA boxes[23].

It is this tight control and detriment/benefit dual nature of MMPs that makes targeting MMPS a difficult path for therapeutic development. MMP activity must be examined in context of the disease state and cannot simply be classified as beneficial or detrimental [18]. While the structures of MMPs and TIMPs have been well studied, there have been minimal successes in developing MMP inhibitors. There are also a large number of similar metalloproteinases such as the ADAMS and ADAMTS which make the production of selective inhibitors difficult [19, 24, 25]. Clinical trials of MMP inhibitors have yielded few results [26]. Early trials were unable to see the early efficacy observed in animal models translated to human patients with cessation or reduction in dose or duration of treatment due to musculoskeletal side effects in patients [18]. Despite the lack of success, there is still much effort dedicated to MMP inhibition because “there can be no doubt whatsoever of the potential contribution of an MMP inhibitor to cancer therapy” [27] and “overwhelming evidence from animal models strongly suggests a number of therapeutic areas that would benefit from MMP inhibition [18].”

Proteins of Interest

Abbreviated descriptions of some of the pivotal MMPs and TIMPs studied in research are below.

MMP-1

Serum MMP-1 has been shown to useful as a component in a battery of tests for diagnosis and monitoring of rheumatoid arthritis progression [28]. MMP-1 mRNA and protein levels have been shown to be elevated by the immunosuppressive compound rapamycin, a process thought to explain the anti-fibrotic effects of rapamycin [18, 20]. In human decidual cells, MMP-1 release is stimulated by protein kinase C activity [29]. Macrophages exposed to MMP-1 in conjunction with MMP-3 have been shown to rapidly release TNF- α [30]. MMP-1 as well as MMP-3 and MMP-9 are thought to have pro-inflammatory roles [19]. MMP-1 and MMP-7 have been postulated to be serum biomarkers of idiopathic pulmonary fibrosis [31]. MMP-1 substrates outside the ECM include the chemokines SDF-1 (CXCL12), MCP 1-3 (CCL2,

CCL8, and CCL7) and the pro forms of the inflammatory cytokines IL-1 β and TNF- α [32]. MMP-1 was thought to be needed for normal collagen remodeling and its inhibition responsible for the musculoskeletal side effects in early MMP inhibitor studies, however MMP-1 is lacking in rats and metal chelation rather than MMP-1 inhibition thought to be responsible for the side effects [18].

MMP-2

MMP-2 may contribute to cardiac damage via myosin light chain and poly(ADP-ribose) polymerase digestion [19]. MMP-2 activity is present in muscle tissue from patients with Duchenne muscular dystrophy [22]. MMP-2 is involved in neurite outgrowth, mesenchymal cell differentiation, generation of vasoconstrictors, and thought to have an anti-inflammatory role [19]. MMP-2 as well as MMP-9 are thought to contribute to cardiac rupture following myocardial infarction [19]. In addition to TIMP inhibition, MMP-2 activity has been shown to be inhibited by β -amyloid precursor, a c-terminal fragment of procollagen c-proteinase enhancer, and RECK, an angiogenesis suppressing glycoprotein [19].

MMP-3

MMP-3 is one the most widely expressed MMPs [17]. Chondrocytes, the only type of cells found in healthy cartilage, have been shown to produce IL-1 β which leads to the production of the chemokines regulated upon activation of normal T cell expression and secretion (RANTES;CCL5) and monocyte chemoattractant protein (MCP-1, CCL2). MCP-1 in turn leads to the production and release of MMP-3 through Phospholipase C (PLC), protein kinase C (PKC) and p38 and ERK MAP kinase pathways with cAMP playing a major role [29]. PLC activation also leads to the release of calcium ions which are required for MMP activity [29]. Eotaxin-1 (CCL11) at low concentrations has been shown to not only result in the upregulation of MMP-3, but it also secretion of MMP-3 from the cell. This upregulation appears to be via the PKC,PLC, and JNK map kinase pathways [29]. MMP-3 is correlative with osteoarthritis by increasing collagen degradation[33]. MMP-3 is tightly regulated on a gene expression level and has been shown to be upregulated by TNF- α , growth factors, phorbol esters, contact with the extracellular matrix, IL-1 β , endothelin-1, β -amyloid, lipopolysaccharide (LPS), 1-methyl-4-phenylpyridine, tetrahydrobiopterin, and inducers of ER stress such as brefeldin A and tunicamycin in a variety of nervous system tissues and cells[13]. MMP-3 is active both intra- and extra-cellularly with extracellular MMP-3 both inducing and being induced by cytokines and free radicals creating a “vicious cycle” in microglial cells [13]. MMP-3 will also activate MMP-9 via proteolytic cleavage [30].

