Matrix Metalloproteinases

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Abstract: Matrix metalloproteinases (MMPs), also known as matrixins, belong to a group of zinc-dependent proteins, which are thought to play a central role in the breakdown of extracellular matrix. Collagen, elastin, gelatin and casein are major components cleaved by MMPs. The breakdown of these components is essential for many physiological processes such as embryonic development, morphogenesis, reproduction, and tissue resorption and remodelling. MMPs also participate in pathological processes such as arthritis, cancer, cardiovascular and neurological diseases. This review summarizes current knowledge regarding these proteins, their participation in physiological and pathophysiological roles, their involvement in activation and inhibition, and their interactions with other metal-binding proteins including metallothioneins.

Keywords: Matrix metalloproteinases, collagen, tissue inhibitors of matrix metalloproteinases, cysteine switch, zinc, metallothionein, tumour disease.

1. MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs), also known as matrixins, are a large group of zinc-dependent proteases responsible for cleaving and rebuilding connective tissue components such as collagen, elastin, gelatin and casein. Found in invertebrates, vertebrates and plants, their phylogenetic origin emerged from lower organisms, namely *Bacteroides fragilis* [1]. According to Web of Science and SCOPUS, “metalloproteinase*” appears in more than thirty thousand published papers within article titles, keywords and abstracts (Fig. **1**). Moreover, in Matrix Biology, more than one hundred and fifty papers (10 % of all papers published in this journal) focused on MMPs [2-8]. A large number of papers reference the biological and biochemical properties of MMPs because of their association with physiological and pathological diseases such as cancer [9-18].

Extracellular matrix assists with the organization and differentiation of cells, helps exchange information between cells and acts as a physical barrier against microorganisms. MMPs play an important role in the degradation of extracellular matrix, a process that takes place during developmental stages such as growth and morphogenesis. Due to their physiological functions, high levels of MMPs activity are observed in diseases and pathological processes involved in connective tissue degradation such as inflammation and cancer (Fig. **2**). These enzymes play crucial roles within organisms and are present in several forms and modifications, which differ in location, substrate specificity and regulation.

1.1. History

MMPs were discovered, in 1962, by Jerome Gross and Charles M. Lapiere while studying the degradation of triple-helical collagen during the metamorphosis of a tadpole tail [19]. The collagen was cleaved by an enzyme known as interstitial collagenase. This enzyme was first isolated from human skin in the inactive form, proMMP (also called MMP zymogen), in 1968 [20]. It was later found in both invertebrates and plants [17]. In 1990, it was discovered a cysteine switch mechanism was responsible for regulating the enzyme in its inactive form [13]. After the complete sequencing of the human genome, it was determined that twenty four different genes encoded a set of all human MMPs [21].

1.2. Classification

MMPs family members have in common roughly 40% of their primary structures. Approximately 20 different types of MMPs have been discovered and classified based on their pre-synthetic region on chromosomes and their various substrate specificities. Number designations MMP-1 to MMP-28 are used for classification [21], but some have still not been identified through this system (Table **1**). Table **1** also shows recent identified loci on DNA.

2. STRUCTURE OF MMPs

MMPs are homologous proteins, which can be classified into six categories according to substrate recognition and cleavage mechanism: collagenases, stromelysins, matrilysins, gelatinases, membrane-associated MMPs and MMPs with no group designation. X-ray crystallography and nuclear magnetic resonance (NMR) studies have made it possible to determine the structures of many MMPs [22]. MMPs are zinc and calcium-dependent endopeptidases, which are synthesized from inactive proMMPs. Commonly secreted from cells in its inactive form, with the exception of membrane-associated MMPs (MT-MMPs), this prevents MMPs from cleaving essential components in cells. The enzyme is divided into three domains: N-terminal propeptide, catalytic domain and C-terminal domain (Fig. **2**) [6].

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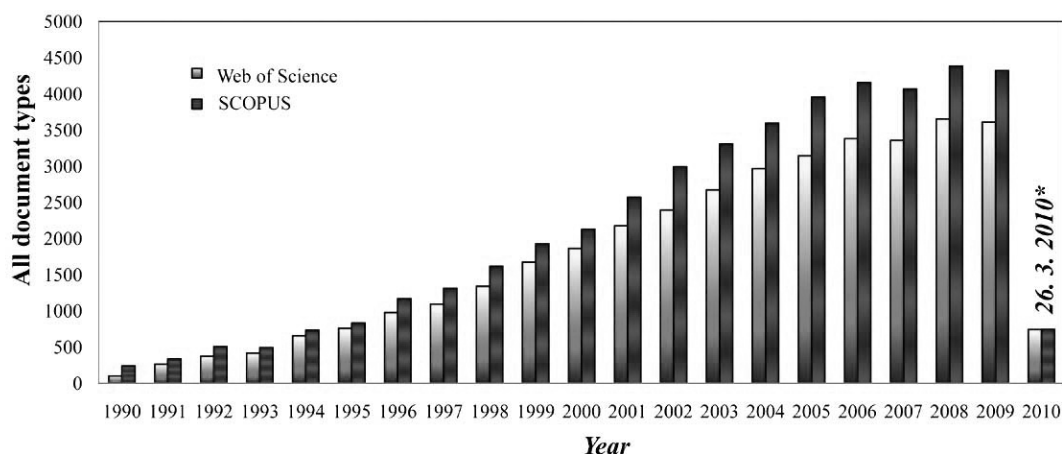

Fig. (1). Number of full length articles, reviews, meeting abstracts and proceedings papers having "metalloproteinase *" in titles, abstracts and keywords per year according to Web of Science and SCOPUS (26. 3. 2010).

