Matrix Metalloproteinases Inhibition

Subjects: Biology

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Matrix metalloproteinases are enzymes that degrade the extracellular matrix. They have different substrates but similar structural organization. Matrix metalloproteinases are involved in many physiological and pathological processes and there is a need to develop inhibitors for these enzymes in order to modulate the degradation of the extracellular matrix (ECM). There exist two classes of inhibitors: endogenous and synthetics. The development of synthetic inhibitors remains a great challenge due to the low selectivity and specificity, side effects in clinical trials, and instability.

matrix metalloproteinases TIMP synthetic inhibitors

1. Introduction

Matrix metalloproteinases (MMPs) are a protein family within the metzincin superfamily, comprising zinc-dependent endopeptidases with similar structural characteristics but with different substrate preferences. MMPs are produced and secreted from cells as inactive proenzymes depending, herein, on a structural alteration for activation [1][2][3][4][5][6]. In human tissues, there are 23 different types of MMPs expressed and they can be subdivided according to their substrate specificity, sequential similarity, and domain organization [1][2][4][7][8][9][10][11][12][13][14][15][16][17] (Table 1).

Table 1. Matrix metalloproteinases (MMPs) classes.

Class	MMP
Collagenases	MMP-1, Collagenase-1, Interstitial or Fibroblast collagenases
	MMP-8, Collagenase-2, or Neutrophil collagenases
	MMP-13 or Collagenase 3
Gelatinases	MMP-2 or Gelatinase A
	MMP-9 or Gelatinase B
Stromelysin	MMP-3 or Stromelysin-1
	MMP-10 or Stromelysin-2
	MMP-11

Class	MMP	
Matrilysin	MMP-7	
	MMP-26, Matrilysin-2, or Endometase	
Membrane-type	Type I transmembrane protein	MMP-14 or MT1-MMP
		MMP-15 or MT2-MMP
		MMP-16 or MT3-MMP
		MMP-24 or MT5-MMP
	Glycosylphosphatidylinositol (GPI)-anchored	MMP17 or MT4-MMP
		MMP-25 or MT6-MMP
Other MMPs	MMP-12	
	MMP-19	
	MMP-20	
	MMP-21	
	MMP-23	
	MMP-27	
	MMP-28	

[1][2][4][5][7][8][10][11][12][13][14][16][18]

1) a pro-
pe MMPs
(MT-MMPs) although some MMPS do not have all the structural features represented in the figure. The pro-domain keeps MMP inactive by a cysteine switch, which interacts with the catalytic zinc making it impossible to connect the substrate. The catalytic domain has two zinc ions, three calcium ions, and three histidine residues, which are highly conserved [1][2][3][4][5][6][7][8][9][11][12][13][14][15][16][17][18][19][20]. In the terminal zone of the catalytic domain, there is a region that forms the outer wall of the S_1' pocket [1][14][17]. This pocket is the most variable region in MMPs and it is a determining factor for substrate specificity [1][2][6][7][11][17][18]. However, there are six pockets (P_1, P_2, P_3, P_1', P_2', and P_3') and the fragments of the substrates or inhibitors are named depending on the interaction with these pockets (R_1, R_2, R_3, R_1' or R_a, R_2', and R_3'). The linker is proline-rich, of variable length, allowing inter-domain flexibility and enzyme stability [4][8][12][13]. The hemopexin-like domain is necessary for collagen triple helix degradation and is important for substrate specificity [3][4][7][9][19].

