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Matrix Metalloproteinases: New Targets in Cancer Therapy

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Abstract

Matrix metalloproteinases (MMPs), the entities responsible for eradicating the structure of extracellular matrix - ECM, are the zinc or calcium ion-dependent enzymes. This family of enzymes embodies a vast spectrum of proteases ranging from collagenases, stromelysins to the membrane type MMPs. The tasks linked with these enzymes are significant not only for a sound and stable development of the body but are also found guilty of carrying out angiogenesis, promoting tumor development and thus providing means to disseminate the cancer cells to the sites other than the primary tumor locale. At each step of angiogenesis, MMPs are operating, thereby featuring endothelial cells and several growth factors like VEGF, FGF, etc. Activation of pro-MMP2 with the involvement of MT1-MMP is one of the key steps that lead to the synthesis of blood vessels from an already existing one. For limiting the action of MMPs, various therapeutic techniques highlighting the mechanism of MMP inhibition have been studied. Several agents have been investigated for phase I, II and III clinical trials in combination with other anti-cancer therapies. Natural endogenous inhibitors of MMPs, TIMPs have a limited half-life and are thus not suitable for the desired outcome. Synthetic agents like Marimastat and BMS-275291 have shown reliable results. Nonetheless, explicit research is required for novel agents being designed and synthesized to attenuate the activity of matrix metalloproteinases that are accountable for cancer metastasis.

Keywords: Matrix metalloproteinases; Angiogenesis; Tumor progression; Cancer metastases; Tumor microenvironment; Matrix metalloproteinase inhibitors

Introduction

The extracellular matrix is likely to undergo degeneration from time to time in order to carry out the crucial processes like tissue repair and remodeling, development of certain components, morphogenesis and assorted signaling activities [1,2]. The elements that are responsible for ECM degradation are the matrix metalloproteinase-MMP. These proteases are involved in decomposing the components of ECM to generate various cellular environments in order to execute precisely coordinated mechanisms. MMPs are intently regulated at different steps ranging from transcriptional level to their activation, communication with other ECM components to their inhibition by specific molecules. If not regulated closely, the unchecked activity of these proteases may lead to several defects including arthritis, cancer, chronic tissue ulcer, fibrosis, aneurysms, nephritis, encephalomyelitis [3], atherosclerosis etc. [2].

Indeed, the discovery of MMPs was based on the fact that for the process of degeneration to take place within the collagen present in the tail of a tadpole, a collagenase activity was required during metamorphosis [4]. First characterized about fifty years ago [5], the matrix metalloproteinases (MMPs), also known as matrixins, are a family of endopeptidases that specifically target the molecules of ECM to conduct important physiological activities like wound healing, apoptosis, cell migration, angiogenesis, ovulation, embryonic development, invasion and proliferation [6]. These enzymes have shared structural and functional sites that are preserved among the various members of the family (Figure 1) and are activated by common processes that work at a neutral pH. The activity of these proteases is based on the presence of Zn^{2+} or Ca^{2+} ion at the active sites [7]. Either secreted or attached to the membrane, these enzymes are produced in a zymogen form that is later activated by the removal of 10 kDa pro-domain at the amino terminal [8]. The inactive state of these enzymes is maintained by the linkage between a cysteine residue of 10 kDa pro-domain with that of a zinc ion at the catalytic site. Based on the sequences, the pro-enzymes undergo a mechanism known as

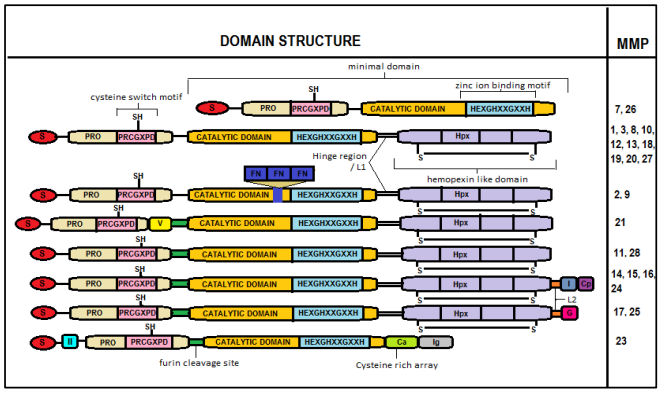

Figure 1: Basic domain structure of a MMPs (modified), consisting of minimal domain (S; signal peptide, Pro; pro-peptide, Cysteine switch motif (PRCGXPD) with a -SH thiol group, Cat; catalytic region with a zinc ion binding motif (HEXGHXXGXXH)), hinge region or linker (L1) of variable lengths, Hpx; hemopexin like domain that comprises of 4 repeats and has a disulfide linkage (S-S) between its first and last sub-domains. FN; fibronectin type II like domain, V; vitronectin insertion (promotes cell adhesion), L2; linker 2 [91], I; type 1 transmembrane domain, II; type 2 trnasmembrane domain, Cp; cytoplasmic domain; Ca; cysteine array, Ig; IgG like domain, G; GpI anchor, Furin cleavage site.

the cysteine switch to change into a proteolytically active state. The cysteine switch is characterized by the action of convertases, involving the removal of the prodomain that leads to disruption of the linkage between the cysteine residue and the zinc ion, either in the extracellular environment by other MMPs or proteinases like plasmin or in the

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intracellular environment by furin (a paired basic amino acid cleaving enzyme). The general structural features of a typical MMP (with some exceptions) includes a pro-domain of almost 80 aa, a catalytic domain of about 170 aa, the hinge region (variable lengths; linker) and about 200 aa long domain of hemopexin (Hpx) [1,2]. The enzymatic activity of MMPs is inhibited by a kind of proteins known as the tissue inhibitors of matrix metallo proteinases (TIMPs). A balance has to be maintained between MMPs/TIMPs in order to carry out regulated functions characterizing MMPs and a disturbance in this balance can eventually result in various abnormal conditions including tumor progression, invasion, angiogenesis, metastasis etc. [9-12].