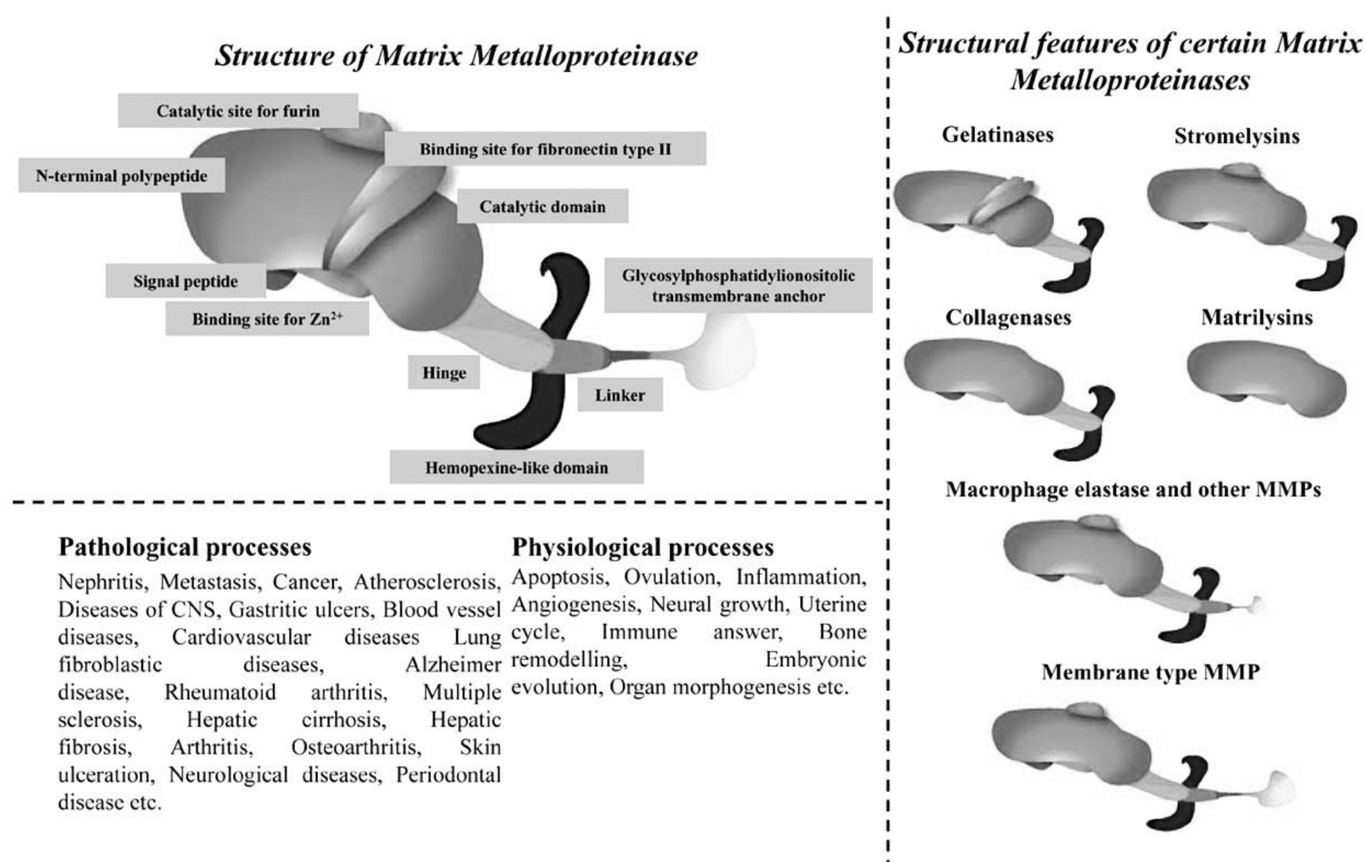

Fig. (2). MMPs domain structures and their roles in physiological and pathophysiological processes (left). Specific structural features of certain MMPs groups (right).

2.1. N-Terminal Propeptide

Containing approximately 80 amino acids, N-terminal propeptide ensures enzyme latency. The most important functional amino acid within the N-terminal propeptide is cysteine, which interacts with catalytic zinc ions through the thiol group and constitutes the cysteine switch (Fig. **3**). In the propeptide a highly conserved sequence (Pro-Arg-Gly-Cys-X-Pro-Asp, where X represents any amino acid) is present. Cleavage of the propeptide triggers proMMP activation [7].

2.2. C-Terminal Domain

The C-terminal domain (or hemopexin-like domain) is structurally similar to proteins of the hemopexin family. The domain has a relatively large surface area for protein-protein interactions e.g. cell membrane receptors. It is ellipsoid-shaped with propeller-like subdomain, where each leaf of the "propeller" is composed of 4 antiparallel β-sheets and one α-helix. The first and fourth leaf are linked by a disulfide bridge [23]. As published for collagenase-1, the catalytic and

Table 1. Classification of Matrix Metalloproteinases

MMP	Metalloproteinase	kDa	EC classification	Locus	Substrates
MMP-1	Collagenase (type I, interstitial)	43	EC3.4.24.7	11q22-q23	Collagens (I,II,III,VIII and X); gelatin; aggrecan; L-selectin; IL-1β proteoglycanes; entactin; ovostatin; MMP-2; MMP-9
MMP-2	Gelatinase A 72 kDa Gelatinase type IV collagenase	66	EC3.4.24.24	16q13	Collagens (I,IV,V,VII,X,XI and XIV); gelatin; elastin; fibronectin;aggrecan; MBP; osteonectin; laminin-1; MMP-1; MMP-9; MMP-13
MMP-3	Stromelysin-1 Proteoglykanase	46	EC3.4.24.17	11q23	Collagens (III,IV,V, and IX); gelatin; aggrecan; perlecan; decorin; laminin; elastin; casein; osteonectin; ovostatin; antactin; plasminogen; MBP; IL-1β; MMP-2/TIMP-2; MMP-7; MMP-8; MMP-9; MMP-13
MMP-7	Matrilysin	20	EC3.4.24.23	11q21-q22	Collagens (IV and X); gelatin; aggrecan; decorin; fibronectin; laminin; entactin; elastin; casein; transferrin; plasminogen; MBP; β4-integrin; MMP-1; MMP-2; MMP-9; MMP-9/TIMP-1
MMP-8	Neutrophil collagenase	58	EC3.4.24.34	11q21-q22	Collagens (I,II,III,V,VII,VIII and X); gelatin; aggrecan; fibronectin
MMP-9	Gelatinase B	92	EC3.4.24.35	20q11.2-q13.1	Collagens (IV,V,VII,X and XIV); gelatin; entactin; aggrecan; elastin; fibronectin; osteonectin; plasminogen; MBP; IL-1b
MMP-10	Stromelysin-2	46	EC3.4.2.22	11q22.3-q23	Collagens (III-V); gelatin; casein; aggrecan; elastin; MMP-1; MMP-8
MMP-11	Stromelysin-3	44	no match	22q11.2	Unknown (the most likely casein)
MMP-12	Macrophage metaloelastase	45	EC3.4.24.65	11q22.2-q22.3	Collagen IV; gelatin; elastin; casein; fibronectin; vitronectin; laminin; entactin; MBP; fibrinogen; fibrin; plasminogen
MMP-13	Collagenase-3	55	no match	11q22.3	Collagens (I,II,III,IV,IX,X and XIV); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; MMP-9
MMP-14	MT1-MMP	54	no match	14q11-q12	Collagens (I-III); gelatin; casein; fibronectin; laminin; vitronectin; entactin; proteoglycans; MMP-2; MMP-13
MMP-15	MT2-MMP	61	no match	16q12.2-q21	Fibronectin; entactin; laminin; perlekan; MMP-2
MMP-16	MT3-MMP	55	no match	8q21	Collagen III; gelatin; casein; fibronectin; MMP-2
MMP-17	MT4-MMP	54	no match	12q24	Unknown
MMP-18	Collagenase-4		no match	unknown	Collagens (I,II,III,VIII a X); gelatin; aggrecan
MMP-19	RASI-1		no match	12q14	Gelatin; aggrecan; fibronectin
MMP-20	Enamelysin		no match	unknown	Amelogrenein; aggrecan
MMP-21*			no match	1p36.3	Unknown
MMP-22*			no match	1p36.3	Unknown
MMP-23*			no match	unknown	Unknown
MMP-24	MT5-MMP		no match	20q11.2	Unknown
MMP-25	Leukolysin/MT6-MMP		no match	16p/3.3	Pro-gelatinase A; fibrin; fibronectin; collagen IV; gelatin
MMP-26	Endometase, matrilysin-2		no match	unknown	Gelatin Iα; P1; fibrinogen; fibronectin; vitronectin
MMP-28	Epilysin		no match	17q11.2	Casein

*… MMP genes were found on chromosomes, but their function and structure have not been identified yet.

