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Overview of MMP Biology and Gene Associations in Human Diseases

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Abstract

Interactions of cell with the extracellular matrix (ECM) are crucial for normal development and functioning of the human organism. By regulating ECM integrity and composition matrix metalloproteinases (MMPs) play the main role in ECM molecules signaling and influence processes such as proliferation, migration, differentiation and apoptosis. ECM remodeling is a highly regulated process. When imbalanced it could contribute to pathophysiology of many diseases. The MMPs actions and activity are regulated through different mechanisms such as regulation of transcription, activation of latent MMPs, inhibition of MMP function by tissue inhibitors of metalloproteinases. MMPs are a family of calcium- and zinc-dependent endoproteinase, which share similar structural domains, but differs in substrate specificity, cell localizations and inducibility. Genetic variations in MMPs have been associated with a number of diseases, still not all findings are reproducible. Nine of 23 human genes encoding MMPs are located in a cluster on chromosome 11, which implicate their haplotype-driven effects. They could be important mediators of disease severity and could trigger acute events. In this chapter, we will review the basics of MMP biology and the most significant associations of MMPs variations with cardiovascular and neurological diseases in humans and MMPs therapeutic potential through synthetic inhibitors.

Keywords: MMP structure, MMP activation, MMP regulation, microRNA, MMP inhibitors, genetic variations, MMP haplotype

1. Introduction

Matrix metalloproteinases (MMPs) are a family of calcium (Ca^{2+})- and zinc (Zn^{2+})-dependent proteolytic enzymes involved in physiological as well as in pathological processes in the human organism. Initially they were thought to degrade only the extracellular matrix (ECM)

components, but nowadays it is well known that they have wider substrate specificity that includes non-matrix proteins of which the vast majority are bioactive molecules. Cell-cell and cell-ECM interactions are inevitable for normal development and functioning of the organism. Various proteinases are implicated in ECM remodeling, but MMPs are playing the key role. Remodeling of the tissue is crucial for physiological processes such as development, tissue homeostasis, morphogenesis and tissue repair. It could be a part of many pathological states such as arthritis, cardiovascular diseases, neurodegenerative diseases or part of the impaired development in congenital anomalies [1–4]. By regulating ECM structure and composition, MMPs are involved in growth factor availability and are playing the main role in the function of cell surface signaling systems and in that way influence proliferation, migration, differentiation and apoptosis [5]. The importance of their role in the physiological functioning of the human organism entails strict regulation of the expression and the activity of MMPs. They are regulated through different mechanisms such as regulation of transcription, activation of latent MMPs, inhibition of MMP function by tissue inhibitors of metalloproteinases (TIMPs) and so on. There is growing evidence that genetic variations in MMPs can influence gene expression or protein activity. Nine of 23 human genes encoding MMPs are located in a cluster on chromosome 11 (11q22.2–11q22.3) [6], which implicate their haplotype-driven effects. It has been shown that MMPs are important mediators of disease severity or could trigger acute events. Since they are involved in a wide spectrum of physiological and pathological processes, there is a need for determination of their precise role in different tissue and cell-specific context as well as in different stages of disease development or progression. Only then the therapeutic potential through the development of the specific inhibitors could be accurately implemented. In this chapter, we will discuss the main structural, substrate and functional properties of MMPs and give a brief review of the genetic associations with cardiovascular, neurodegenerative diseases and congenital anomalies in humans.

2. Matrix metalloproteinases

MMPs or matrixins belongs to the large family of proteinases called metzincin superfamily. Regarding its structural characteristics metzincins are subdivided into five subgroups. Other members of this superfamily are adamalysins, including a disintegrin and metalloproteinase (ADAMs) and ADAM with thrombospondin-like motif (ADAMTS), astacins, serralyins and pappalysins [7]. MMPs are expressed as zinc-dependent endopeptidases and have a wide spectrum of biological substrates that are overlapping. There are 24 genes encoding MMPs in humans, including duplicated MMP-23 gene. So, there are 23 different MMPs in humans [8]. They were named as MMP by the International Union of Biochemistry and Molecular Biology and each member of the enzyme family was assigned by a number (MMP-1, -2, -3 etc.) [9]. All MMPs contains the Zn^{2+} binding motif, HEXXHXXGXXH, in their catalytic domain and a conserved methionine forming a 'Met-turn'; are secreted in pro-pre enzyme form; need Ca^{2+} for its stability; function at neutral pH; are inhibited by TIMPs. The MMPs can be and are classified in different ways. The most common classification is based on their substrate specificity and basic domain structure. According to these criteria MMPs are subdivided into

collagenases, gelatinases, stromelysins, matrilysins and membrane type-MMPs (MT-MMPs). However, there are MMPs that do not belong to any of this group specifically so they are grouped as “others” (**Figure 1**).

2.1. Structure of MMPs

MMPs domain composition and arrangements are presented in **Figure 1**. MMPs protein contains at least three homologous domains: signal peptide responsible for protein secretion, pro-peptide domain containing a consensus cysteine-switch sequence and is needed for activation of the enzyme, catalytic domain which contains a zinc-binding consensus sequence and is responsible for the proteolytic activity.

Amino-terminal signal peptide is composed of 17–29 amino acids and is responsible for targeting the enzyme to the endoplasmic reticulum and Golgi complex and for the later excretion out of the cell. The most of the MMPs are extracellular proteins except MT-MMPs that are bound to the cell surface by a transmembrane domain or glycosylphosphatidylinositol anchor.

The next, pro-peptide domain consists of 77–87 amino acids and have conserved ‘cysteine switch’ motif. All MMPs except MMP-23 have this motif. The thiol group from the unpaired cysteine molecule could bind to the Zn^{2+} in the catalytic domain, making the pro-MMPs inactive [10]. After the proteolytic cleavage of the bite region of the protein (serine protease, MMPs and furin) the pro-peptide domain become destabilized and the interaction between Zn^{2+} and cysteine disrupts which turns zymogens into the active MMP form. The modification of the cysteine thiol group with physiological (oxidation) or non-physiological agents (heavy metal ions) could lead to irreversible activation of the MMP by autolysis [11].