In this review, the widely expressed MMPs and their role in angiogenesis, as well as different techniques that are targeting MMP's as anti-cancer therapies, particularly those involving MMPIs will be focused on. A tremendous amount of progress has been made in the recent years regarding the role of MMP's and that how these molecules are not only involved in the degradation of ECM but also take part in many other processes.

Matrix metalloproteinases: Grouping

>20 matrixins are expressed in humans (Table 1) [13] making up the matrixin family and are classified on the basis of substrate selectivity, domain assembly, and conservation within the sequences. The general features on the basis of which a proteinase is assessed before designating it as an MMP are sequence similarity with that of collagenase 1 (MMP-1), the factor found in the pro-peptide that is responsible for maintaining the matrix metalloproteinases in a zymogen form, the cysteine-switch motif (PRCGXPD), and a catalytic domain comprising of a zinc ion binding motif (HEXGHXXGXXH) [2].

Based on the previously mentioned aspects, the MMPs are labeled into the following categories.

Collagenases: this category includes interstitial collagenase; collagenase 1 (MMP-1), neutrophil collagenase; collagenase 2 (MMP-8), collagenase 3 and collagenase 4 designated as MMP-13 and MMP-18 (Xenopus) respectively. The substrate targeted by these peptidases is the fibrillar type collagen i.e. type I, II and III collagen. MMP-1 enzyme usually catalyzes the degradation of type III collagen, MMP-13 is involved in degeneration of type II collagen whereas MMP-8 shows its activity against type I collagen [14]. The characteristic feature of this group of proteinases is that they cleave their substrate at a specific site with an output of ¾ (from the N-terminus) and ¼ triple helical portions [1,2,15]. Apart from these substrates, collagenases can act on several other molecules as well that may or may not belong to the

Enzyme (Alternative names)	MMP	Chromosomal location (*human*)	Sequence length	Mass (Da)	Stromal cell expression	Biological activities	Diseases associated
Collagenases							
Interstitial collagenase; Collagenase 1	MMP-1	11q22-q23	469	54,007	Macrophages, fibroblast, dendritic cells	Collagen catabolic process, ECM disassembly, etc.	Autosomal recessive dystrophic epidermolysis bullosa, severe generalized recessive dystrophic epidermolysis bullosa
Neutrophil collagenase; Collagenase 2	MMP-8	11q21-q22	467	53,412	Neutrophils	Collagen catabolic process, endodermal cell differentiation, etc.	Gingivitis and gum cancer.
Collagenase 3	MMP-13	11q22.3	471	53,820	Fibroblast	ECM disassembly, bone mineralization and morphogenesis, etc.	Spondyloepimetaphyseal dysplasia, Missouri type, and metaphyseal anadysplasia.
Collagenase 4 (*Xenopus*)	MMP-18	Not found in human	467	52,813	-	Collagen catabolic process	-
Gelatinases							
Gelatinase A	MMP-2 (72 kDa type)	16q13	660	73,882	Macrophages, mast cells, endothelial cells, fibroblast, dendritic cells, HPCs	Embryo implantation, endodermal cell differentiation, ephrin receptor signaling pathway, ECM disassembly, etc.,	Torg Winchester syndrome, multicentric osteolysis of torg.
Gelatinase B	MMP-9	20q11.2-q13.1	707	78,458	Neutrophils, macrophages, lymphocytes, mast cells, fibroblast, dendritic cells, HPCs	ECM disassembly, ECM organization, leukocyte migration, macrophage differentiation, etc.	Metaphyseal anadysplasia 2, Intervertebral disc disease
Stromelysins							
Stromelysin 1	MMP-3	11q23	477	53,997	Lymphocytesendothelial cells, fibroblast, dendritic cells	Cellular response to nitric oxide, collagen catabolic process, ECM disassembly, etc.,	Coronary heart disease 6
Stromelysin 2	MMP-10	11q22.3-q23	476	54,151	-	ECM disassembly, ECM organization, proteolysis, regulation of cell migration, etc.,	Middle cerebral artery infarction, epidermolysis bullosa dystrophica.
Matrilysins							
Matrilysin 1	MMP-7	11q21-q22	267	29,667	Macrophages, endothelial cells	Aging, cellular response to mechanical stimulus, collagen catabolic process, etc.,	Light chain deposition disease, brain glioblastoma multiforme.
Matrilysin 2 Endometase	MMP-26	11p15	261	29,708	-	Collagen catabolic process, negative regulation of inflammatory response, proteolysis, etc.,	Endometrial cancer

Stromelysin 3	MMP-11	22q11.2	488	54,590	Fibroblast	Basement membrane organization, collagen catabolic process, collagen fibril organization	Aleutian mink disease, ophthalmomyiasis
Membrane-type MMPs							
(A) Transmembrane-type							
MT1-MMP	MMP-14	14q11-q12	582	65,894	Macrophages, endothelial cells, fibroblast, HPCs	Angiogenesis, astrocyte cell migration, ECM disassembly, etc.	Winchester syndrome (WNCHRS)
MT2-MMP	MMP-15	15q13-q21	669	75,807	-	Cellular protein modification process, collagen catabolic process, endodermal cell differentiation, etc.	Arthritis, metastasis, etc.
MT3-MMP	MMP-16	8q21	607	69,521	-	Chondrocyte proliferation, collagen catabolic process, craniofacial suture morphogenesis, etc.	Osteochondrosis, seminoma
MT5-MMP	MMP-24	20q11.2	645	73,231	-	Cell-cell adhesion, cell-cell adhesion mediated by cadherin, proteolysis, etc.	-
(B) GPI-anchored							
MT4-MMP	MMP-17	12q24.3	603	66,653	-	Positive regulation of catalytic activity, etc.,	-
MT6-MMP	MMP-25	16p13.3	562	62,554	-	Hard palate development, inflammatory response, proteolysis, etc.	-
Others							
Macrophage elastase	MMP-12	11q22.2-q22.3	470	54,002	Macrophages	ECM disassembly, ECM organization, positive regulation of epithelial cell proliferation involved in wound healing, etc.	Mid-dermal elastolysis, pulmonary emphysema.
--	MMP-19 RASI-1	12q14	508	57,357	Endothelial cells, fibroblast, dendritic cells	Angiogenesis, cell differentiation, collagen catabolic process, etc.	Cavitary optic disc anomalies, anthracosis
Enamelysin	MMP-20	11q22.3	483	54,387	-	Amelogenesis, collagen catabolic process, ECM disassembly, etc.	Amelogenesis imperfecta, hypomaturation type, 2A2
XMMP (*Xenopus*)	MMP-21	-	569	65,043	-	Coronary vasculature development, determination of left/right symmetry, HPC differentiation, etc.,	-
CA-MMP	MMP-23	1p36.3	390	43,935	-	Proteolysis, reproduction, etc.,	-
CMMP (*Gallus*)	MMP-27	11q24	513	59,026	-	Collagen catabolic process, etc.,	-
Epilysin	MMP-28	17q21.1	520	58,939	-	Negative regulation of macrophage chemotaxis	-