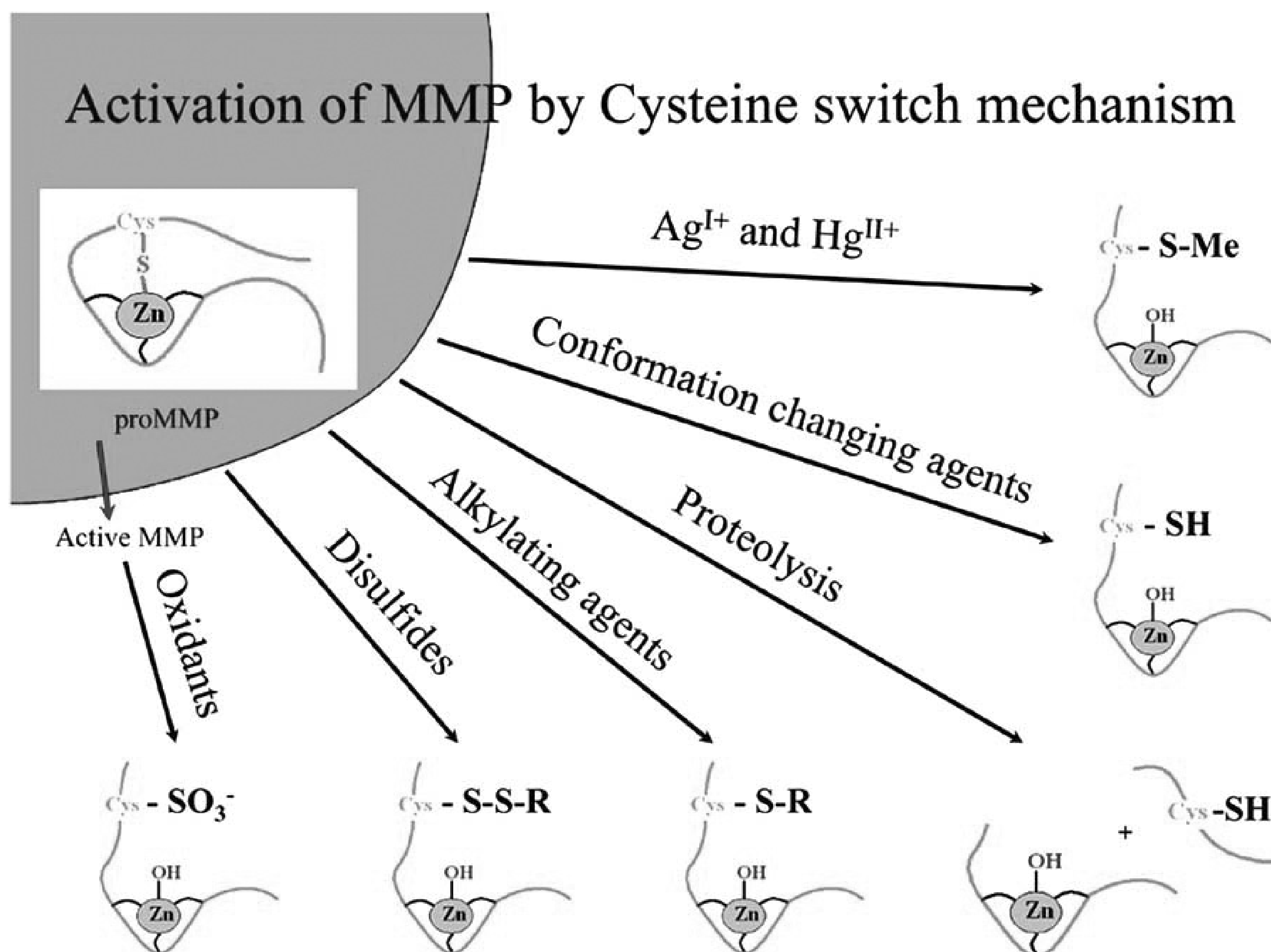

Fig. (3). Activation of MMPs by cysteine switch mechanism [74]. Activation of MMPs through cysteine switch can be achieved by several ways i) treatment with oxidants, ii) disulfides and iii) alkylating agents, iv) proteolytic cleavage, v) usage of agents changing conformation, and vi) silver(I) and mercury(II) ions.

C-terminal domains [23], are freely attached by a flexible proline-rich peptide linker (hinge). The length of the hinge is extremely variable 16 amino acid residues in collagenase to 65 amino acid residues in MMP-15. The specific function of the hinge is not fully understood (Fig. **2**).

2.3. Catalytic Domain

The catalytic domain consists of five β-sheets, three α-helices and connecting loops. It is composed of 170 amino acids and contains zinc-binding motif (His-Glu-His-XX-XX-XX-Gly-His, where X represents any amino acid) associated with methionine, which forms a unique structure known as the methionine loop. The catalytic domain contains two zinc(II) ions and two or three calcium(II) ions. The first Zn^{2+} ion present in the active site directly participates in catalytic processes. The second Zn^{2+} ion (also called structural) and Ca^{2+} ions are approximately 12 nm far from the Zn^{2+} ion in the catalytic site [24]. Calcium ions are necessary to stabilize the domain structure [25].

The hemopexin-like domain is characteristic for collagenase and it is necessary for the degradation of specific amino acid sequences in interstitial collagen. The catalytic domain of MMPs has proteolytic activity [26]. Matrilysin MMPs, however, do not contain this domain. MT-MMPs posse this catalytic domain. However, deletion of the hemopexin domains in MT1-, MT2-, MT3-, MT5-, and MT6-MMP does not impair their abilities to activate proMMP2 [27]. A transmembrane domain, this domain contains one hydrophobic chain composed of approximately 25 amino acids with a purine-like convertase specific recognition motif (Arg-X-Lys-Arg, where X represents any amino acid) , except MT4-MMP and MT6-MMP, which are are connected to the cell surface by a glycosylphosphatidylionositol (GPI) transmembrane anchor [8,17,25,28]. Gelatinases contain domains showing structural similarities with matrix proteins, three tandem copies of the domain with a sequence similarity to fibronectin type II (58 aminoacid moieties long) are present in all gelatinases[29].

Matrilysins (MMP-7 and MMP-26) belong to the smallest members of MMPs and do not contain the hemopexin-like domain. For MMP-23 the hydrophobic N-terminal signal anchor is specific [30,31]. MMP-19, MMP-20, MMP-27 and MMP-12 are considered to be the first members of a new MMPs group . Structures of other domains of matrilysins are similar to other MMPs, i.e. a signal sequence, the latent domain, catalytic domain and C-terminal propeptide-like hemopexin, but lack structures specific for other groups

[32,33]. Some MMPs, such as MT-MMPs and stromelysin-3, contain protein convertase specific recognition motifs (Arg-X-Arg-X-Lys-Arg, where X represents any amino acid) [34].

3. SUBSTRATE-CLEAVING MECHANISM AND SUBSTRATE-SPECIFITY

MMPs can degrade the majority and minority components of the extracellular matrix. With a few exceptions, namely MMP-11 and MMP-23, most of these enzymes have broad substrate specificity. MMPs not only break down extracellular matrix components, but also act as activators for biologically important molecules. For example, MMP-2, MMP-3 and MMP-7 have the ability to cleave decorin, a protein which interacts with transforming growth factor β-1 [35]. Consequently, the growth factor is released into surrounding tissue initiating other processes that may not be directly related to the degradation of extracellular matrix [36].

3.1. Collagenases

This group of MMPs includes MMP-1, MMP-8 and MMP-13. These MMPs degrade α-helices of interstitial collagen (type I, II, III) at two locations, specifically ¼ and ¾ fragments, in the place of uncoiled triple-helix (thanks to absence hydroxyproline and higher abundance of hydrophobic groups). Collagenases cleave the α_1 chain of collagen type I in sequence Gly775/Ile776 while the α_2 chain of collagen type I in Gly775/Leu776 regions [37,38]. Collagenases differ from other MMPs, in that they are only able to cleave collagen helices in the native state at neutral pH by a non-denaturing mechanism. Collagenases significantly differ in their substrate specificity. Neutrophil collagenases (MMP-8) cleave collagen type I substrates, while interstitial collagenases (MMP-1) cleave collagen type III substrates. Although MMP-3 binds to collagen type I, it does not cleave it [39-42]. MMP-13 cleaves collagen type I and III, but at a slower rate than type II [43].

In addition to its catalytic properties, De Souza *et al.* assume that a proline-rich linker in interstitial collagenase mimics collagen conformation [44], which destabilizes the quaternary structure of collagen triple-helix by forming the proline zipper between linker and substrate. This destabilizing step is critical for further cleavage. After destabilization, the catalytic domain cleaves only one strand of the uncoiled triple-helix in collagen. Afterwards, other MMPs can participate in further cleavage of collagen. Hemopexin-like domains are important in this process, because they can indirectly stabilize pro-collagen-like domains of collagenases. Further studies have shown this domain initially provides non-functional binding sites for substrates over hinge domain to ensure proper spatial arrangement of substrates before their cleavage [7,45].