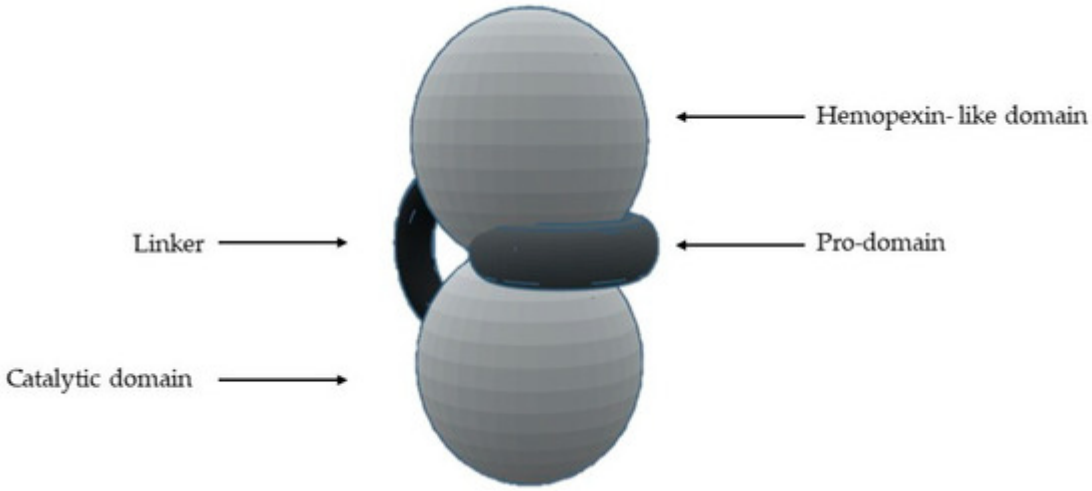

Figure 1. Schematic representation of the general structure of MMP.

The MMPs can process ECM proteins and glycoproteins, membrane receptors, cytokines, hormones, chemokines, adhesion molecules, and growth factors [1][3][4][6][7][9][10][11][13][14][20][21][22][23][24][25][26]. However, the presence and the activity of MMPs have been demonstrated to be intracellular [25][26]. For example, some studies show intracellular localization of MMP-2 in cardiac myocytes and colocalization of MMP-2 with troponin I in cardiac myofilaments [23]. The MMP-2 activity has also been detected in nuclear extracts from the human heart and rat liver [23]. The MMPs are involved in many biologic processes, such as tissue repair and remodulation, cellular differentiation, embryogenesis, angiogenesis, cell mobility, morphogenesis, wound healing, inflammatory response, apoptosis, ovulation, and endometrial proliferation [1][2][4][6][8][10][11][13][16][17][18][20][27]. The deregulation of MMPs activity leads to the progression of various pathologies depending on which enzyme is involved [1][6][10][13][14][15][16][17][20][27]: cancer and metastasis, inflammatory processes, arthritis, ulcers, periodontal diseases, brain degenerative diseases, liver cirrhosis, fibrotic lung diseases, otosclerosis, atherosclerosis, multiple sclerosis, dilated cardiomyopathy, aortic aneurysm, or varicose veins.

Although therapeutic strategies for specific inhibition of MMPs have been long researched, they are difficult to develop because these enzymes are involved in a myriad of pathways [2][5]. However, this inhibition can be done at the biomolecular expression and active enzyme terms [2][5][18]. The MMPs inhibitors can be divided into endogenous inhibitors, which can be specific or non-specific, and synthetic inhibitors [1][2][4][7][10][12][13][14][16][20][28][29] (Table 2).

Table 2. MMPs inhibitors classification.

	Specific Inhibitor	Tissue Inhibitor of Metalloproteinases (TIMP)
Endogenous inhibitor	Non-specifics inhibitors	α2-macroglobulin
		Tissue factor pathway inhibitor (TFPI)
		The membrane-bound β-amyloid precursor protein

	Specific Inhibitor	Tissue Inhibitor of Metalloproteinases (TIMP)
		C-terminal proteinases enhancer protein
		Reversion-inducing cysteine-rich protein with Kasal domain motifs (RECK)
		GPI-anchored glycoprotein
Synthetic inhibitor	Hydroxamate-based inhibitors	
	Non-hydroxamate-based inhibitors	
	Catalytic domain (non-zinc binding) inhibitors	
	Allosteric and exosite inhibitors	
	Antibody-based inhibitors	

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2. Specific Endogenous Inhibitor-Tissue Inhibitors of Metalloproteinases (TIMPs)

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous proteins responsible for the regulation of MMPs activity, but also of families such as the disintegrin metalloproteinases (ADAM and with thrombospondin motifs ADAMTS) and therefore maintaining the physiological balance between ECM degradation and MMPs activity [1][2][8][9][18][30].

There are four TIMPs (TIMP-1, -2, -3, and -4) (Table 3), with 22–29 KDa and 41%–52% sequential similarity [2][4][12][13][16][20][31].

Table 3. Tissue inhibitors of metalloproteinases (TIMPs) classification.