Table 1: MMPs: Trivial names and chromosomal location (human) [2]. Numerous stromal cells are found in tumor microenvironment including macrophages, neutrophils, mast cells, lymphocytes, endothelial cells etc. These cells express a variety of MMPs to degenerate the extracellular matrix and participate in tumor growth, invasion, metastases and angiogenesis. MMPs sequence lengths along with their mass (expressed in dalton, Da), biological activities and diseases associated with these MMPs have been mentioned [10-12].

extracellular matrix [2]. Studies have suggested that collagenases are involved in tumor cell invasion and metastasis. Elevated expression of MMP-13/collagenase 3 has been reported in breast carcinoma, with reduced expression in the normal tissue, suggesting its involvement in the tumor cell invasion [16]. MMP-1 has been noted to participate in the release of bFGF by degrading the endothelial derived perlecan [17].

Gelatinases: this group has two members namely gelatinase A and gelatinase B designated as MMP-2 and MMP-9 respectively. These peptidases act upon gelatin i.e. denatured collagen, several types of collagens including type IV (degradation within the triple helical structure), V, VII, IX and X [18] along with other ECM molecules like aggrecan core protein etc. MMP-2 and MMP-9 have three repeats of fibronectin type II, within their catalytic domain (Figure 1) that has an affinity for gelatin/collagen. MMP-2's catalytic activity resembles that of collagenases in denaturing the collagen type I, II and III [1]. The basement membrane has type IV collagen in excess and MMP-2/MMP-9 may catalyze the degradation of the basement membrane, suggesting their role in tumor metastasis. Both of these gelatinases have been reported to be participating in angiogenesis when secreted by tumor-associated macrophages (TAMs) in the tumor microenvironment [19,20].

Stromelysins: stromelysin 1 (MMP-3), stromelysin 2 (MMP-10) and stromelysin 3 (MMP-11) are members of a class that belongs to matrix metalloproteinases family. Stromelysin 1 and stromelysin 2 are alike, in the context of domain structure (Figure 1) and substrate selectivity with their genes located on chromosome 11 along with the genes of other MMPs including MMP-1, MMP-8, MMP-20, etc. The substrates targeted by MMP-3 and MMP-10 include proteoglycans, fibronectin, laminin etc. [21,22]. However, stromelysin 3 (MMP-11) is distantly related because of differences in chromosomal location

(chromosome 22), structure as well as substrate specificity. It usually targets serpins as well as α1 proteinase inhibitor [23]. MMP-11 is characterized by the presence of a furin cleavage site near C-terminus of the pro-domain and is usually changed into an active state in the intracellular environment by furin, whereas the other two stromelysins (MMP-3, -10) are released into the extracellular environment in the zymogen form and are later activated. An isoform of MMP-11 (β stromelysin 3) found intracellularly in the placenta with an unknown function has been reported [1]. MMP-11 is usually grouped with "other" MMPs due to the above-mentioned characteristics. These stromelysins are also active constituents that participate in tumor metastasis and invasion. Expression of MMP-3 and MMP-10 along with other MMPs in various types of carcinomas particularly in breast, head, esophageal squamous cell etc. have been recorded in various studies [24,25]. Higher grade tumor in NSCLC is characterized by the overexpression of MMP-10 and MMP-3 [26].

Matrilysin: proteinases belonging to this group are MMP-7 (matrilysin 1) and MMP-26 (matrilysin 2; also known as endometase). These matrilysins constitute the minimal domain (Figure 1) that comprises a signal peptide, pro-domain and the catalytic domain. Hemopexin domain is absent. Synthesized by the epithelial cells and targeting the cell surface molecules like E-cadherin, pro-TNF-α, Fas ligand, pro-α-defensin etc. is the MMP-7 enzyme that also acts on a number of extracellular matrix components as well [2]. The expression of MMP-7 is elevated by the tumor-associated macrophages-TAMs when present in the hypoxic areas of the tumor microenvironment [27]. This proteinase is involved in endothelial cell migration and proliferation and thus supports tumor angiogenesis [20]. MMP-26 (matrilysin 2), usually found in the intracellular environment degrades several components of the extracellular matrix and is frequently expressed by endometrium cells [28]. Endometase/MMP-26 is expressed is certain carcinomas such as breast ductal carcinoma [29] and endometrium tumor cells [30].

Membrane-type MMPs: this group is further divided into two types of MMPs, transmembrane type and GPI (glycosyl phosphatidyl inositol) anchored. MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16) and MT5-MMP (MMP-24) are included in transmembrane type MMPs whereas MT4-MMP (MMP-17) and MT6-MMP (MMP-25) are GPI-anchored. All of these enzymes have furin cleavage site near the C-terminus of the pro-domain (Figure 1) and are most likely to be cleaved by the action of furin in the intracellular environment and expressed in active form at the cell surface [1]. Except MMP-17, all the members of this category are capable of activating pro-MMP2 [2]. MT1-MMP has been well described and is known to catalyze the denaturation of several extracellular matrix molecules, activates pro-MMP2 and functions when the plasmin is unavailable [22]. Collagens I, II and III can be acted upon by MT1-MMP due to its collagenolytic activity [1]. MMP-14 is also involved in angiogenesis and promotes tumor metastasis [31]. MT5-MMP is expressed in the cerebellum of the brain whereas widely expressed in the peripheral blood leukocytes and brain tumors is the MT6-MMP enzyme [32,33].