3.2. Gelatinases

Gelatinases include MMP-2 and MMP-9 proteins. Gelatinases primarily cleave denatured collagen and intact collagen type IV in basal membranes. They are also able to cleave denatured collagen type V, VII, X, XIV, fibronectin, elastin and aggrecan. MMP-2 is known to cleave native collagen type I [39]. Other studies have shown that MMP-2 binds to intact collagen to prevent autolytic inactivation [46]. In addition to gelatin and other forms of denatured collagen, MMP-9 cleaves a number of other physiological substrates [47-51]. Although sequences cleaved by gelatinases are still not fully identified, repeated motifs with Pro-XX-Hy-(Ser/Thr), where X is any amino acid residue and Hy is a hydrophobic amino acid residue (Ala, Ile, Leu, Met, Phe, Trp, Tyr and Val) were found to be frequently cleaved. Another group of sequences cleaved by gelatinases include sequence motif Gly-Leu-(Lys/Arg). Substrates containing arginine residues were preferred by MMP-9 [52].

3.3. Stromelysins

Stromelysins have relatively broad substrate specificity. The majority of stromelysins cleave non-collagenous extracellular matrix proteins (proteoglycans, glycoproteins, fibronectin and laminin). Collagen type IV is cleaved in globular rather than helical conformation by stromelysins. Moreover, these enzymes can cleave other MMPs. Stromelysin 2 (MMP-10) can degrade the ends of propeptide domains of neutrophil collagenases (MMP-8), cleaving it at the Gly^{78}-Phe^{79} site, leading to the activation of MMP-8 only [43].

3.4. Membrane-Associated MMPs

Membrane-associated MMPs (membrane-type MMPs, MT-MMPs) exhibit similar substrate specificity analogous to free MMPs. They degrade mainly collagen but also other substrates as well. The main difference to other MMPs is their association with the cell membrane. In addition to its location, MT-MMPs also differ in its activity from other types of MMPs. MMP-14 (MT1-MMP) is five to seven times less effective in cleaving hydrolysed collagen type I than its analogue MMP-1. It is however eight times more effective in cleaving gelatin in comparison to MMP-1 [53]. MT4-MMP can cleave gelatin and synthetic substrates, but can not cleave collagen type I and IV, fibronectin and laminin [54]. MT2-MMP, MT3-MMP and MT4-MMP can cleave proMMPs prior to activation.

3.5. Macrophage Elastase and other MMPs

Macrophage elastase (MMP-12) shares its ability to cleave elastin with other MMPs (gelatinases and matrilysins). It is also able to cleave fibronectin, laminin, collagen, basal membrane, entactin, chondroitin sulphate etc. [55]. This enzyme enables macrophages to penetrate basal membrane and, thus, rebuild the inflammatory tissue. MMP-19 also degrades basal membrane [33]. In addition, MMP-20 can degrade tooth enamel, specifically emalogenin, [33]. Many MMPs (MMP-3, MMP-13, MMP-8 and MT1-MMP) cleave human aggrecan containing globular domain G1 and G2 at Asn^{341}-Phe^{342} [28].

4. ACTIVATION AND INHIBITION OF MMPs

From inhibition and activation of latent MMPs through the regulation of secretion of enzyme molecules to regulating gene transcription and inhibition of MMPs by tissue inhibi-

tors of metalloproteinases (TIMPs), MMPs are regulated at many levels. Genes coding for tissue specific MMPs are induced by biologically active molecules such as growth factors, oncogenes and tumour promoters.

4.1. Regulation of MMP Gene Expression

Most of the genes responsible for MMPs transcription is inducible and can also be activated by various chemicals such as phorbol esters [56]. Factors which may suppress the expression of MMP genes are transforming growth factor beta, glucocorticoids and retinoic acid [57,58]. Some key transcription-binding sites involved in the regulation of MMP genes are activator proteins (AP) -1 and -2 sites, the polyomavirus enhancer-A binding protein-3 (PEA3) site, the NF-κB site, and the STAT site [59]. Interestingly, MMPs that are co-regulated in their expression under certain conditions share several transcription-binding sites in their promoter sequences, whilst functionally related MMPs, such as gelatinases (MMP-2 and -9) or collagenases (MMP-1 and -8), differ greatly in the composition of the cis-elements present in their respective promoter regions [60]. The AP-1 site appears to be the major mediator of the regulation of MMP genes. Thus, most MMP promoters harbour an AP-1 site in the proximal promoter, located close to a typical TATA box. However, the composition of the AP-1 complex itself, as well as the juxtaposition of transcription factor binding sites, may determine the specificity among different genes [61]. Another responsive element, PEA3 site, binds members of the Ets family of oncoproteins. In several MMPs, the PEA3 site, located adjacent to the AP-1 site, may act cooperatively with the AP-1 site to promote MMP production by cancer cells resulting in their migration and invasiveness [62]. Ets1 increases MMP1 expression through c-Jun, which indicates MMP gene expression may specifically be modulated in both physiological and pathophysiological events [60,63]. The NF-κB pathway is involved in the regulation of several MMPs, upon activation by a number of growth factors and cytokines in pathological conditions, such as arthritis, muscular disorders and cancer [64-66]. In addition, this family of transcription factors, including NF-κB1 and 2, RelA, c-Rel, and Rel-B, interact with other proteins to increase MMP expression. Family STAT proteins frequently associates with different factors to induce MMP gene-specific expression [60].

MMP genes are expressed only when the tissue is remodelled under physiological or pathological conditions, remodelled. However, regulation of MMP-2 takes place at the level of direct activation and inhibition of enzyme activity [67]. Natural sequence variation of MMP gene promoters may alternatively modify the expression of MMP genes in different organisms. MMP genes may also be induced through various signalling pathways. Inflammatory cytokines (tumour necrosis factor and interleukin-1) indirectly influence the expression of genes for MMP and trigger the ceramide signalling pathway. Ceramide-dependent expression of MMP-1 in human skin fibroblasts is influenced by three distinct MAP kinase pathways: ERK1/2, stress-activated protein kinase/c-Jun N-terminal kinase and protein p38 [68-70]. An example of an inducible factor is ultraviolet B radiation which can increase the expression of MMP-1, MMP-3 and MMP-9 in human dermal fibroblasts [71,72].

4.2. Regulation of MMP Activity by Cysteine Switch

MMPs are secreted from cells as inactive forms known as proMMPs. Some inactive MMPs are found bound to the extracellular matrix, which allows their quick activation. MMP-2 binds to extracellular matrix containing elastin. MMP-3 binds to the basal membrane and occasionally to collagen fibrils while MMP-13 binds to proteoglycans, collagen and elastin [73]. Forms of "storage" are not the same for all MMPs. For example, MMP-8 is stored in specific cell granules, while MMP-1 and MMP-3 are constitutively produced by the activities of cytokines and inflammatory mediators [25].

Extracellular activation of the enzyme involves two steps. In the first step, MMP propeptide is cleaved releasing one zinc(II) ion from the containing cysteine complex (Fig. **4**). Propeptide contains a highly conserved sequence (Pro-Arg-Gly-Cys-X-Pro-Asp where X represents any amino acid) containing one cysteine residue. The thiol group of cysteine in the propeptide interacts with a Zn^{2+} ion in the enzyme's active site. This interaction, [Zn^{2+}-cysteine], keeps the proMMP inactive [74,75]. A mechanism known as the cysteine switch mobilizes proMMP's activation [17].

With the assistance of other MMPs, the second step involves cleaving the propeptide, which converts the enzyme into its active form. Activation pathways vary among MMPs. MT3-MMP and stromelysin-3 acquire active sites from a consensus containing Arg-X-Lys-Arg (where X can be any amino acid), which is directly activated by endopeptidases associated with the Golgi apparatus [76]. MT1-MMP as an inactive proMMP is located on the membranes of cells. Gelatinase A (MMP-2) and collagenase-3 can be activated on the cell surface by active membrane-associated MMP fulfilling both the function of the enzyme and the membrane receptor. This activation process requires active MT1-MMP and the binding of tissue inhibitor of MMP-2 and MT1-MMP [77-79].