Catalytic domain contains approximately 170 amino acids and has the highest sequence homology between the metalloproteinases. It comprises Zn^{2+} binding motif HEXXHXXGXXH and a conserved methionine, forming a ‘Met-turn’. This domain contains additional Zn^{2+} and Ca^{2+} ions that maintain the three-dimensional MMP structure needed for MMPs stability and enzymatic activity [12]. MMP-2 and -9 also contains three tandem fibronectin II type repeats that are responsible for elastin and gelatin binding. Typically in MMPs, carboxy-terminal end is linked to hemopexin domain with linker peptide called ‘hinge region’. Hemopexin domain consists of about 200 amino acids and modulates substrate recognition. The MMP-7, -26 and -23 do not possess hinge region and hemopexin domain. MMP-23 has a unique carboxy terminal domain rich in cysteine and an immunoglobulin-like domain after the C terminus of the catalytic domain.

2.1.1. Collagenases

There are three collagenases: interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8) and collagenase 3 (MMP-13). Their main characteristic is to cleave fibrillar collagens type-I, -II and -III at the specific site of the triple helices, specifically three-fourths from the N-terminus. In that way they are making characteristic $\frac{1}{4}$ and $\frac{3}{4}$ fragments. Beyond the fibrillar collagens they also degrade a number of ECM and non-ECM substrates (**Table 1**).

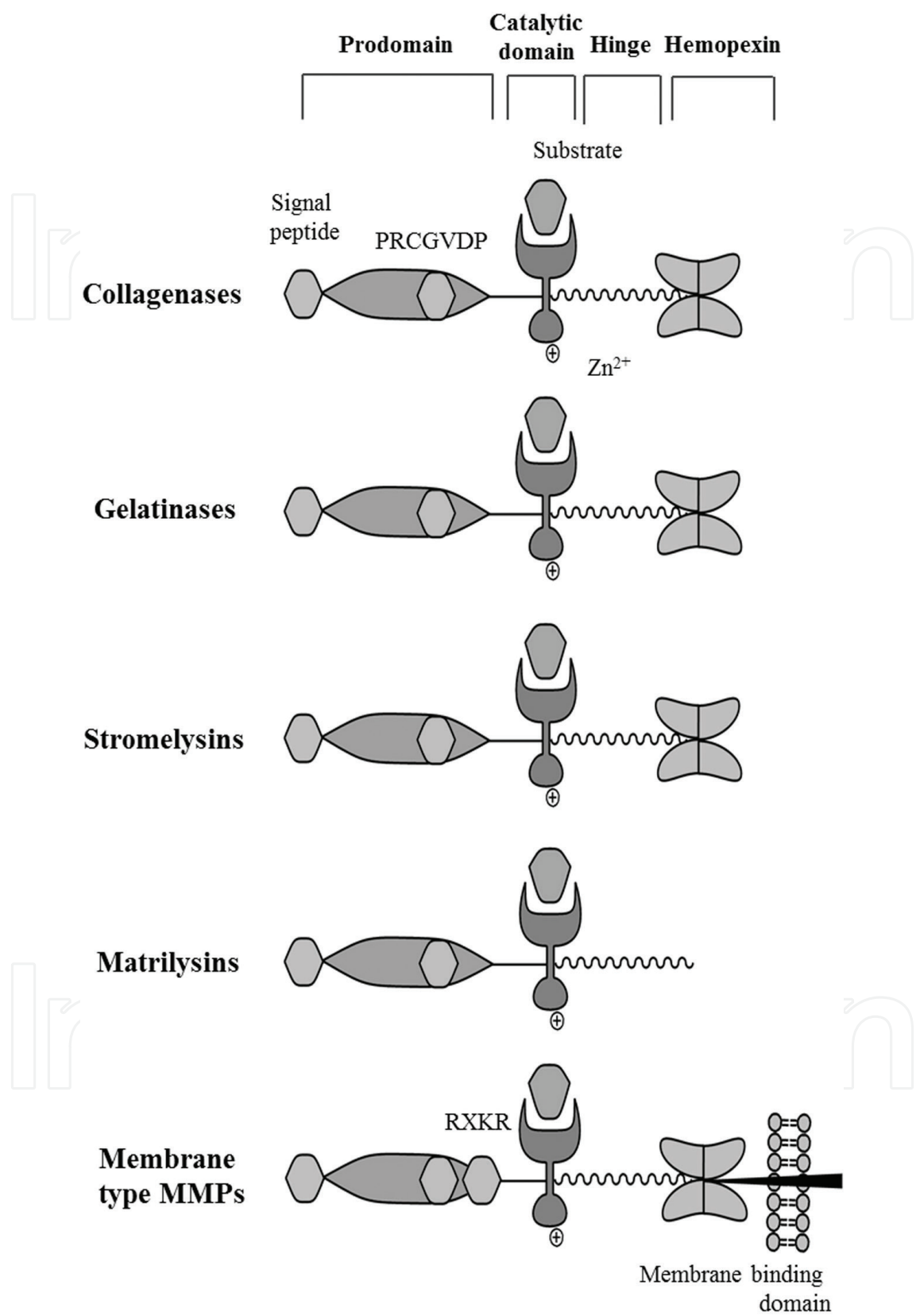


Figure 1. The domain composition and structural features of the MMPs subgroups.