TIMP	Expression	Inhibition	Inhibition Mode
1	Several tissues with transcription inducible by cytokines and hormones	Strong interaction with MMP-1, -2, -3, and -9 Weak interaction with MT1-MMP, MT3-MMP, MT5-MMP, and MMP-19	TIMP-1 forms a complex with pro-MMP-9 by binding to the hemopexin domain
2	Constitutive expression	Strong interaction with MMP-2	TIMP-2 has four residues in the *N*-terminal domain and an adjacent CD-loop region, which allows interaction between TIMP and the active center of MMP-2
3	In response to mitogenic stimulation and during cell cycle progression	MMP-1, -2, -3, -9, and -13	The inhibition mode is different from the other TIMPs for its unusual localization, as it is largely sequestered into the extracellular matrix or at the cell surface via heparan sulphate proteoglycans

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TIMP	Expression	Inhibition	Inhibition Mode
4	Especially abundant in the heart, but is also expressed in injured tissue	MMP-2 and -14	- [2][4][7][8][9][12][31][32]

and

aining six
08, 29
a). The *N*-

terminal domain is an independent unit, which can be inhibited by MMPs, in a 1:1 ratio [2][4][8][9][10][12][13][16][20]. This domain has two disulfide bonds, which are important for TIMP activity [7][12]. This is the main domain responsible for MMP inhibition through its binding to the catalytic site in a substrate-like manner [31]. The several domains allow the TIMP and pro-gelatinases interactions [4].

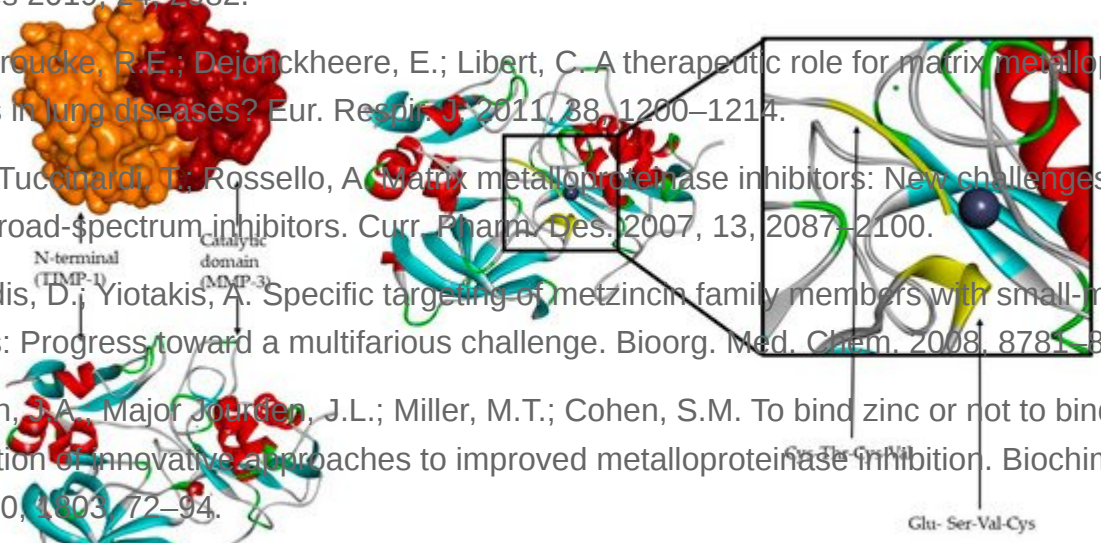

Figure 2. (**a**) MMP-1 catalytic domain of the MMP-3 complex. (**b**) MMP-1 catalytic domain of the MMP-3 complex, where two conserved groups, Cys-Thr-Cys-Val and Glu-Ser-Val-Cys, are represented in yellow.

3. Non-Specific Endogenous Inhibitors

Non-specific, endogenous inhibitors have been reported to inhibit MMPs (Table 4), however, the inhibition mechanism details have only been partially discovered [7][12].

Table 4. Non-specific endogenous inhibitors [7][32][33][34].

Non-Specific Inhibitor	Inhibition
α2-macroglobulin	MMP-2 and -9
Tissue factor pathway inhibitor	MMP-1 and -2
Membrane-bound β-amyloid precursor protein	MMP-2

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kaleidoscope of cell surface substrates processed by matrix metalloproteinases. Crit. Rev.

Non-Specific Inhibitor	Inhibition
C-terminal proteinase enhancer protein	MMP-2
Reversion-inducing-cysteine-rich protein with Kasal motifs (RECK)	MMP-2, -9, and -14
GPI-anchored glycoprotein [2]	[2][31]

pathway inhibitor (TFPI) is a serine proteinase inhibitor, which targets MMP-1 and -2, but this inhibition mode is still unknown [7][12]. The *C*-terminal proteinase enhancer protein and tissue factor pathway inhibitor have sequences with certain similarities to the *N*-terminal domain of TIMPs [31].

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