Other MMPs: some of the MMPs couldn't be placed into any of the above-mentioned categories. These include MMP-12, -19, -20, -23, -27 and -28. Among these, MMP-12, -20 and -27 share the same structure as well as the location of their genes on the similar chromosome as that of stromelysins (Figure 1) (Table 1) [1]. MMP-12 (macrophage elastase) is mainly expressed by the macrophages and is involved in their migration, supporting their role in tumor angiogenesis and metastasis [20]. MMP-19 is also known as RASI (rheumatoid arthritis synovial inflammation), since it is found in plasma and lymphocytes from rheumatoid arthritis patients, as a T-cell autoantigen [34]. It is also expressed by proliferating keratinocytes at the wound healing site [35]. MMP-20 is associated with a genetic disorder named amelogenin imperfecta that is caused due to the mutation of the cleavage sites where MMP-20 is supposed to act. MMP-20 digests amelogenin and is expressed by newly build tooth enamel, hence named enamelysin [2]. First found in Xenopus, and later in humans and mice, is the matrix metalloproteinase [21]. Little information is known about the activity of this enzyme in the ECM, however, it's known to digest gelatin [1] and is found in certain carcinomas [36]. An exclusive member that belongs to the family of matrixins is the MMP-23. It has a unique structure with a cysteine array followed by an Immunoglobulin-like domain, whereas it lacks the hemopexin domain. It's suggested that MMP-23 is a transmembrane protein with a transmembrane type II domain at the N-terminal part of the pro-peptide. However, it has a furin cleavage site and is therefore cleaved intracellularly and released in the extracellular space. In humans, the genes coding for MMP proteins is 24 in number whereas only 23 MMPs are found in humans. This is because MMP-23 is coded by two genes, both of which are located on chromosome number 1 [37]. Limited information about MMP-27 is known MMP-28 (epilysin), the most recent addition to the matrixin family, is known to express in a number of tissues like placenta, testis, heart, lung etc. [1]. MMP-28's role in wound healing has been reported [38] and is also expressed in patients with osteoarthritis [39].

Other relatable molecules to MMPs are the ADAMs (a disintegrin and metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs), belonging to the metzincin proteinase superfamily [5]. These are actively involved in maintaining the cell composition by regulating a vast array of activities ranging from cell signaling to its migration, adhesion, etc, within various types of cellular environments [40]. The domains shared by most of the ADAMs are the signal peptide domain, pro-domain, MMP and disintegrin domain, a cysteine-rich array, EGF-like sequence along with a transmembrane and a cytoplasmic region. An MMP domain is possessed by almost all, but the protease activity is displayed by a few of the ADAM members particularly ADAM-10, -12, -17 [22]. ADAM-17, also known as TACE, participates in converting pro-TNFα to its active form and has important functions in epithelial tissue regulation. ADAM-1 monitors the gene transcription by displaying its sheddase activity that yields intracellular domains which travel to the cell nucleus and regulate the transcription process [40]. ADAMTS, on the other hand, are usually secreted and share the proteinase, disintegrin as well as thrombospondin domains among their members. These are engaged in events like ovulation, cancer, and extracellular matrix modeling (Table 2).

The MMP inhibitors

The proteolytic activity of the matrix metalloproteinases can be suppressed by α-macroglobulin. Composed of four 180 kDa subunits,

Stromal Cells	ADAMs/ADAMTS Expressed
Macrophages	ADAM-9, -15, -17 ADAMTS-4
Fibroblasts	ADAMTS-5
Endothelial cells	ADAM-15, -17
Lymphocytes	ADAM-17, -28
Mast cells	-
Neutrophils	ADAM-8, -17 ADAMTS-1
Dendritic cells	-
HPCs	-

Table 2: Stromal cells found in tumor microenvironment express a variety of ADAMs and ADAMTS, indicating their role in tumor progression and metastasis.

Figure 2: Evaluation of angiogenesis [31].

the 725 kDa α-macroglobulin is a plasma protein found in human. The catalytic activity of MMP is inhibited by the receptor-mediated endocytic operation of the α-macroglobulin.

The other molecules that inhibit MMPs are the tissue inhibitors of matrix metalloproteinases-TIMPs. Comprising of 184-194 aa, TIMPs have been subdivided into a C and N-terminal sub-domain, where each domain retains three conserved disulfide bonds [1]. The TIMPs bind to their active substrates (MMPs) in a 1:1 ratio forming non-covalent complexes. Some of the TIMPs, for instance, TIMP-1 and TIMP-2, can bind in complexes (involving the C-terminal of both MMP and TIMP) with the latent form of MMPs and inhibit their proteolytic activity. Up till now, four members have been identified that belong to the endogenous inhibitor TIMP family. These are TIMP-1, TIMP-2, TIMP-3, and TIMP-4. All of them are involved in the inhibition of several MMP and ADAM molecules [22]. Besides their inhibitory action against the MMPs and ADAMs, TIMPs also participate in other activities, acting as multifunctional entities. For example, TIMP-1 and -2 suppress the apoptotic mechanism and are involved in promoting cell proliferation *in vitro* [41,42]. TIMP-3, on the other hand, promotes apoptosis [43].

Angiogenesis: An overview

Angiogenesis, a well-defined process for the formation of new blood vessels from an existing vessel, by certain mechanisms which involve endothelial cell proliferation and capillary formation [44]. Angiogenesis is required to carry out several activities like embryonic development (vascular remodeling), wound healing and female reproductive cycle [45]. It can be distinguished from vasculogenesis based on the fact that in angiogenesis, the participating endothelial cells do not differentiate from the stem cells. Rather, these endothelial cells crop up from the existing blood vessels. Angiogenesis comprises of several key steps, beginning from the degradation of the ECM along with endothelial cell division and migration and ending up with the assembly of the new vessel and extracellular matrix elements. Angiogenesis exhibits a well-coordinated interplay among the effector and regulatory elements, that is mediated by ECM proteinases and proteins found in the integral membrane. The process of angiogenesis is triggered only when stimulated by inflammatory or hypoxic stress conditions (the female reproductive cycle being an exception) characterized by several tumors [46,47]. Therefore, in order to learn about the details of MMPs roles in angiogenesis, an overview of the angiogenic process has been specified.