MMP	Collagenous substrates	Noncollagenous ECM substrates	Non-ECM substrates
MMP-1	Collagen types I, II, III, VII, VIII, X and gelatin	Aggrecan, casein, serpins, versican, perlecan, proteoglycan link protein and tenascin-C	α 1-antitrypsin/ α 1-antichymotrypsin, IL-1 β , latent TNF- α , MCP-1,-2,-3,-4, IGFBP-2, -3, SDF-1, VEGF
MMP-2	Collagen types I, IV, V, VII, X, XI, XIV and gelatin	Aggrecan, elastin, fibronectin, laminin, perlecan, proteoglycan link protein and versican	IL-1 β , Pro-IL-1 β , SDF-1, MCP-3, IGFBP-3, latent TGF- β , latent TNF- α , FGFR1, pleiotrophin, CTGF
MMP-3	Collagen types II, IV, IX, X and gelatin	Aggrecan, casein, decorin, elastin, fibronectin, laminin, perlecan, proteoglycan, proteoglycan link protein and versican	α 1-antitrypsin/ α 1-antichymotrypsin, IL-1 β , Pro-IL-1 β , MCP-1,-2,-3,-4, SDF-1, IGFBP-1, -3, latent TGF- β , latent TNF- α , Pro-HB-EGF, osteopontin, VEGF
MMP-7	Collagen types I, II, III, V, VI and X	Aggrecan, casein, elastin, entactin, laminin and proteoglycan link protein	α 1-antitrypsin, Pro-HB-EGF, Latent TNF- α , syndecan-1, osteopontin, cellular membrane bound FasL, VEGF
MMP-8	Collagen types I, II, III, V, VII, VIII, X and gelatin	Aggrecan and laminin	α 1-antitrypsin, CXCL5, IL-8
MMP-9	Collagen types V, VI, VII, X and XIV	Fibronectin, laminin, proteoglycan link protein and versican	α 1-antitrypsin, IL-1 β , Pro-IL-1 β , CXCL5, IL-8, SDF-1, latent TGF- β , latent TNF- α , IL-2R α , IGFBP-1, VEGF
MMP-10	Collagen types II, IV, V and gelatin	Fibronectin and laminin	
MMP-11	None known	Laminin	α 1-antitrypsin, IGFBP-1
MMP-12	None known	Elastin	Latent TNF- α
MMP-13	Collagen types I, II, III, IV, V, IX, X, XI and gelatin	Aggrecan, fibronectin, laminin, perlecan and tenascin	α 1-antichymotrypsin, latent TGF- β , latent TNF- α , MCP-3, SDF-1
MMP-14	Collagen types I, II, III and gelatin	Aggrecan, dermatan sulfate proteoglycan, fibrin, fibronectin, laminin, perlecan, tenascin and vitronectin	MCP-3, SDF-1
MMP-15	Collagen types I, II, III and gelatin	Aggrecan, fibronectin, laminin, perlecan, tenascin and vitronectin	
MMP-16	Collagen types I, III and gelatin	Aggrecan, casein, fibronectin, laminin, perlecan and vitronectin	VEGF
MMP-17	Gelatin	Fibrin and fibronectin	TNF- α
MMP-19	Collagen types I, IV and gelatin	Aggrecan, casein, fibronectin, laminin and tenascin	VEGF
MMP-20		Aggrecan, amelogenin and cartilage oligomeric protein	
MMP-23	Gelatin	Chondroitin sulfate, dermatan sulfate and fibronectin	

MMP	Collagenous substrates	Noncollagenous ECM substrates	Non-ECM substrates
MMP-24	Gelatin	Fibrin and fibronectin	
MMP-25	Collagen type IV and gelatin	Casein, fibrinogen and fibronectin	
MMP-26	Collagen type IV and gelatin	Casein	α 1-antitrypsin
MMP-28			
MMP, matrix metalloproteinase; ECM, extracellular matrix; IL-1 interleukin 1; TNF, tumor necrosis factor; MCP, monocyte chemoattractant protein; IGFBP, Insulin-like growth factor-binding protein; SDF, stromal cell-derived factor; VEGF, vascular endothelial growth factor; TGF, tumor growth factor; FGFR, fibroblast growth factor receptor; CTGF, connective tissue growth factor; EGF, epidermal growth factor; CXCL5, C-X-C motif chemokine ligand 5; IL-8, interleukin 8; IL-2R, interleukin 2 receptor.			

Table 1. MMP substrates.

2.1.2. Gelatinases

So-called gelatinase A (MMP-2) and gelatinase B (MMP-9) belongs to this group. Both of them have three repeats of a fibronectin type-II motif in the catalytic domain. They degrade denaturated collagens as well as native collagens type-IV, -V and -XI. They also denaturate gelatins, laminin and aggrecan and number of other ECM molecules. MMP-2, but not MMP-9 could cleave collagens type-I, -II and -III [13, 14]. Nevertheless, its collagenolytic activity is weaker than that of collagenases. Still, because of ability of pro-MMP-2 to recruit to the cell surface and to be activated by the MT1-MMP, it can accumulate extracellularly and have higher collagenolytic potential locally.

2.1.3. Stromelysins

Stromelysin 1 (MMP-3), stromelysin 2 (MMP-10) and stromelysin 3 (MMP-11) belongs to this group. Their name reflects the capability of degrading the wide spectrum of ECM proteins. MMP-3 and -10 degrade proteoglycans, laminin, fibronectin, vitronectin and some types of collagens but not interstitial collagens, whereas MMP-11 has a very weak affinity for ECM molecules (**Table 1**). It is located on chromosome 22, while MMP-3 and -10 are in the cluster with seven more genes on the chromosome 11 [6]. MMP-3 has the highest proteolytic efficiency in the group and is capable of activating many other pro-MMPs. It plays the main role in full activation of pro-MMP-1 [15].

2.1.4. Matrylisins

The main characteristic of matrylisins is that they lack hemopexin domain. MMP-7 and -26 belongs to this group. Both of them degrade ECM components, while MMP-7 degrade some of the cell surface molecules such as E cadherin, pro-tumor necrosis factor alpha, Fas ligand, syndecan 1 and pro-alpha defensin.

2.1.5. Membrane-type MMPs

There are six MT-MMPs that are divided into two groups: MMP-14, -15, -16 and -24 belongs to the type-I transmembrane proteins, while MMP-17 and MMP-25 are glycosylphosphatidylinositol-anchored proteins. All of them have a furin-like proprotein convertase recognition sequence and are activated intracellularly. All, but MT4-MMP, can activate pro-MMP2 [16]. They degrade ECM molecules, whereas MT1-MMP14 can cleave collagen type-I, -II and -III [17] and can activate proMMP-13 on the cell surface [18].

2.1.6. Other MMPs

Seven MMPs belong to this group. Three of them (MMP-12, -20 and -27) have a similar domain arrangement and are part of the cluster of nine genes on the chromosome 11 [6]. MMP-12 is called metalloelastase. It is mainly produced in macrophages [19] but has been found in hypertrophic chondrocytes [20] and osteoclasts [21], as well. Besides elastin it degrades other ECM proteins and is essential for macrophage migration [22]. MMP-19 is expressed in human tissues [23] and degrades basement membrane as well as other ECM molecules [24]. It is involved in tissue remodeling and migration of epithelial cells by degrading laminin 5 gamma 2 chain [25]. MMP-20, enamelysin is expressed in newly formed tooth and degrades amelogenin [26]. A mutation in this gene causes genetic disorder called amelogenin imperfecta [27]. MMP-21 is expressed in human tissues. It was found in basal and squamous cell carcinomas [28]. Annotation of its action toward ECM molecules is still not known. MMP-23 is different from other MMPs because it lacks the cysteine switch motif in the prodomain and the hemopexin domain. It possesses a cysteine-rich domain which is followed by an immunoglobulin-like domain. It is mainly expressed in reproductive tissues [29]. MMP-27 is expressed in B lymphocytes [30], but the function of this enzyme in mammals is not known, yet. MMP-28, or epilysin, is expressed in many human tissues [31]. It is involved in wound repair [32] and its expression was elevated in patients with osteoarthritis [33] and rheumatoid arthritis [34]. MMP-28 overexpression up-regulated MT-MMP1 and MMP-9 in A549 lung adenocarcinoma cells [35].