4.3. *In Vitro* Mechanisms of MMP Activation

For the *in vitro* activation of MMP, sodium dodecyl sulphate is probably the most important agent, because it is used in zymograms to show proteolytic activity even of pro-MMP forms. MMP activation can be also induced *in vitro* by thiol-modifying reagents and oxygen radicals [73] which can change the affinity interaction between the thiol groups of cysteine in the propeptide and the zinc cation in the activation site. Similar mechanisms of activation were observed in MMP-2 and MMP-9 *in vitro* in the presence of metallothionein [80,81]. Change of physio-chemical conditions such as temperature or decrease in pH, may trigger MMP activity [17].

4.4. Tissues Inhibitors of Matrix Metalloproteinases

Tissue inhibitors of matrix metalloproteinases (TIMP) are major endogenous regulators of MMP in tissue. These proteins' molecular masses range from 21 to 30 kDa. Four homologous proteins known as TIMP-1, TIMP-2, TIMP-3 and TIMP-4 have been identified. MMPs bind with TIMPs in a 1:1 ratio forming binary noncovalent complexes with high dissociation constant K_d (10^{-9}–10^{-10}). MMP-TIMP

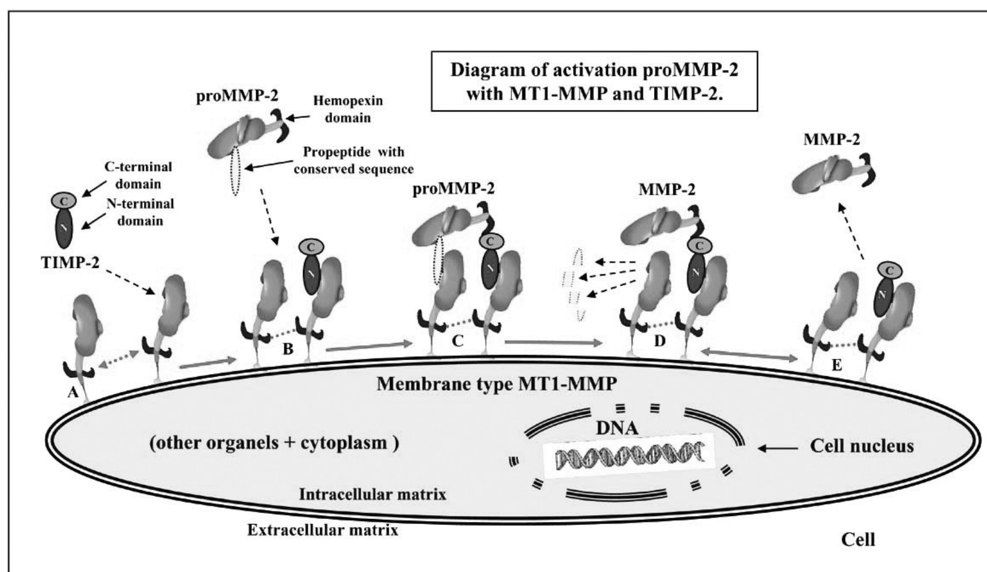

Fig. (4). Mechanisms of pro-MMP-2 activation by trimer formation with TIMP-2 and MT1-MMP on a cell membrane. Adopted and modified according to Visse and Nagase [220]. (**A**) Two membrane type MT1-MMPs are anchored on the cell membrane. (**B**) One molecule TIMP-2 selectively interacts with one MT1-MMP and forms MT1-MMP-TIMP2 complex. (**C**) This complex binds proMMP-2. (**D**) The second MT1-MMP anchored on the cell membrane free of TIMP-2 cleaves propeptide from proMMP2. (**E**) This process leads to activation of MMP-2, which is released into extracellular matrix.

Table 2. Classification of Tissue Inhibitors of Metalloproteinases

	Inhibited MMP	kDa	Expression	Tissues	Localization
TIMP-1	All except MMP-14	28.5	inducible	bones, ovary	diffusible
TIMP-2	All	21	constitutive	placenta	diffusible
TIMP-3	MMP-1,-2,-3,-9 a -13	21	inducible	kidney, brain	ECM associated
TIMP-4	MMP-1,-2,-3,-7 a -9	22	?	heart	diffusible

formations block substrate cleavage binding sites [82]. Classification and characterization of the data associated with TIMP are shown in the Table **2** adopted from [83].

TIMP-1 preferentially forms a complex with MMP-9, while TIMP-2 preferentially forms a complex with MMP-2. The crystal structure of complex TIMP-1 and catalytic domain of MMP-3 was previously described [84]. The critical points in MMP inhibition by TIMPs are centred on the disulfide bonds at Cys^1 and Cys^{73} [74]. The N-terminal α-amino group and carbonyl group of Cys^1 coordinate the position of the catalytic zinc ion. TIMPs catalysis of disulfide bond formation between cysteines prevents autocatalytic MMPs activation through the cysteine switch mechanism. In addition, TIMPs in complexes with other molecules involve in the inhibition of already active MMP as it was known in MT-MMP. Activation of proMMP-2 by MT1-MMP and TIMP-2 have widely been studied [74]. MT-MMP can form dimers and multimers on the cell surface *via* hemopexin domains (Fig. **4**). ProMMP-2 binds its hemopexin domain to the C-terminal domain of TIMP-2, which is linked *via* the N-terminal domain to MT1-MMP (Fig. **4**), while simultaneously inhibited by TIMP. Complex (MT1-MMP)-(TIMP-2)-(ProMMP-2), subsequently leads to splitting the conservative sequence of the propeptide located in the catalytic site of proMMP-2 by MT1-MMP (Fig. **4**).

A catalytic amount of MMP-3 significantly activates proMMP-9 in the absence of TIMP-1, but not complex (proMMP-9)-(TIMP-1) [85,86]. If complex (proMMP-9)-(TIMP-1)-(MMP-3) forms, the interaction between proMMP-9 and TIMP-1 weakens and the complex partially dissociates into free proMMP-9 and (TIMP-1)-(MMP-3). Therefore, a higher concentration of MMP-3 is needed for the activation of proMMP-9, which is inhibited by TIMP-1; however, TIMP-1 must also be saturated by other MMPs [87].

The expression of TIMP-1 and TIMP-2 is regulated differently by cytokines (e.g. tumour necrosis factor-α), which may modify the expression of TIMP-1 through the induction of nuclear transcription factors NF-κB [88,89]. TIMP-3 detected in the myocardium of mouse embryos is likely to be involved in embryonic extracellular matrix remodelling and heart development [90]. It differs from other TIMPs in that it is directly linked to components of the extracellular matrix (TIMP-1 and TIMP-2 is free in the interstitial space). Therefore, TIMP-3 modulates the activity of MMP more effectively than other TIMPs [91]. TIMP-4 was detected in low concentrations in the kidney and colon and is absent in the lungs, liver and brain. By contrast, high expressions of TIMP-4 were detected in the heart [92]. TIMPs also have other biological functions. TIMP-1 and TIMP-2 have mito-

genic activity in different cell types. TIMP-2 affects the interaction between the molecules from the extracellular matrix and cells [93,94]. Overproduction of TIMP inhibits the growth of various types of tumour cells. Decreasing the concentration of TIMP in the remodelling process during repair of damaged tissue increases the collagenolytic activity and allows tumour cells to disrupt the extracellular surroundings and migrate to neighbouring tissues. Strict regulation and maintaining specific levels of collagenase and its inhibitors is necessary for tissue remodelling. Its disruption can significantly alter the functional characteristics of tissue [95].

5. RELATION OF MMPs TO OTHER NATURALLY ZINC-CONTAINING PROTEINS

Zinc plays an essential role in biological systems with a variety of functions performed by zinc-binding proteins. Up to 10 % of the human proteome is potentially capable of binding zinc *in vivo*, with zinc-fingers being the most abundant class of metalloproteins [96,97]. The zinc(II) ion displays suitable properties for catalytic and structural functions within proteins. Amongst these properties are: (a) a great stability towards redox reactions; (b) a d^{10} electronic configuration where the coordination geometry (4–6) is not dependent on ligand-field stabilization; (c) an intermediate polarizability or borderline hardness allowing coordination of N, S, and O donor atoms; and (d) a Lewis acid useful in activating coordinated substrates, while still maintaining ligand nucleophilicity [98,99]. These properties make zinc the most common transition metal observed in proteins [97,100].