Firstly, the activation of angiogenic process is driven by various stimulating factors which include several growth factors like VEGF (vascular epidermal growth factor), EGF (Epidermal growth factor), PDGF (platelet derived growth factor), TGF β (transforming growth factor), TNF α (tumor necrosis factor), FGF (fibroblasts growth factor), proangiogenic cytokines like IL-18, angiopoietin, angiogenin, etc. These factors are released by keratinocytes, macrophages, pericytes, mast cells or tumor cells that are present at the site of inflammation, injury or under hypoxic conditions [44]. At the beginning of the vessel assembly, layers underneath endothelial cells i.e. type IV collagen and laminin, basement membrane, type I, II and III collagen and interstitial matrix are all degenerated and dissolved, illustrating the role of several proteinases involved in the extracellular matrix degradation leading to angiogenesis [48]. As the ECM degradation proceeds, the endothelial cells begin to invade the extracellular matrix in the form of a stable column known as the vascular sprout [49]. This vascular sprout is dependent on the activity of proteinases to degrade ECM but also at the same time, the proteinase inhibitors counteract against proteinases to maintain steady state ECM degeneration, creating a balance to perform a controlled proteolytic activity and thereby not degrading excessive ECM. In response to the growth factors, the endothelial cells migrate and proliferate resulting in the formation of capillary lumen. Later on, these endothelial cells arrange themselves tightly near the differentiating zone; a new basement membrane is constituted. ECs cease their proliferation and form a vessel wall [31]. With the passage of time, the components mature and a new blood vessel is now in a running condition, providing oxygen, nutrients and several types of cells to the distant sites. A large range of effector elements plays their role in carrying out a well-organized process, the angiogenesis. The part that these factors play has a significant impact on the outcome; therefore, they are precisely regulated throughout the process. MMPs perform many roles in angiogenesis, from ECM degradation to the assembly of the new vessel, which are discussed in the upcoming sections (Figure 2).

Angiogenic role of matrix metalloproteinase

The capability of cancer to advance to the surrounding organs or tissues sets up an even greater risk for lowering the life expectancy. Tumor cellular environment requires nutrients, oxygen and removal of waste products and this could be only achieved if it has a source of circulating blood and lymphatic vessels. Angiogenesis provides tumors with newly formed vessels which can carry nutrients and waste products to and from the tumor sites respectively [50]. The conversion of a tumor to a malignant form is highly dependent on its ability to carry out angiogenesis. When angiogenesis occurs in the primary tumors, it carries tumor cells, macrophages and infiltrating immune cells to other locations to form secondary tumors. Without angiogenesis, the tumor growth is limited by a diameter of about 0.2 mm [51]. Vessel formation within the tumor, by means of which the tumor cells enter the bloodstream, can be done by several other sources apart from

MMP/s involved	Factors involved	Anti-angiogenic activity of MMP
MMP-2	FGF	Cuts the ectodomain of FGFR1; in spite of ligand binding, signals are not transduced. No angiogenesis and other activities of FGF.
MMP-12	uPA	MMP cleaves the uPA receptor. Minimizing the signaling activities related with uPA ligand and hinders angiogenesis.
MMP-2	PEX domain	Binding of PEX domain (MMP-2) with αvβ3 integrin following the degradation of gelatinase A, resulting in inhibition of angiogenesis.
MMP-3, -9, -12, -13, -20	Endostatin	Binding of Endostatin with various receptors (eg. VEGFR, α5β1 integrin as well as proteoglycans) leading to apoptosis of ECs or deactivating MMPs like MMP-2, -9, -14, preventing angiogenesis.
-	Canstatin	Ties up with α3β1 and αvβ3. Regulates the activities that oppose angiogenesis.
MMP-9, -2, -3, -13	Tumstatin	Binding with αvβ3 integrin within the vessels that are found in tumor environments and leads to EC apoptosis and inhibits EC growth, division, and angiogenesis.
-	Arrestin	Binds to α1β1 integrin and modulates anti-angiogenic processes.
MMP-9	Thrombospondin 1	Elevated expression of TSP-1 correlates with overexpression of MMP-9 but shows reduced cell invasion and adhesion.
MMP-2	Thrombospondin 2	TSP-2 governs the endocytosis of gelatinase A by fibroblasts promoting anti-angiogenic activity

Table 3: MMPs participate in several anti-angiogenic activities while collaborating with several other factors.

angiogenesis. For instance, the precursor endothelial cells (bone marrow) directed vasculogenesis within the tumor. Human breast, prostate tumors, and uveal melanoma have been described to possess channels of tumor cells organized to form networks within the tumor, demonstrating vasculogenic mimicry. Occasionally, tumor cells opt to gather around the already existing vessels and generate a transitory pass way to enter the circulating blood, hence, known as vessel co-option [45]. Our focus is on the function of matrix metalloproteinases that they perform during angiogenesis, hence spreading the tumor cells to the distant locations elsewhere from the site of the primary tumor microenvironment.

At the beginning of angiogenesis, pro-angiogenic stimulators must provoke the initiation of the process. These signals turn on the "angiogenic switch" within the tumor, usually just before the metastasis. The signals that trigger the onset of angiogenesis may include peptidases and other pro-angiogenic elements expressed by the inflammatory cells. Conditions, like mutations in the genes leading to the activation of certain oncogenes and suppression of tumor suppressor genes, hypoxia, etc may also lead to the onset of angiogenesis [44].

The basement membrane is rich in type IV collagen and its degradation is a part of initial stages of angiogenesis. The gelatinases i.e gelatinase A (MMP-2) and gelatinase B (MMP-9) carry out the type IV collagenase activity leading to its degradation [52,53]. A study conducted by Zeng and co-workers reported that the basement membrane type IV collagen is lost as a result of MMP-2 and MMP-9 overexpression, *in vitro*. These readings are valid for a number of tumors, where metastasis involves the denaturation of basement membrane [54].