2.2. Regulation of MMPs

Since they have the potential to degrade ECM and wide spectrum of non-ECM substrates and to activate other MMPs or release growth factors, matrix metalloproteinases have been stringently regulated at different levels. They are regulated at a transcriptional and translational level, by activation of the zymogen forms, by the extracellular or endogenous inhibitors, by subcellular or extracellular localization and internalization by endocytosis.

Cellular expression of MMPs is based on successive activation of multiple signaling pathways leading to synergistic effects of more transcriptional factors on the MMP promoter. Some of the most important are NF- κ B, activating protein (AP)-1 and Sp-1. Recent studies have shown that endogenous miRNAs are able to recognize complementary genomic sites within human gene promoters, and in that way regulate gene transcription [36, 37]. There are multiple factors that can trigger different signaling pathways modulating MMP gene expression. They could be

cytokines, chemokines or growth factors such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). The expression could also be modulated by reactive oxygen species, mechanical injury, shear or tensile stress, contact with cell bound ligands, etc. The expression of MMPs could be down-regulated by the anti inflammatory molecules such as nitric oxide (NO), transforming growth factor beta (TGF- β), Interleukin-4 (IL-4), Interleukin-10 (IL-10), interferon- γ and peroxisome proliferator-activated receptor (PPAR) (reviewed in Ref. [38]). The cell-cell and cell-matrix interactions established through adhesion molecules or integrins also have an impact on the MMPs expression [39]. Transcription could be also modified by genetic variation within the MMPs gene promoters. In the past two decades, the SNPs identified in the promoter of the MMP-1, -2, -3, -7, -9, -12, and -13 genes has been denoted as functional and associated with cardiovascular disease phenotypes (reviewed in Ref. [40]).

2.2.1. Activation of MMPs

Almost all MMPs are secreted as an inactive form. One of the mechanism of activation is, earlier mentioned, a 'cysteine switch' mechanism where the thiol group of the unpaired cysteine is replaced by the water. This mechanism is the first step of the stepwise activation process. It enables further pro-peptide hydroxylation of partially activated MMP or other proteases until the final step of its removal and activation [41]. Most of the MMPs are activated after the secretion, extracellularly. But the MT-MMPs and MMP-11, -23 and -28 are activated intracellularly. They have a furin recognition sequence that allows them to be activated in the Golgi apparatus by pro-protein convertase within the secretory pathway [42–45]. One of the most significant activator of MMPs in vivo is considered to be a serine protease plasmin [46]. It is shown that it activates MMP-1, -3, -7, -8, -9, -10 and -13 [46]. Other serine proteases such as mast cell proteases, chymases and tryptases also have the potential to activate pro-MMPs. Human tryptase could activate pro-MMP-3 and pro-MMP-1 but the activation of the latter is dependent on the activation of the former [47, 48].

Moreover, once activated MMPs are able to activate other pro-MMPs. For example, MMP-3 could activate pro-MMP-1, -7, -8, -9 and -13. Then, activated MMP-7 could activate zymogens pro-MMP-1, -9 and -13. Pro-MMP-2 and -3 could be activated by MMP-12 as well, while MMP-2 can activate pro-MMP-9. So, a very complex network of positive feedback loops exist and it could trigger proteolytic cleavage of the complete ECM (**Figure 2**). That is why the strict and multilevel regulation of MMPs must exist for the physiological functioning of the human organism. Inactive form of MMP-2 has somewhat specific activating mechanism which involves MT1-MMP and TIMP-2 [49]. Long story short, low or moderate levels of TIMP-2 activate pro-MMP-2 while higher levels saturate MT1-MMP and in that way inhibit activation of pro-MMP-2 [50]. Also, it was shown that other MT-MMPs (MT2-MMP, MT3-MMP, MT5-MMP and MT6-MMP) can activate pro-MMP-2 as well [51, 52]. Agents that do not have proteolytic feature, but could activate pro-MMPs, are a thiol group modifying agents oxidized glutathione and reactive oxygen species [53].

2.2.2. microRNA and MMP

In the last decade the novelty in the research has emphasized the role of microRNAs (miRNAs) on posttranscriptional regulation of the expression. The number of studies that have focused