MMPs interact with biologically active metal binding proteins such as metallothioneins (MTs). Human MTs belong to the group of intracellular and low molecular mass proteins (from 5 to 7 kDa). Their primary structure is rich in cysteine and contains no aromatic amino acids. Although the naturally occurring protein has Zn^{2+} ions in both binding domains, which are able to bind up to seven divalent and/or twelve monovalent ions, these ions may be substituted for other metal ions that have higher affinity for thiolate such as Pb, Cu, Cd [101,102]. In mammals, these proteins may serve as a metal reservoir (mainly zinc and copper) for the synthesis of apoenzymes and zinc-finger transcription regulators. Moreover, new roles of these proteins have been discovered including those needed in carcinogenic processes [101,103, 104]. Fibrosarcoma tumour cells exhibit increased invasiveness in *in vitro* experiments with lung and ovary tissue of micerelated to MMPs and MTs activity [105,106]. It was also found that MT is able to activate MMP *in vitro* [80,81]. Progelatinase B, purified from conditioned medium of human fibrosarcoma HT-1080 cells, and gelatinase A, isolated from human fibroblast WI-38, were enhanced in a concentration- and time-dependent fashion by addition of MTs. One may suggest that MTs can contribute to MMP activation *in vivo*. The probable mechanism of MMP activation using MT is similar to the activation by thiols or surfactants, in which a Zn^{2+} ion in the active centre of the enzyme is more likely to interact with thiols moieties of MTs leading to the release of N-terminal propeptide. MTs affect the activity of MMP by indirectly and directly controlling levels of metals in an organism (Fig. **5**). Although metals are bound with high affinity in MTs, it was found that individual binding sites in MTs differ in their affinity for metals, and that MTa are capable of yielding different metal ions depending on pH under physiological conditions [107]. It was shown that MTs are able to deliver Zn^{2+} to metalloenzyme aconitase thereby activating them [108]. Additionally, MTs were able to deliver Zn^{2+} ions to carbonic anhydrase [109] and porphobilinogen synthase [110]. In other paper, it has been shown activation sorbitoldehydrogenase, by supplying Zn^{2+} and inhibiting tyrosine phosphatase-1B by the same ions coming from MTs.

Cytosolic Zn^{2+} concentrations can be regulated through the balance of MTs/apoMTs concentrations [111]. Although, MTs are intracellular proteins, they are also present in the extracellular space. Elevated levels of MTs found in the blood serum of cancer patients compared with control subjects [101,112,113] suggest the activation of MMPs in the body is not yet fully understood.

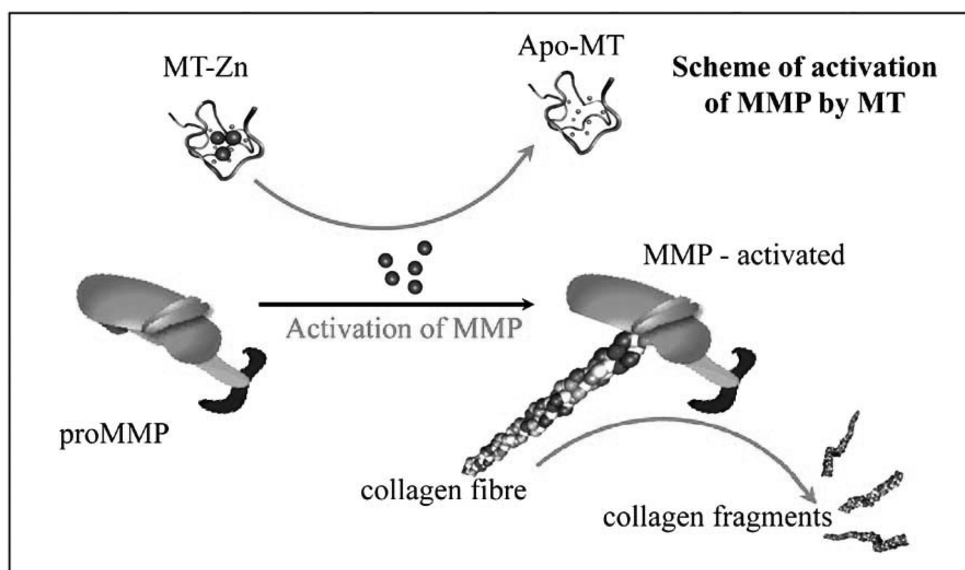

Fig. (5). MMPs activation by MT *via* transporting Zn^{2+} ions to MMPs.

6. MMPs PARTICIPATION IN PHYSIOLOGICAL PROCESSES AND DISEASES

6.1. Physiological Processes

MMPs primary role is to breakdown and remove extracellular matrix molecules from the tissue. However, it has been increasingly evident that the breakdown of extracellular matrix or cell surface molecules alters cell–matrix and cell–cell interactions and the release of growth factors bound to the extracellular matrix. A number of non-extracellular matrix molecules also become potential substrates of MMPs [114]. Fig. (**2**) lists examples of MMP actions that may affect many processes. Specifically, embryonic growth and tissue morphogenesis are fundamental events that require disruption of ECM barriers to allow cell migration and matrix microenvironment remodelling (Fig. **6**). The ability of MMPs to degrade structural components of ECM and basement membranes has supported their direct implication in these processes [115]. Most MMP genes are overexpressed in a number of reproductive processes, including menstrual cycle, ovulation, and uterine, breast and prostate involution [13,116]. In addition, the expression patterns of several MMP genes have been analyzed during gonadotropin induced ovulation, in order to identify those members responsible for follicular wall degradation [13,117]. The relevance of MMPs in embryonic development has prompted the identification and characterization of new members of this family in model organisms such as *Drosophila*, where developmental processes have been extensively studied [118,119]. The role of MMPs in tissue remodelling has also been studied by others [120-122]. The role of MMPs in angiogenesis is also broad in scope and complex. Many MMPs are produced by endothelial cells and is important in the formation of new blood vessels in physiological conditions [123].

In addition to physiological processes, MMPs effects are also observed in a number of pathological processes such as arthritis, Alzheimer's disease, atherosclerosis, vascular disease, gastritis ulcer disease, central nervous system disease, liver cirrhosis, tumours and their metastasis (Fig. **2**). MMPs can be studied from different perspectives as markers of some cancer, neurodegenerative, immune and cardiovascular diseases [14].

6.2. Cancer

The ability to invade tissues and establish colonies at remote sites is a defining characteristic of malignant neoplasms. Since metastases are the principal cause of death in cancer patients, a greater understanding of the process of tumour invasion and metastasis is essential in leading to the identification of new therapeutic targets. Tumour invasion is considered to be a dynamic, complex and multi-step process, which involves detachment of malignant cells from their point of origin, traversal of the extracellular matrix and basement membranes, and invasion into lympho-vascular channels. Proteolytic degradation of components of the basement membrane and extracellular matrix are essential steps in tissue invasion. The complexity of proteolytic sys-