The conversion of pro-MMP2 to activated form involves its attachment to the MT1-MMP and this in turn forms complex with TIMP-2 and activated MMP-2 is released by the catalytic activity of MT1-MMP [55]. When type IV collagen is degraded by the activity of MMPs, exposes the integrin αvβ3 on the endothelial cells which then binds to a certain domain of MMP-2 to activate it [44]. Other studies have also stated the activation of the pro-MMP2 i.e. reaction of thrombin with thrombomodulin (present on the cell surface of endothelial cells) leads to the activation of protein C, which in turn activates the latent MMP-2 [56]. The specific activity performed on laminin 5 (γ^2 chain) by gelatinase A/MMP-2 and MMP-14 (MT1-MMP) [57] induces cell migratory properties within endothelial cells [17].

MMP-9 is elevated in a number of carcinomas that are metastasized, including those of head and neck. When these tumors lack MMP-9 expression, the density of microvessels is lessened as compared to when MMP-9 is expressed, suggesting its role in vessel formation as well as vessel maturation [58,51]. MMP-9 may be released time to time from the vesicles within the cells to carry out its proteolytic activity to degrade basement membrane [56]. The type I, III collagen found in the interstitial matrix and type II collagen found in the cartilage are degraded by the MMPs during angiogenesis. Type I-III collagens in the interstitial matrix are denatured by MT1-MMP that is triggered by VEGF. MMP-2 has proteolytic activity against type I collagen (interstitial matrix) when activated by MT1-MMP, whereas MMP-1, MMP-8, and MMP-14 are involved in degrading the type I, II and III collagens [48]. Several studies involving the knock-out mice are described to demonstrate the role of certain MMPs in angiogenesis. MMP-2, MMP-9 and MT1-MMP (MMP-14) null mice displayed developmental defects and deformities in vessel formation. This depicts the participation of MMPs in metastasizing tumors via angiogenesis [45].

MMPs can perform a dual action during angiogenesis and in order to carry out the extracellular matrix degradation, the equilibrium between these actions governs the outcome. Via their proteolytic activity, MMPs can activate pro-angiogenic elements, for instance, growth factors like VEGF and FGF. On the other hand, they may also activate angiostatin, a potent inhibitor of angiogenesis. MMP-9 is involved in the release of VEGF-A, a potent inducer of angiogenesis, from extracellular matrix [51]. When cleaved, VEGF-C may bind to the VEGFR-2 and helps in angiogenesis [59]. The participation of FGF in angiogenesis is illusory yet it exhibits its proteolytic activity on perlecan (derived from endothelial cells). MMP-1 and MMP-3 along with heparanase and plasmin are involved in dispensing the basic fibroblast growth factor [60,61]. Degrading extracellular matrix proteoglycan, decorin, are MMP-2, -3 and -7 and release the pro-TGF 1 [62,63]. Intriguingly, MMPs like MMP- 3, MMP-7, MMP-9, and MMP-12 are able to form angiostatin by degrading plasminogen and generating fragments that oppose the division of endothelial cells (ultimately leading to the inhibition of angiogenesis) and probably participate in carrying out apoptosis and EC migration and division [17]. Apart from angiostatin, other elements can hinder the process of angiogenesis. These may be synthesized by the activity of MMPs themselves or by other sources (Table 3) [44].

By lending their docking sites to soluble MMPs, growth factors are then administered by these MMPs under particular conditions and this interaction, in turn, embellishes the performance of MMPs. For example, TGF-β is activated by MMP-9 (mice mammary carcinoma) which has been bounded by CD44 receptor and ultimately inducing

angiogenesis [64]. The other role played by MMPs is their sheddase activity. This activity is performed by transmembrane proteases that cut the extracellular portions of other transmembrane proteins yielding fragments that have other functions. MMP-14 (MT1-MMP) is a membrane-bound enzyme that cleaves receptors like syndecan 1, CD44, tissue transglutaminase, etc. and governs cell migration and other activities. Another notable capability of MMPs is their participation in vascular regression, an important step in angiogenesis. Matrix metalloproteinases like MMP-1, MMP-2 and MMP-9 are involved in vascular regression (featuring apoptosis) within the lately developed capillaries [65]. MT1-MMP has been found in parallel to overexpression of VEGF in certain tumor microenvironments which are characterized by vascular forms of vessels, indicating the role of MT1-MMP (MMP-14) in the production of a growth factor (VEGF) and their collective participation in cancer angiogenesis [45].

Plasminogen activator-plasmin is concerned with the fibrinolysis within the extracellular matrix-ECM. Anyhow, the studies (using null mice) conducted to confirm the role of PA-plasmin system involvement in angiogenesis revealed that fibrinolysis during angiogenesis is not carried out by the PA-plasmin system and in fact some other proteinases are involved to assist endothelial cells in invading the fibrin matrix [66]. Hence, in order to consider other proteinases, several MMPs were investigated for their role in fibrinolysis *in vitro*. MMP-1, MMP-2, MMP-3 and MT1-MMP (MMP-14) were proven to carry out the fibrinolytic activity [67,68]. Therefore, endothelial cells invade the fibrin comprising the ECM in a way that is not reliant on PA-plasmin system but does depend on the activity of certain MMPs.

In view of the growing information regarding the functions of MMPs, limiting these enzymes just to the degradation of the extracellular matrix is, indeed, an injustice to their actuality. MMPs act not only act on the ECM substrates but also target many non-ECM molecules, expanding their range of action to a much broader spectrum [69]. During the recent years, assorted reports have cast the role of MMPs in various angiogenic activities. The purpose of stating above mentioned information about MMPs in angiogenesis is to draw the attention towards the solution required for a substantial problem, Cancer. The formerly mentioned data clearly depicts the jobs done by MMPs in the complex mechanism of angiogenesis. Researchers have made efforts to counteract this process by several therapeutic techniques (mentioned below) in order to limit the spread of tumor and increase the life expectancy.