MMP-7

MMP-7 activity has been detected in serum from patients with Duchenne muscular dystrophy [22] and contribute to adipocyte differentiation, Fas-receptor mediated apoptosis, disrupted cell aggregation and increased cell invasion, osteoclast activation, vasoconstriction and cell growth [19]. In murine vertebral disk resorption the activity of MMP-7 is required for generation of signaling proteins that activate and recruit macrophages, a process independent of ECM degradation [18]. MMP-7 knockout mice have phenotypes exhibiting defects in innate immunity and prostate involution, and impairments in tracheal wound repair, migration of neutrophils and herniated disc resorbtion in addition to reduced intestinal adenoma formation [32].

MMP-9

MMP-9 expression is low in normal tissues and is markedly elevated during neoplasia, inflammation, and wound healing [30]. MMP-9 cleaves type IV collagen and elastin [19] and is itself activated by MMP-3 [30]. In an interesting interplay between genetics and its manifestation in disease states, it has been demonstrated that in patients with coronary artery disease a C/T transition in the MMP-9 promoter-1562 results in a large increase in mRNA but not in healthy patients possessing the same mutation [18]. In human decidual cells, MMP-9 release is stimulate by protein kinase C activity [29]. MMP-9 is also thought to degrade and alter cytokine, chemokine, and growth factor activity [30]. MMP-9 is induced by TNF- α , LPS, and ECM components requiring increases in cyclooxygenase-2 (COX-2) and prostaglandin E₂ [30]. MMP knockouts also have growth plate defects [21].

MMP-13

MMP-13 (aka collagenase-3) is upregulated in chondrocytes via the IL-1 associated mitogen activated kinases p38, and JNK and via ERK in osteoblasts [29]. It is involved in skeletal development as well as contributing to joint tissue destruction playing a dominate role in the progression of rheumatoid and oseto arthritis [17, 34]. MMP-13 is involved in osteoclast activation and plays an anti-inflammatory role [19] as well as a promising biomarker for postoperative relapse in colorectal cancer [35].

TIMP-1

TIMP-1 is expressed in many tissues including the reproductive system and in the nervous system at the more dynamic locations such as the hippocampus [23]. It has been shown to be antiaopoptotic in some cell types, perhaps via posphoinositide-3-kinase [23]. TIMP-1 concentrations have been shown to be elevated in breast and colorectal cancer patients and have been indicators of poor outcome [23]. It looks to be a promising biomarker for colorectal, breast, and other cancers of the blood [23]. TIMP-1 has been shown to be an important component of plasma cell development as well as in tumors of pertaining to these cells [36]. However, inhibition of TIMP-1 enhances angiogenesis and cell migration [37].

TIMP-2

TIMP-2 has been shown to have antiproliferative effects that are MMP independent [22]. It is constitutively expressed in most tissues but is not induced by common growth factors [23]. TIMP-2 binds to $\alpha 3 \beta 1$ integrin leading to G1 arrest. It is upregulated by IL-1 (in conjunction with TIMP-1) in rheumatoid arthritis [38]. TIMP-2 has been shown to reduce angiogenesis and invasion but also inhibited apoptosis in melanoma cells [39]. TIMP-2 concentrations have been shown to be markedly different in the vitreous humor of diabetic patients with retinal detachments and diabetic retinopathy [40].

Summary

The continued research on the ECM and MMPs has revealed important information on the progression of many diseases, even though the research has not translated to safe and effective therapies that inhibit MMPs. However, the potential for MMP inhibitors is vast and research has continued unabated. Whether you are looking for the elusive MMP inhibitor with limited side effects, or examining the role of the ECM

and ECM modification in your favorite diseases, there are multiplex technologies that will prove helpful in your research. The Quansys MMP array contains ELISAs to quantify MMPs 2, 3, 7, 9, and 13. The Quansys Angiogenesis array contains the antiangiogenesis MMP inhibitors TIMP-1 and TIMP-2 as well as multiple growth factors involved with MMPs providing you with powerful tools to quantify protein concentrations saving you time, precious sample, and money.

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