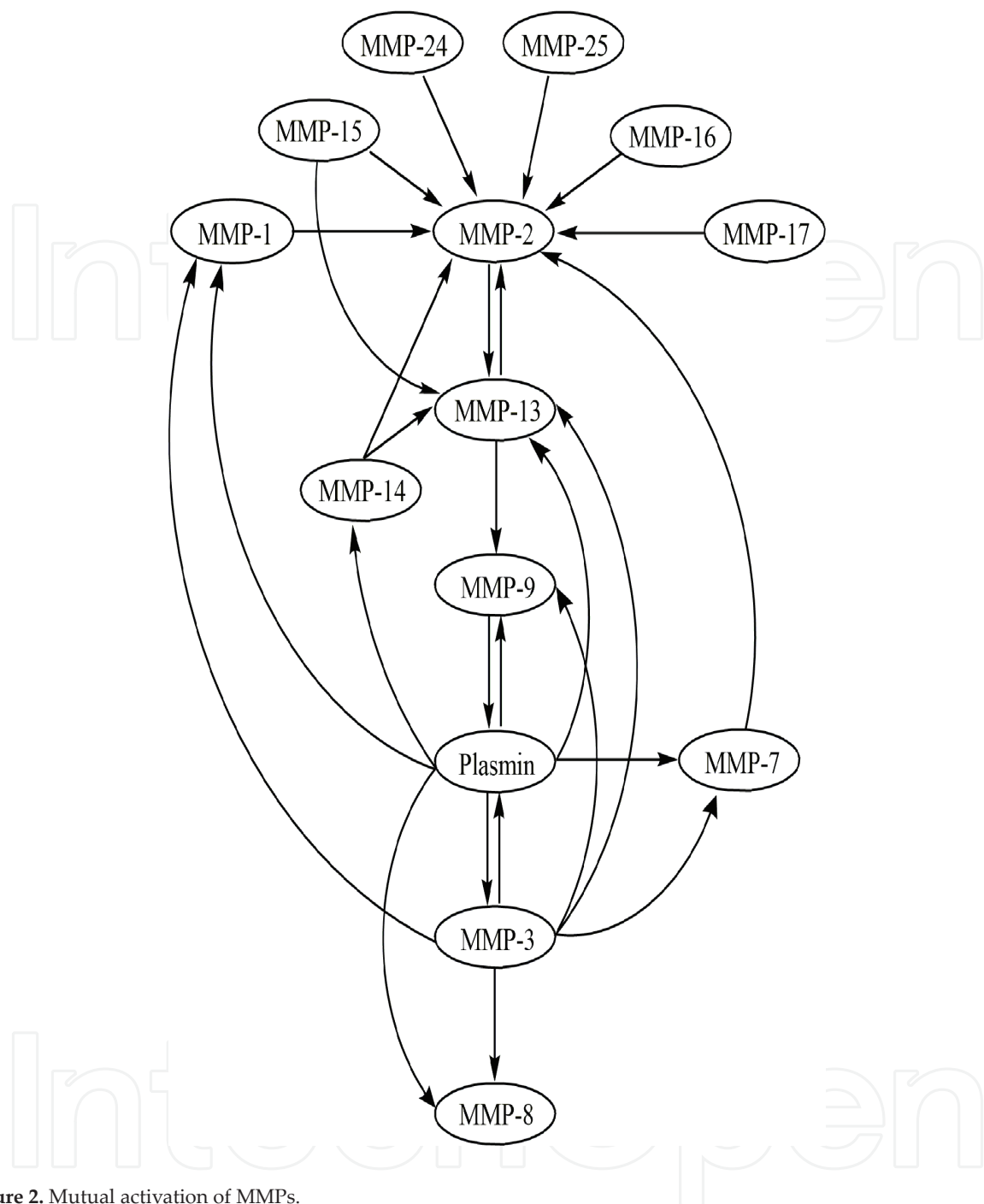


Figure 2. Mutual activation of MMPs.

on this step of MMPs regulation is growing. In this chapter, we will briefly discuss the most recent studies.

Since MMPs have an important role in the progression, metastatic potential and aggressiveness of the cancer, numerous studies have analyzed the MMP regulation by miRNAs in this disease. A recent study has investigated miRNA-489 effect on migration and invasion of hepatocellular carcinoma (HCC) cells. They have found that miRNA-489 overexpression reduced the expressions of MMP7 mRNA and protein. Additionally, miRNA-489 overexpression decreased the luciferase activity of wild type MMP7 3'-UTR but not mutated MMP7 3'-UTR in

HEK293T and HCCLM3 cells. The following rescue experiments suggest that miR-489 inhibits the migration and invasion of HCC cells, possibly by targeting MMP7 [54]. Another study has analyzed the functional background of miRNA-204-5p association with better prognosis in patients with melanoma. miRNA-204-5p is down-regulated in melanoma tissues and cells, and confers a protective effect that improves the prognosis of those patients. The binding sites of miRNA-204-5p matched the 3'-UTR of MMP-9. Up-regulation of miRNA-204-5p led to a decrease in the expression of endogenous MMP-9 and their correlation was negative. The authors have demonstrated that MMP-9 is the functional target of miRNA-204-5p in melanoma and concluded that miRNA-204-5p inhibits melanoma growth *in vivo* by regulating the expression of MMP-9 [55]. Using computational algorithm programs and chromatin immunoprecipitation datasets, Zheng et al. identified neighboring binding sites of myeloid zinc finger 1 (MZF1) and miRNA-337-3p within the MMP-14 promoter. They have found higher MZF1 and MMP-14 levels in gastric cancer cell lines compared to normal gastric epithelial cells. Their research results indicated that miRNA-337-3p significantly decreased the growth, invasion and angiogenesis of gastric cancer cells through repressing MZF1-facilitated MMP-14 expression *in vitro*, as well as *in vivo* on animal model [56]. Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive cancers with very poor 5 year survival rate. The research has revealed that miRNA-375 is downregulated in several types of ESCC and that ectopic expression of miRNA-375 suppressed cancer cell aggressiveness in several types of cancer cells. The authors have shown significantly upregulated expression of MMP-13 in 25 ESCC specimens and ESCC cell lines compared with that in 13 normal specimens. They revealed that MMP-13 is directly regulated by antitumor miRNA-375 and acts to regulate several cell cycle promoting genes having the role in the ESCC aggressiveness [57].

With regard to the cardiovascular phenotypes a recent study has investigated miRNA-516a-5p in vascular smooth muscle cells (VSMCs) explant cultured from human abdominal aortic tissues. They have generated stable overexpression and knockdown of miRNA-516a-5p in those VSMCs. The relative MMP-2 protein expression in VSMCs with miRNA-516a-5p-overexpression was significantly higher than that in control VSMCs while the TIMP-1 levels were significantly lower. When miRNA-516a-5p was knockdowned, the opposite results were seen. Additionally, the changes in protein expression of collagen type I alpha 1 chain (COL1A1), TIMP-2 and MMP-9 have not been observed in VSMC. The authors suggested that miRNA-516a-5p may regulate MMP-2 and TIMP-1 expressions in human VSMCs, possibly promoting the proteolytic degradation of elastin for abdominal aortic aneurysm formation. [58]. Another study showed that shear-sensitive miRNA-181b binds to the TIMP-3 3'-UTR and downregulates it when overexpressed in human aortic valve endothelial cells. Additionally, it increases gelatinase/MMP activity. Through specific rescue of TIMP-3, they have clearly shown that the decreased matrix degradation results from anti-miRNA-181b treatment [59]. The miRNA-155 has been considered to be a pro-inflammatory agent, because its major target is the suppressor of cytokine signaling-1(SOCS1). It has been linked to pro-atherogenic processes in humans, as well. A recent study has shown that the SNP in the angiotensin II receptor type 1 AT1R 3'-UTR has significantly changed the miRNA-155 expression in human carotid plaques whereas rare allele homozygotes has a significantly higher expression compared to the subjects carrying wild type allele containing genotypes [60]. Also, miRNA-155 has been reported to participate in cell migration and transformation, but its function in skin

wound healing was unknown. Jang et al. have been investigating the function of miRNA-155 on keratinocytes in wound healing. The results of the study showed that the protein level of MMP-2 significantly increased after miRNA-155 overexpression, while the level of TIMP-1 obviously decreased, whereas the levels of MMP-9 and TIMP-2 did not change. The authors concluded that miRNA-155 induced acceleration of keratinocyte migration is mediated at least partly through MMP-2/TIMP-1 pathway in the process of wound healing [61].