Anti-Cancer therapies targeting MMPs

The tissue inhibitors of matrix metalloproteinases-TIMPs have shown their ability to inhibit the activity of matrix metalloproteinases and indicated their selectivity for anti-cancer therapies. For example, TIMP-1 can inhibit angiogenesis caused by the destructive conduct of MMPs. However, these biological molecules have a limited half-life when tested *in vivo* to validate their operation as a pharmaceutical agent [70]. Apart from this, TIMPs feeble methods concerned with gene delivery system led to its withdrawal as a potent option for MMP inhibition. Acknowledging this fact, studies are now focused on attaining an alternative solution to minimize the role of MMPs in cancer progression, hindering their functionality within the sophisticated arena of the tumor microenvironment. The concept that tumor cells secrete surplus amount of MMPs that results in cancer progression has grabbed the attention of many researchers. Several other strategies that target MMP inhibition have been designed and investigated in the recent years. Here, a brief description has been reviewed.

A generalized concept of the steps involved in bringing about the catalytic activity of matrix metalloproteinases has been highlighted by Hidalgo and co-workers. It refers to the activation of an MMP from the genomic level when an MMP gene is transcribed into an MMP mRNA, which is then later translated into an inactivated protein form of an MMP. Secretion and activation of pro-MMP then result in the degradation of the extracellular matrix [71]. These regulatory steps characterizing MMPs have been investigated in different ways to obstruct transcription of MMP genes. Cytokines like IFN-α, IFN-β and IFN-γ have shown to inhibit the MMPs at the transcriptional level [72]. Targeting the receptors of cytokines involved in increasing the expression of MMP genes, results in reducing the tumor burden in certain cancers [73]. Alternative options to interrupt MMP gene transcription includes blocking MAPK or ERK pathways or nuclear factors like NF-κB, ultimately resulting in lowering the MMP expression [15,74-76]. Besides targeting the MMP genes transcription, another strategy is to decrease the conversion of pro-MMP into activated form. This can be done by focusing on MT1-MMP, due to its capability to activate a number of pro-MMPs. Activation mechanism by MT1-MMP can be blocked by several compounds, ranging from naturally occurring to the synthetic ones. These include thrombospondin 1 and 2 [77,78], endostatin (Table 3), green tea catechins [79], α1-PDX [80], etc.

When pro-MMPs have been converted to active forms, the next task at hand is to block the proteolytic activity of MMP. Among the four endogenous inhibitors-TIMPs, none of them targets a specific MMP. Besides TIMPs, multiple therapeutic agents' i.e MMPIs (matrix metalloproteinase inhibitors) have been designed, synthesized and implanted at specific checkpoints within the MMP activation and action cascade [71]. These agents can target a specific MMP and reduce the risk of unchecked inhibition. Different generations of several synthetic compounds have been tested for various clinical trials of Phase III in humans.

Batimastat and Marimastat (developers: British Biotech) [71] are matrix metalloproteinase inhibitors that belong to the pseudo-peptides category (also referred as peptidomimetic inhibitors). These inhibitors directly interact with the zinc at the active site of an MMP via a zinc ion binding hydroxamate domain and imitate the MMP substrates [81]. Among these, Batimastat (BB-94) was the first synthetic inhibitor that was tested on patients with cancer for inhibiting the MMP proteolytic activity. It inhibits matrix metalloproteinases like MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 [71]. Batimastat reduced tumor metastases when tested in human models for colon carcinoma [82]. However, its administration in several clinical trials didn't lead to the desired outcome. Marimastat, the next peptidomimetic agent that could be taken orally, inhibits a wide range of MMPs like gelatinases, collagenases as well as MMP-12, MMP-13 etc. Regardless of its great advantages, Marimastat has been associated with side effects such as musculoskeletal syndrome [83,84].

Since the first generation of MMP inhibitors lacked the specificity required, non-peptidomimetic inhibitors were then designed to overcome this problem. The production of these agents was based on the conformational outline of the MMP 3D structure focusing on the zinc ion binding site within the catalytic domain of an MMP. Compounds like BAY12-9566 (tanomastat), AG3340 (prinomastat), BMS-275291 and CGS27023A belong to this category. Developed by Bayer Corporation, BAY12-9566 has a relatively longer half-life when found in plasma and actively inhibits metalloproteinases like MMP-2, -9, -11, -13, -3, -14 [85,86]. In a number of cases, on the account of the unlikely outcome followed by the administration of BAY12-

9566, the production of BAY12-9566 was ceased in September 1999. AG3340, also referred as prinomastat and developed by Agouron Pharmaceuticals [87], was reported to be analyzed in combination with chemotherapy in August 2000. The agent has been tested in phase I, II and III clinical trials and has expressed several side effects. For details see [71]. BMS-275291 inhibits MMP-2 and MMP-9 and was developed by Bristol-Myers Squibb, New York. This agent lacks the toxic side effects characterizing musculoskeletal syndrome [81]. When added to chemotherapy in phase III clinical trials in patients with non-small-cell lung cancer (NSCLC), BMS-275291 resulted in increased toxicity and no better outcomes were reported [88]. Novartis Pharmaceuticals developed CGS27023A, a broad range matrix metalloproteinase inhibitor that is involved in hindering the process of angiogenesis in certain tumors [89].

The derivatives of tetracycline have been modified chemically in order to make them lack the expression of antimicrobial properties. They inhibit the proteolytic activity of MMPs and are also involved in the inhibition of MMP synthesis by targeting the genes at the transcriptional level. This category includes compounds like minocycline, the classic doxycycline, and metastat (COL-3). These agents act by targeting the zinc or calcium ions present at the catalytic sites of matrix metalloproteinases. Other novel compounds like INCB7839 [90] and SB-3CT [91] have been developed for the same purpose i.e MMP inhibition. SB-3CT selectively hinders the activity of gelatinase A (MMP-2) and gelatinase B (MMP-9) by amending the basic structure of these gelatinases [83].

The next class belongs to the group of inhibitors that do not primarily inhibit MMPs but in the course of directing their primary targets, these agents tend to aid in MMP inhibition. Bisphosphonates are a group of drugs that inhibit mevalonate pathway (concerned with steroid production in eukaryotes) and are linked with the treatment of disorders characterizing calcium homeostasis and bone metastasis in certain cancers. They have shown to hinder MMP catalytic activity, particularly that of MT1-MMP, MMP-2, -9, -7 and MT2-MMP [71,92-94]. An inhibitor of P450 aromatase, letrozole can also inhibit the activity of MMPs. Letrozole inhibits gelatinases A/B and exhibits anti-invasive action against tumor cells [95].