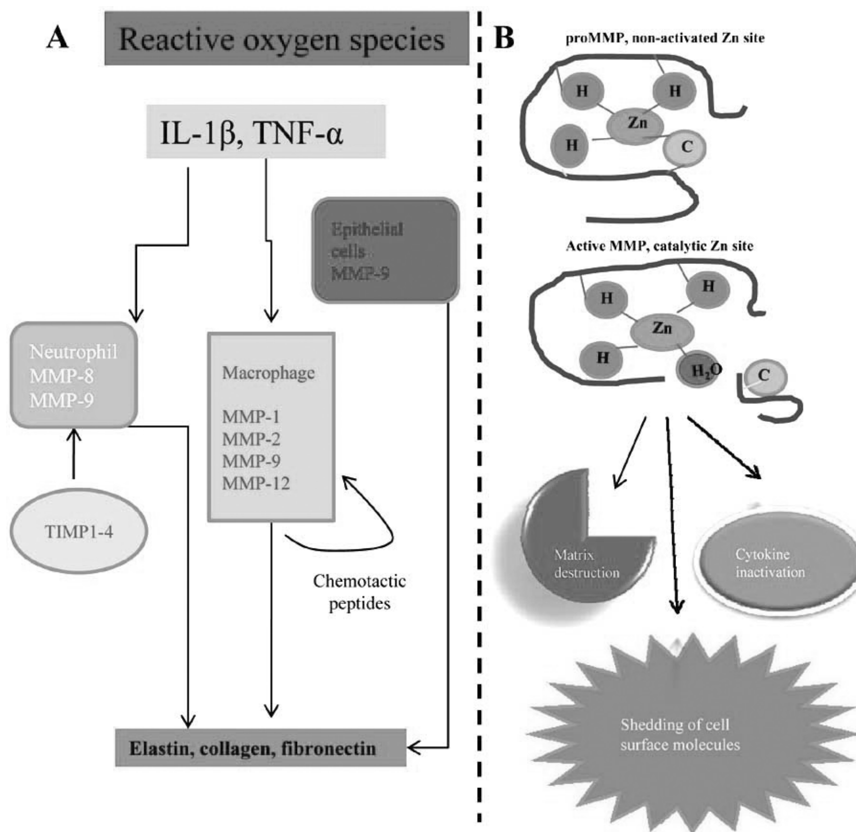

Fig. (6). (**A**) Activation of MMPs *via* molecules inducible by oxidative stress. (**B**) Activation of MMPs by cleaving of the signal propeptide and roles of the activated enzyme.

tems operating in human tissues is impressive, as assessed by the finding that more than 500 genes encoding proteases or protease-like proteins are present in the human genome [21]. Proteolytic breakdown of substances such as collagen, laminin, and fibronectin, which constitute major components of the extracellular matrix, requires specific proteases. There are four major groups of proteases – the aspartate and cysteine enzymes (including the cathepsins) which function at low pH and are involved mainly in intracellular proteolysis within lysosomes, and the serine and metal-dependent enzymes, which are active at neutral pH and are responsible for extracellular proteolysis. Although representative of all four classes of proteolytic enzymes have been implicated in tumour invasion and metastasis, the plasminogen activators (serine proteases) and the matrix metalloproteinases (MMPs) have been the subject of extensive study [124].

MMPs and their impact on tumour diseases emerged in the early nineties, when they were studied in relation to stomach, colon [125] and prostate cancer [126]. Isolating MMPs and studying their enzyme activities were published a year later [127]. In the same year it was found that stromal cells synthesize MMPs along with neoplastic epithelial cells to degrade the basal membrane, a characteristic of invasive tumour proliferation [128]. Thus, the relationship between MMPs and tumours were intensively studied [129]. In 1995, MT-MMPs (membrane type) in colorectal, chest, head and neck cancer was found [130]. Since then the mechanistic process of extracellular matrix degradation mediated by MMPs has been the focus of many investigations for years. Recent studies have shown the role of MMPs in cancer progression is much more complex (Fig. **7**) than that derived from their direct degradative action on extracellular matrix components [15,131,132]. Growth-factor receptors, cell adhesion molecules, chemokines, cytokines, apoptotic ligands, and angiogenic factors are some of many versatile substrates targeted by MMPs. The recent characterizations of new MMP substrates as well as the generation of genetically modified animal models with and without MMP function have demonstrated the relevance of MMP activities in the early stages of cancer development [123].

6.2.1. Tumour Growth

There is increasing evidence supporting the participation of MMPs in the regulation of tumour growth by favouring the release of cell proliferation factors such as insulin-like growth factors bound to specific binding proteins [133]. MMPs may also target and activate growth factors whose precursors are anchored to the cell surface or sequestered in the peritumour extracellular matrix [134]. Furthermore, a study has illustrated the direct effect of MMP matrix remodelling activity on cell growth. However, the authors found that the overproduction of a number of soluble MMPs did not have an effect on tumour cell growth [135]. Nevertheless, it is interesting to find that tumour cells may develop protease-independent migratory mechanisms in response to pericellular proteolysis blockage [136]. A recent study based on more than 2 600 determinations on cancer specimens from 133 patients with clinically localised prostate carcinoma, 20 patients with prostatic intraepithelial neoplasia and 50 patients with benign prostate hyperplasia and controls shows that MMPs 1, 2, 7, 9, 11, 13, 14 had higher expression. Tumours with higher concentrations of MMP-11 and MMP-13 have a significantly greater probability of relapse [137].

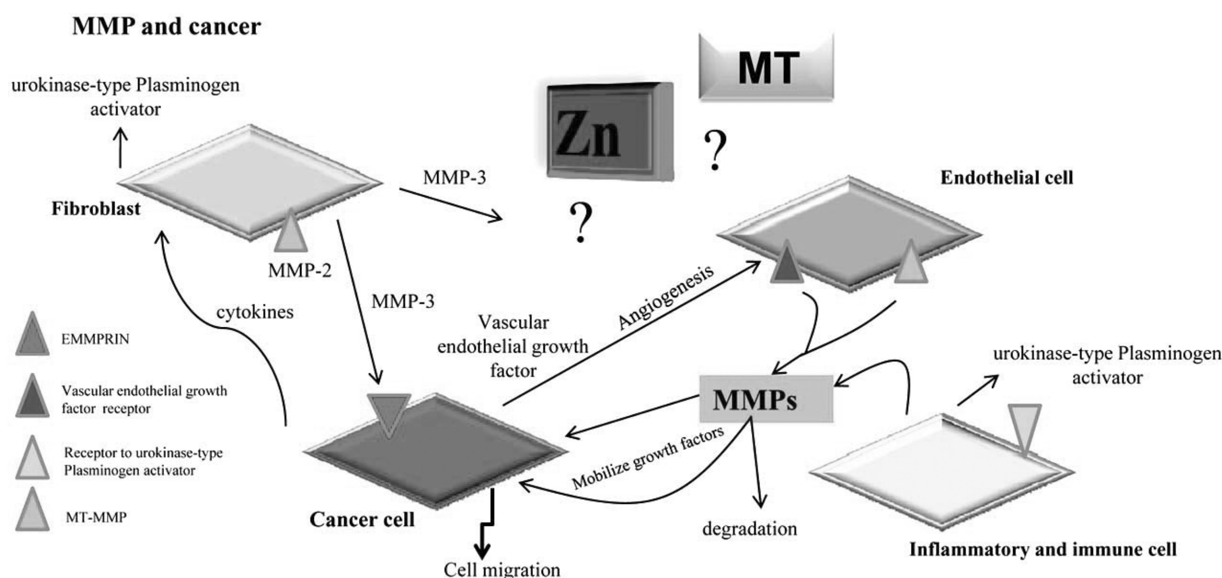

Fig. (7). Schematic illustration of the tumour interaction with surrounding tissues mediated by MMPs and possible role of zinc and metallothionein adopted and modified according to Zucker and Cao [221]. Cancer cells express cytokines and other factors, such as the extracellular MMP inducer that induce stromal cells (fibroblasts and endothelial cells) and inflammatory cells to produce proteinases. These include uPA, MMP-1 and MMP-2. Cancer cells are believed to accumulate the secreted MMPs in their cytoplasm after cell-surface uptake. Proteinases localized to the cancer cell surface, such as MT-MMPs and receptor-bound uPA play an important role in substrate degradation and cell migration. The displayed cells also effectively use these mechanisms. In addition to degrading the extracellular matrix, MMPs are involved in releasing sequestered growth factors and generating protein-cleavage products that have diverse biologic functions. Moreover, the connection with metallothionein and zinc(II) ions should be considered.