2.2.3. Tissue inhibitors of matrix metalloproteinases (TIMPs)

There are a lot of physiological inhibitors of MMPs in the organism. However, in tissues they are primarily regulated by TIMPs that bind MMPs in a 1:1 stoichiometry. Four mammalian TIMPs have been revealed and characterized. They are named TIMP-1, -2, -3 and -4 [62–65]. TIMP-1 and -3 are glycoproteins, while TIMP-2 and -4 do not contain carbohydrates. TIMPs inhibit all MMPs but TIMP-1 is a poor inhibitor of three membrane-type MMPs (MT1-MMP, MT3-MMP and MT5-MMP) and MMP-19 [66].

TIMPs have an N-terminal domain of approximately 125 and C-terminal domain of 65 amino acids. Both of these domains contain three conserved disulfide bonds [67, 68]. It is thought that the N-terminal domain is responsible for their binding to MMPs [67]. But there are some exceptions, C-terminal domain of TIMP-1 is shown to bind pro-MMP-9 [69]. Certain TIMPs inhibit different MMPs better than other TIMPs. Additionally, TIMPs do not inhibit only matrixins, several studies have shown that they can inhibit adamalysins as well [70, 71]. It has been shown that expression of TIMP-1 and -3 could be regulated by cytokines and growth factors such as: IL-1, fibroblast growth factor 2, platelet-derived growth factor BB, tumor growth factor-beta and tumor necrosis factor-alpha [72, 73].

TIMPs exert other functions except inhibition of MMPs. For example TIMP-1 and -2 have mitogenic activity for different type of cells [74, 75] and both have antiapoptotic activity [76, 77], while TIMP-3 has proapoptotic activity in tumor cells [78]. Solely, TIMP-2 is shown to have antiangiogenic activity [79].

Several other molecules have been reported to inhibit different MMP-s. The serpine family member, alpha2-macroglobulin, can irreversibly inhibit active MMPs in the circulation [80]. Secreted form of beta-amyloid precursor protein can inhibit MMP-2 [81]. Reversion-inducing cysteine-rich protein with Kazal motif (RECK), a GPI-anchored glycoprotein inhibits MMP-2, MMP-9 and MT1-MMP [82].

3. Synthetic MMP inhibitors

The first efforts in developing synthetic MMPs inhibitors were based on a peptide sequence recognition of the desired MMP and introduction of the group that chelated its catalytic Zn^{2+} ion. This first generation of the MMP inhibitors was called hydroxamate-based MMP inhibitors. Despite the promising results in animal models regarding their antitumor effects [83–85], following clinical studies were unsuccessful [86, 87]. The major concern was unselectivity in MMPs inhibition and serious side effects. It became clear that the knowledge of the MMPs

activity in different stages of the disease and spatio-temporal expression needs to be followed in future development of the synthetic inhibitors of MMPs. Nevertheless, although hydroxamate-based MMP inhibitors have not shown the desirable effects the efforts toward their improvement had continued.

The second type of the MMP inhibitors that were developed are non-hydroxamate MMP inhibitors. The hydroxamate was replaced with other Zn^{2+} binding groups that were more metabolically stable and had higher specificity for MMPs alone. But, again the results were not satisfactory, they all had side effects in different stages of trials. From the other hand, tetracycline antibiotics have an innate ability to inhibit MMPs. The only inhibitor approved by the US Food and Drug Administration for any human disease is collagenase inhibitor doxycycline hyclate, which is a tetracycline analogue [88].

The new approach in synthetic inhibitor development has focused on targeting less conserved sites in MMPs compared to the catalytic one. This should enable more specific targeting and reduce off-target effects that the clinical trials have shown so far. As a result, inhibitors with a much stronger inhibition capacity of target MMPs have been developed [89].

The next alternative strategy has focused on the use of specific antibody fragments. Up to date, functional blocking antibodies that specifically target MT-MMPs have been developed. What is the most important, it seems that antibodies could target specific function of MMP rather than its broad proteolytic activity [90].

Part of the research has investigated the use of endogenous MMP inhibitors as potential therapeutics [91]. Nowadays, it is known that TIMPs have many of non-MMPs functions in the organism and it is very difficult to make them selective and specific to the target MMP inhibition. It could be hard to keep the balance between MMPs and TIMPs which could have serious impact on the overall MMPs activities.

So, there are few important issues to be solved before the efficacious metalloproteinase inhibitors could be made. First of all, there is a need for knowledge of precise MMP functioning and activity in cells, tissues and different stages of the disease. Also, their function in maintaining the tissue and cell homeostasis should be analyzed in details. An additional concern is their overlapping expression patterns and successive activation as well as context-dependent functioning. It seems that they could be good therapeutics for many of diseases, but the designing criteria for synthetic inhibitors are very demanding. We should combine refined and validated experimental and theoretical knowledge in order to raise the selectivity and specificity of the inhibitors toward target MMPs. Another important issue is administration of synthetic MMP inhibitors in order to avoid unnecessary toxicity of the inhibitors in the circulation. It would be of interest to determine the location (cells, tissue and organ) and temporal framework of the adverse MMP activity and develop site-specific delivery systems (detailed review in [92]).

4. MMP genes in human disease

The functions of MMPs are implicated in a variety of diseases, including those of respiratory system, central nervous system, liver, kidneys, muscles, and joints as well as the cardiovascular

system [93]. Accordingly, genetic variations in genes that codes for MMPs were investigated in many of them, but only the limited number of genetic variations was thoroughly investigated. Herein, we will review mainly the findings of the genetic influence of MMPs in coronary artery disease (CAD), atherosclerosis and neurodegenerative disease.

4.1. Genetic association of variants in MMPs with vascular disease

Among all the MMPs genes only few were repeatedly investigated in a gene candidate association studies. In the year of 1996 and 1999, the two papers that investigated the functional role of promoter variants in MMP-3 [94] and MMP-9 [95] gene were published, respectively. Since then, the most investigated genetic variants in any of the MMP gene have been the MMP-3 5A/6A (rs3025058) and MMP-9-1562 C/T (rs3918242) variant, based on their role to influence gene transcription.