Apart from TIMPS, other natural inhibitors of MMP have been found. Neovastat, an active inhibitor of MMP 1, -2, -7, -9 and -13, is extracted from the cartilage of shark. Together with blocking the catalytic activity of MMP, this compound displays anti-angiogenic properties as well as anti-metastatic properties by inhibiting endothelial cell proliferation [96]. Genistein is a soy isoflavone naturally occurring compound that has anti-cancerous features and is involved in obstructing the progression of the tumor by blocking MMPs [83].

Extracellular matrix is a crucial component for maintaining the structure of the extracellular environment. The degradation of ECM by matrix metalloproteinases is required for bringing about normal developmental processes. However, MMPs involvement in cancer progression is evident and inhibiting their activity or synthesis is the only way to end their deleterious effects. The tumor microenvironment is a diverse region comprising of a number of constituents. This environment keeps on changing and progressing with the passage of time and as a fallback, the expression of MMPs from the tumor cells or other cells in the tumor environment keeps altering. Therefore, administration of broad range MMP inhibitors might show better results as compared to those that target specific MMPs. But there lies another limitation to this notion i.e. administering broad range MMPIs might inhibit the proteolytic activity of those MMPs that are present in the surroundings and carrying out their function to bring about the ordinary physiological activities. Therefore, the designing of MMPIs and their consumption requires further research in order to provide the finest product.

Discussion

The tumor microenvironment is a complex arena where various different activities are taking place. Based on the secretion of particular cytokines, several immune cells are being recruited at the tumor site. In order to expand its operation beyond the point of its origin, the invariably active tumoral units need to infiltrate the surrounding tissues. This mission is accomplished by the matrix metalloproteinases. These enzymes, when expressed by certain cells, participate in promoting tumor metastasis by their active involvement in angiogenesis. The formation of new blood vessels serves as means for the transport of tumor cells and other constituents of the primary tumor to the secondary metastatic locality. MMPs begin by degrading the underlying basement membrane, forming a defect in the wall of the capillary and aiding the endothelial cells to invade the surrounding tissues. The secretion of MMPs also ensures the stable up keeping of the angiogenic phenotype by involving several other elements, for instance, generating the angiogenic inhibitor angiostatin as well as casting growth factors and their receptors [22]. Endothelial cells then arrange themselves to form a well-developed vessel wall that matures later as the tumor advances.

Matrix metalloproteinases are widely expressed in cancerous settings, not only from the tumor cells but also from the nearby stromal cells. Prostate cancer, breast cancer, ovarian cancer, oral cancer etc., marks the expression of MMPs [97]. Initially, the secretion of matrix metalloproteinases in certain tumors was linked to the degradation of the ECM aiding in tumor metastasis to the blood and lymphatic vasculatures. However, MMPs have been associated with an expansive role in tumor growth and progression. From initiating the growth of tumor at primary and secondary sites, particularly with the assistance of growth factors, to the regulation of angiogenic activities, the role of MMPs has been discussed in several reviews [18,98]. The mode of action of matrix metalloproteinases in different tumors depends on various factors, mainly on the type of cells or tissues, phases of endothelial cell maturation, host interactions with the local tumors etc. [48]. MMPs target many non-extracellular components like receptors found on the cell surface and growth factors, which in turn then regulate the activity of MMPs in tumor progression.

The emerging stream of details about the process of angiogenesis and the comprehensive data available regarding the role of MMPs in tumor invasion and metastasis has unlocked the doors to the development of potential neutralizing mechanisms. The meticulous 3D structure of the MMP catalytic sites and details of interactions between the MMPs and their substrates has provided essential grounds for acknowledging the molecular mechanisms involved in carrying out the MMP activities. With this excessive information, several novel and innovational therapeutic pathways have been proposed. In order to inhibit MMPs, agents like MMPIs have been investigated in several clinical trials. Many of the phase III clinical trials testing the efficacy of synthetic inhibitors have been terminated, since they were not potent enough to raise the life expectancy of cancer patients. Development of MMPIs based on the catalytic structure and their use in combination with other anti-cancer therapies has been practiced in many research studies. However, most of them have failed to get the satisfactory results. The failure in these clinical trials has highlighted the need to better understand the mechanisms involved in MMPs and tumor

interaction along with the other factors that participate *in vivo* to carry out the underlying process of tumor progression.

The activity of MMPs in the early stage of cancer is quite different than their action in the later stages and different MMPs are activated at various stages of cancer progression. Therefore, administration of broad range matrix metalloproteinase inhibitors may be favored. However, the use of broad-spectrum MMPIs has certain shortcomings as well. On their way to block the MMPs that are actively involved in cancer development, these MMP inhibitors may even hinder the anti-tumor activities of MMPs. Utilizing such MMPIs may not implement the desired outcomes. In order to better evaluate the process of MMP inhibition, novel imaging techniques need to be developed that could highlight the interaction of MMP with their inhibitors as well as the ongoing activities within the tumor environment. Developing MMPIs with fluorescent or radioactive particles may help in visualizing the MMPI interaction with that of MMPs. Also, it may contribute in denaturing the structure of specific MMPs *in vivo*, with the assistance of radioactive rays being emitted from the MMP inhibitor. A number of radioactive MMP inhibitory agents have been developed which are being investigated for their potential role as an MMP tracer for providing visual assistance [99-101].

On the account of data cited in this review, information provided by several phase I, II and III clinical trials being conducted, 3D techniques being used to elucidate the multi-domain structure of MMPs and development of various pharmaceutical techniques to counterbalance the destructive role of MMPs, it is concluded that considerable amount of research work is required in the upcoming years to study the possibilities of using widely proposed therapeutic strategies. The modern approach towards the schematic designing of remedial agents may outline an improved version of MMP inhibitors and other neutralizing capacities, for overcoming the controversial mechanism of cancer metastases [102,103].

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