6.2.2. Tumour Protection

MMP activities have also been traditionally associated with a variety of escaping mechanisms that cancer cells develop to avoid host immune responses [138-140]. Some MMPs, such as MMP-9, can suppress the proliferation of T lymphocytes through IL-2Rα signalling disruption [141]. Likewise, MMP-11 decreases the sensitivity of tumour cells to natural killer cells by generating a bioactive fragment from α1-proteinase inhibitor [142]. MMPs can also modulate antitumour immune reactions by efficiently cleaving several chemokines or regulating their mobilization [143-145]. MMPs, additionally, protects it host by stimulating protective and adaptive immune responses [146].

6.2.3. Apoptosis

The ability of MMPs to target substrates that influence apoptotic processes is also relevant to cancer studies. MMP-3 has pro-apoptotic actions on neighbouring epithelial cells [147], whereas MMP-7, which is able to release membrane-bound Fas ligand, induces epithelial cell apoptosis [148]. Fas ligand cleavage promotes tumour progression by protecting cancer cells from chemotherapeutic drug cytotoxicity [149]. It was shown that MMP-1, MMP-9 and MT1-MMP are involved in autophagic cell death and apoptosis of breast tumour cells [150]. Other MMPs, such as MMP-11, suppress tumour cell apoptosis inhibiting cancer cell death [151].

6.2.4. Angiogenesis

The role of MMPs in angiogenesis is also of great interest [152]. The relevance of these enzymes as positive regulators of tumour angiogenesis has largely been demonstrated [153]. Several pro-angiogenic factors such as vascular endothelial growth factor, basic fibroblast growth factor or transforming growth factor-β are induced or activated by these enzymes, triggering the angiogenic switch during carcinogenesis and facilitating vascular remodelling and neovascularization at distant sites from a tumour [134,154-157]. Vascular endothelial growth factor and vascular endothelial growth factor receptor-2 interaction down-regulates MMP-9 *via* STAT1 activation and inhibits B chronic lymphocytic leukaemia cell migration [158]. Similar results on the interaction of growth factors and its receptors were published by Machado *et al*. [159]. In addition, Dommez *et al*. showed expression of MMP-9 and vascular endothelial growth thrombospondin-1 in urothelial carcinomas. These recently published results show a close relationship between vascular endothelial growth factor and MMP-9. Connections between angiogenic factors and MMPs were also discovered when MMP-9 induced tumour macrophages, endothelial cells and promoted lung metastasis [160]. Furthermore, host-derived MMP-9 contributes to the malignant behaviour of ovarian carcinomas by promoting neovascularization [161]. However, and contrary to proangiogenic roles of MMPs, the recent discovery of the mechanisms by which these enzymes negatively regulate angiogenesis has contributed to increased functional complexity of this proteolytic system in cancer. Thus, a number of MMPs are able to cleave the precursors of angiostatin and endostatin, and generate the active forms of these endogenous inhibitors [162,163].

6.2.5. Metastasis

During tumour proliferation and developing metastases, MMPs are responsible for tissue reconstruction near proliferating cells of malignant neoplasm, and participate in tumour growth in surrounding tissue [15,164]. For example, studies show MMP-9's involvement in lung tumour metastasis [165-167]. Furthermore, MMP-2 and MMP-14 selectively cleave the γ2 chain of laminin 5, consequently turning on chemotactic activity and stimulating cell migration *in vitro* [168,169]. Studies have also shown MMP-9 and MMP-14 cleave collagen type IV in lung tumours [167,170-172] and MMP-2, MMP-9 and MMP-12 degrade elastin-associated microfibrils in various types of tumours [173-175]. Several MMPs have opposing actions. MMP-3, MMP-7, MMP-9 and MMP-12 dismantle plasminogen to generate an N-terminal fragment that inhibits endothelial cell proliferation, known as angiostatin [162,176].

6.3. Other Diseases

Matrix metalloproteinases in synovial membranes, cartilage, tendon and bone of synovial joints in both ***rheumatoid arthritis*** and ***osteoarthritis*** are well documented and often correlated with tissue destruction [177,178]. In both diseases, inflammatory mediators stimulate the production of MMPs that degrade most components of the extracellular matrix. MMPs such as MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, MMP-13, MMP-14 and MMP-19 are also elevated in arthritis and, along with MMP-13, these enzymes degrade non-collagen matrix components of the joints [179]. Many MMPs have been associated with bone matrix turnover, involving virtually all cell types. Collagenolytic MMPs play a role in osteoblastic activities, but the major collagenolytic proteinase in osteoclastic bone resorption appears to be the cysteine proteinase, cathepsin K, which can function in the acidic phagolysosomal resorption zone of the osteoclast [180].

MMPs are key players in most ***vascular diseases*** [181-183]. MMPs have been implicated in intimal thickening, a repair response to wall damage of large arteries in human atherosclerotic pathologies, as well as in plaque rupture [180]. Studies using MMP gene knockout mice have indicated MMP-2 and MMP-9 play key roles in cardiac rupture after myocardial infarction [184-187]. Other studies have shown positive association between MT1-MMP (MMP-14) and ischemia-reperfusion [188], the importance of MMP-2 and MMP-9 for the development of abdominal aortic aneurysm [189] and the protective roles MMP-3 and MMP-9 have on atherosclerotic plaque stability [190].

There are several ***hereditary disorders*** caused by autosomal mutations in human MMP genes. All of them result from the loss of function of the corresponding protease activity, which leads to marked deficiencies in the turnover of specific extracellular matrix components. The first MMP mutation associated with a human inherited disease was identified in two consanguineous Saudi Arabian families with nodulosis–arthropathy–osteolysis syndrome (NAO), an autosomal-recessive form of multicentric osteolysis [191]. To date, several MMP2 mutations have been reported in three different skeletal disorders, collectively known as inherited osteolysis syndrome and characterized by progressive

resorption of bones. Like NAO, Winchester syndrome [192] and Torg syndrome [193] also showed similar observations. Mutations in the MMP13 gene are responsible for the Missouri type of human spondyloepimetaphyseal dysplasia (SEMD), an autosomal dominant disorder characterized by defective growth and remodelling of vertebrae and long bones [194]. Mutations in MMP-20 changed nucleic acid base sequence leading to total lack of proteolytic activity on amelogenin, the *in vivo* substrate for this MMP [195-197].

7. OUTLOOKS AND FUTURE PERSPECTIVES

Matrix metalloproteinases, a popular category among zinc-dependent proteins, have attracted the attention of many investigators. A recent special issue devoted to these proteins was published in Biochimica et Biophysica Acta [60,152,198-204]. Possessing multiple functions in various pathways, understanding their participation in carcinogenesis and other tumour-related processes is critical in tackling perilous diseases. Future investigations on specific pathways, their interactions with metal binding proteins and protein action inhibition diversify their study to include research areas in a variety of academic disciplines [205-209]. Moreover, there is great potential in the drug development to inhibit MMPs and on studying of the effects elicited by such drugs [61,132,138,205,207-211]. Some of the approaches how to inhibit MMPs activity involve the generation of protease-activated retroviral vectors which contain engineered MMP-cleavable linkers [212,213]. Other strategies employ macromolecular carriers that are linked to anticancer drugs released from the carrier by the proteolytic activities of MMPs present in the tumour environment [214,215]. Finally, a mutated cytotoxin has been engineered by replacing the furin protease cleavage site that is involved in lethal-factor activation with sequences that are selectively cleaved by MMPs [123,216]. Another interesting alternative to synthetic MMPs inhibitors is the use of gene therapy approaches aimed at delivering TIMPs at tumour sites [217,218]. Moreover, there is a new field in noncatalytic targeting of MMPs *via* substrate-targeted inhibitors [123]. The possibility to regulate transcription, activation and inhibition of MMPs may help in designing new strategies to block their unwanted activity in cancer and also in optimizing the current approaches. *In vivo* imaging is of great potential in this field [219].

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