4.1.1. MMP-3

The common 5A/6A (rs3025058) variant in the promoter of the MMP-3 gene has been shown to affect the level of gene expression in both in vitro [94] and in vivo [96] conditions. The 5A allele was associated with higher and the 6A allele with lower transcriptional activity [94, 96]. In general, the 6A allele was mostly associated with stenosis and coronary disease progression. It was associated with greater progression of coronary artery disease (CAD) in men [97, 98] and women [99] and with the greater number of coronary arteries with significant stenosis [100, 101], but not with susceptibility to coronary heart disease [100, 102]. The 6A/6A genotype was associated with greater progression of coronary atherosclerosis [97] and the number of coronary arteries with stenosis >50% [100]. Also, it was associated with carotid stenosis >70% [103] and greater intima-media thickness (IMT) [103–105]. One or more 6A alleles had significantly higher risk for development of carotid atherosclerosis compared to 5A/5A homozygotes [106]. Besides its association with definite cardiovascular phenotypes the 5A/6A polymorphism has been linked to their risk factors such as elevated blood pressure [107], stiffer large arteries [108] and, in combination with angiotensin I-converting enzyme DD genotype, with hypertension in men [109]. On the contrary, the 5A allele as the high activity allele was predominantly associated with acute clinical events such as plaque rupture and consequently myocardial infarction (MI) [100, 110, 111]. The combination of MMP-9 and MMP-3 genotypes was found to be potentially significant for presentation of atherosclerosis. Patients with “high activity genotypes” of both SNPs had larger area of complicated atherosclerotic lesions compared to other genotypes [112]. One of the first meta-analysis that aimed to realize the effect of MMP variants on atherosclerosis found significant effect of the 5A allele on acute MI [113]. The newer meta-analysis of 15 studies (10,061 cases, 8048 controls) in coronary disease displayed no significant overall risk of coronary disease for the carriers of the 5A allele and 6A/6A genotype of rs3025058 [114]. Similarly, the haplotype-tagging approach for several SNPs (rs522616, rs650108, rs569444 and rs635746) and rs3025058 did not show significant difference in genotype distribution in MI patients compared to controls [114]. The meta-analysis of 8 SNPs selected from the studies in which 58 SNPs within MMPs and TIMPs were investigated in abdominal aortic aneurism (AAA) pinpoint the significant association of only MMP-3 rs3025058 with AAA presence [115]. Another one, published the same year, which was investigated several genes in AAA presented the similar results for MMP-3 rs3025058 [116].

4.1.2. MMP-9

The first main role of MMP-9, which gave the rationale for the investigation in atherogenesis is the degradation of basement membrane, which surrounds each VSMC and is primarily composed of type-IV collagen, laminin and fibronectin [117]. The MMP-9 gene possesses several single nucleotide polymorphisms, the most widely studied of which is the -1562 C/T gene polymorphism (rs3918242) in the promoter of the MMP-9 gene [118]. It was suggested that this polymorphism has a functional capacity to regulate MMP-9 expression, since luciferase reporter assays showed higher promoter activity of the T allele in vitro [95]. Although in this study authors have not found the significant effect of the rare allele on the susceptibility to MI they suggested its role in coronary artery severity [95]. Recently, another study challenged the functional role of this SNP [119]. In cells with different -1562C/T genotypes there was neither difference in MMP-9 expression level nor in MMP-9 promoter activity [119]. Nevertheless, this variant was extensively and repeatedly studied in CAD. Both positive [120, 121] and negative [122, 123] association of -1562T allele with the disease were presented. The meta-analysis of previous studies showed no association of MMP-9-1562 C/T polymorphism with coronary heart disease [113]. The other one, which included 11 polymorphisms from MMPs showed that Glu45Lys in MMP3 gene and -1562C/T in MMP9 gene had an overall significant association with CAD [124]. In one of the biggest gene association studies of MMP genes in MI and CAD the composite genotypes of MMP-9 variations CT/RQ had greater risk for MI after full adjustment for covariates [125].

Arterial stiffness and MMP-9 levels were explored in healthy subjects in association with common risk factors and MMP-9 variations. Mean aortic pulse wave velocity (PWV) values were significantly higher in the carriers of the 1562 T and 279 Q alleles compared with common homozygotes, as well as serum MMP-9 levels [126]. The rs3918242 and exon 6 R279Q A/G (rs17576) polymorphisms were not associated with the presence of CAD or MI, but R279Q was associated with hypertension [127]. Among several MMP-2, MMP-7 and MMP-9 variations the MMP-9 R668Q genetic variant was associated with left ventricular dysfunction [128].

In order to overcome a simplistic mechanistic interpretation of the -1562T allele roles in regulation of MMP-9 gene expression, the five promoter and nine exon SNPs, which change amino acid in the encoded protein, were analyzed. The functional consequences of these SNPs were investigated [129]. Three exon SNPs altered the specific enzymatic activity while altered promoter activity was shown for four promoter SNPs among which was the -1562C/T [129]. Still, for promoter SNPs the explanation of how they exert their effect is not known, yet.

Recently, several variants in the 3' UTR of the MMP-9 gene were analyzed in association with atherosclerotic cerebral infarction (ACI) in Chinese population. They found a significant association of the rare C allele and CC genotype of rs1056628 with ACI. Also the haplotype rs20544C-rs1056628C-rs9509T showed significantly increased risk for ACI. Further findings indicated that miR-491 directly targets MMP-9 and that the A-C transition in rs1056628, which is located in the miR-491 seed sequence, could influence the miR-491 binding. Moreover, the miR-491 decreased MMP-9 protein expression in cotransfected HUVEC, but did not show the influence on the mRNA expression [130].

4.1.3. MMP-2

The other gelatinase, MMP-2, contrary to MMP-9 is constitutively expressed in many of the connective tissue cells that have a role in the vascular system. It is also functionally implicated in the processes of cell invasion, migration of smooth muscle cells (SMC) and destabilization of atherosclerotic plaque. The 15 novel sequence variants in the MMP-2 gene were firstly described in the year of 2001 [131] among which six were in the promoter of the gene and six in the coding region. Among three promoter variants that map onto cis-acting elements the one that disrupts SP-1 type of promoter site (-1306 C/T, rs243865) showed the lower promoter activity in rare allele [131]. The -790 T allele was associated with triple vessel disease [132].

4.1.4. MMP-1

Similarly to other MMP genes the study of MMP-1 genetic variants started with a definition of potentially functional SNPs. First, the 2G-allele of -1607 1G/2G variation in the MMP-1 promoter has been noted to increase transcriptional activity by creating an E26 transcription factor binding site [133]. Next, in vitro analysis in human macrophages showed that the A-519-C-340 and G-519-T-340 haplotypes compared with the A-519-T-340 haplotype, had lower promoter activity, whereas the G-519-C-340 haplotype had greater promoter strength [134]. At the same time that study was one of the first which investigated the genetic variations in MMP-1, solely and in haplotype, in association with cardiovascular disease. It revealed both, risk (G-519-C-340) and protective (A-519-C-340 and G-519-T-340) MMP-1 haplotypes in MI [134]. Recently, the -340 T/C, -519 A/G and -1607 1G/2G variations, separately and in haplotype were associated with the occurrence of carotid plaques (CP). Compared to the referent haplotype 2G-1607-T-340-A-519, the haplotypes 1G-1607-T-34-A-519, 1G-1607-T-340-G-519 and 2G-1607-C-340-A-519 had statistically significant protective effect on CP presence. The MMP-1-1607 2G allele had significantly increased allele dose-dependent risk for CP presence [2]. Previously, the 2G allele also appeared to favor carotid artery stenosis [103].

SNPs in genes encoding MMP-1, -2, -3 and -9 and TIMP-1, -2 and -3 were associated with MI and CAD and combinations of MMP-1 1G/2G and MMP-3 5A/6A genotypes were significantly associated with CAD, but not MI [125]. Recently, the MMP-1/MMP-3 less active haplotype 1G-1607-6A was described as a significant risk factor for obstructive uropathy, which is characterized by collagen accumulation [4]. Others did not find the association of the selected SNPs in MMP1, MMP2, MMP3, MMP9 or MMP10 with either acute MI compared with angina, or with coronary disease compared with controls [135]. Recently, the genome-wide association analysis was performed using 500 K SNPs to identify genes influencing variation in serum levels of MMP-1 [136]. The cluster of 179 SNPs in the cluster on chromosome 11 were associated with MMP-1 serum levels, with the peak of association on rs495366, which is located between the MMP-1 and MMP-3 genes [136].

4.1.5. MMP-8

The investigation of the genetic variations in MMP-8 started with the identification of several polymorphisms in the MMP8 gene, at -799 C/T (rs11225395), -381 A/G (rs1320632) and +17 C/G. Their

functional capacity was suggested after the study showed significantly higher promoter activity of the construct that contained the minor alleles compared to the construct with major alleles [137]. Many of the forthcoming studies investigated the role of MMP-8 in atherosclerosis. After analysis of selected 16 SNPs in the MMP-8 gene the rs1940475, in the coding region of the MMP-8 gene, was associated with the extent of coronary atherosclerosis [138]. Also, the minor T allele of rs1940475 was associated with a protective effect against carotid atherosclerosis progression in a 10-year follow-up [138]. In another study the significantly higher frequency of the -381 G allele was found in female patients with carotid atherosclerosis compared to controls [139]. The significantly higher expression of MMP-8 mRNA was found in carotid plaques of the G-381 T-799 haplotype compared to the reference A-381C-799 haplotype [139].

One of the not so commonly investigated MMP gene, the MMP 14, was significantly associated with ultrasonographically defined plaque phenotype suggesting protective effect of rs2236307 major T allele for vulnerable plaque, in Chinese Han population [140].

It seems that a precise definition of particular phenotypes of interest is necessary to get the reproducible findings about genetic influence of a variant in complex disease. The CAD endpoints, the study design as well as a selection of the controls in association studies might influence the findings. The good example of previous is the particular meta-analysis performed for MMP family gene variants [124].

4.1.6. Serum levels of MMPs in atherosclerosis

Over the past 15 years the protein, plasma and serum levels of MMPs were investigated in association with cardiovascular and atherosclerotic plaque phenotypes (mainly MMP-1, MMP-2, MMP-9, MMP-13 and recently MMP-8). MMP-1 but not MMP-9 serum levels were associated with the total plaque burden [141]. Both, MMP-9 and MMP-8 serum and plasma levels were associated with cardiovascular outcomes in CAD patients [142–144]. Although MMP-8 cleaves collagen type-I three times more potently than two other interstitial collagenases, MMP-1 and MMP-13 [145], its role in CAD was lesser investigated in comparison to other MMPs, until recently. MMP-8 plasma levels were associated with unstable angina [146] and with the occurrence of carotid plaque [147]. Among the serum levels of MMP-1, -2, -3, -8, -9, -13, and TIMP-1, -2, -3, -4 analyzed prospectively after the MI only the baseline levels of MMP-8 were significantly associated with changes in left ventricular end-diastolic volume after the adjustment for covariates [148].

4.2. Genetics of MMPs in brain disease

In the central nervous system, MMPs have an important role and may influence proteolysis of basement membranes, extracellular matrix molecules, precursors of the cytokines, cell surface molecules and myelin components. In healthy central nervous system (CNS), they also have a role in synaptic plasticity, learning and memory. It is known that MMPs play a significant role in Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS). Their role in neuroinflammation and neurodegeneration was reviewed in detail in Brkic et al. [149]. Thus, as reasonable candidate genes the variations in MMPs were investigated in several neurological diseases.

The results in this field were also partly inconsistent. While one of the first studies that investigated MMP-9 polymorphism in multiple sclerosis had not found that it is a susceptibility marker for MS [150] two other studies showed the significant decrease in the MMP-9 rs3918242 rare T allele carrier ship in female patients with MS [3, 151]. The same allele was found to be more often in patients with PD and amyotrophic lateral sclerosis [152]. The haplotype formed by the -1562 T allele and the L allele ((CA)(≤ 20)) of -90 (CA)_n repeat polymorphism in MMP-9 was over-represented in patients with MS in comparison to controls [153]. Others suggested that haplotypes of these two polymorphisms might modulate disease severity, expressed through expanded disability status scale (EDSS) [154]. The MMP-3 6A/6A genotype was also associated with disease severity, showing significantly higher mean multiple sclerosis severity score (MSSS) values in comparison to other genotypes [155]. In another study, the MMP-2 -1575 G/A variation was shown to influence the age of disease onset in MS patients with optic neuritis as a first symptom [156]. The four polymorphism haplotypes in the gene encoding MMP-3 was associated with changes in amyloid beta levels in non-demented subjects [157] but no evidence was found that the MMP-3 gene is causally involved in dementia or AD [158].

4.3. Genetic epilogue

In the last few years the explosion of the data regarding the genetic variations and their association with disease happened as a consequence of the use of high throughput technologies, genome wide association studies, bioinformatical databases, etc. Thus, the results of the candidate gene association studies should be combined with the findings of the different genetic analysis approaches. Some of the genetic variations mentioned above cannot even be found on the arrays, as they are the insertion/deletion variation type, for example, MMP-3 5A/6A or MMP-1 1G/2G. The majority of studies that consider the role of MMPs in different pathologies did not include the genetic component, thus a lot is to be done yet in specifying the genetic architecture of MMPs in health and disease.

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