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Original Research Article

Matrix remodeling and collagen disintegration in Acute Myocardial Infarction

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Abstract

Coronary artery diseases manifest in different forms. The pathophysiology of coronary artery diseases are complex in origin and mechanism. There are several factors involved and are contributing together for the atherosclerotic event and its complications. The plaque formation and plaque rupture are the two crucial events which may lead to acute myocardial infarction, one of the manifestations of coronary artery diseases. The matrix are involved from the very beginning of the formation of plaque via collagen formation and deposition of lipid particles in the plaque by transforming the smooth muscle cells. An equally crucial event is the plaque rupture, which occurs by the action of matrix degrading metalloproteinases on fibrous plaque leading to extracellular matrix changes subjected to thrombus formation and subsequently to infarction

Keywords: Coronary Artery Diseases, Atherosclerosis, Acute Myocardial Infarction, Extracellular Matrix, Collagen, Plaque, Matrix Metalloproteinases.

INTRODUCTION

Coronary Artery Diseases (CADs) continue to be a major public health issue to many people all over the world irrespective of the measures taken in the diagnosis and management. In the last decade, it was observed that there was a drastic reduction in the death rate due to CADs by about 30 percentage, however its development is still a fatal event in many patients and life threatening too. It is alarming that most of the deaths due to CADs occur within one hour of the incidence and are mainly due to the complications like arrhythmias, most often ventricular fibrillation. Acute Myocardial Infarction (AMI) commonly referred as "Heart Attack" is a progressive outcome of CADs and can strike an individual during the most productive years, it can have profound deleterious psycho-social and economic implications.

The underlying causes especially the pathophysiological mechanisms involved in coronary diseases and its consequences and complications have also been studied over the past two decades all over the world. Pathologic observations proved that formation of an atheromatous plaque leading to narrowing of the arteries and hence the hindrance of blood flow or erosion of the vulnerable plaque due to various stimuli may be the initiating mechanism of occlusion of the arteries leading to thrombus formation and haemorrhage which may ultimately manifest in the form of Acute Coronary Syndromes (ACS). Further studies have subsequently suggested that plaque erosion or rupture more frequently occurs in lipid-laden plaques with the endothelial cap weakened by internal metalloproteinase activity derived primarily from macrophages [1]. When the plaque ruptures, elements in the blood stream are exposed to plaque matrix elements, including collagen and the intensely thrombogenic lipid core with its associated macrophage derived tissue factor. The result is stimulation of platelet adhesion, activation and aggregation [2]. Secretion of vasoconstrictive and thrombogenic mediators leading to thrombin generation and fibrin formation, causing vasospasm and the formation of a platelet and fibrin rich thrombus. The result is reduction (non ST elevation) or interruption (ST elevation) of coronary blood flow, with rapid onset of myocardial cell dysfunction and death [3]. The Extra Cellular Matrix (ECM) of the myocardium is a dynamic multifunctional complex system that undergoes constant exchange of signalling molecules and proteins between the interstitium and the cellular components, thus playing a fundamental role in myocardial remodeling [4, 5]. It is interesting to note that the ECM undergo constant changes during each of the events in CADs due to the changes occur to the collagen, a very important connective tissue protein present in vasculature and myocardium.

Collagen

Collagen is a connective tissue protein and triple helical in structure. It is classified into different types like type I, type II etc based on the triple helical nature and the type of helices involved. Most often synthesised by the smooth muscle cells (SMCs) of the arteries, is the major component of the fibrous cap of the plaque and is the one involved in the strengthening of the cap. Collagen synthesis also requires vitamin C for the crosslinking of the helices. Studies revealed that the plaque mainly contains interstitial collagen Types I and III [6] and even type V and IV. Hence it is evident that collagen contributes to the plaque growth and narrowing of the arteries. The major factors modulating collagen biosynthesis are the cell types, phenotype of the SMCs and other local factors like Transforming Growth Factor-Beta (TGF-β) and Platelet Derived Growth Factor (PDGF). While Fibroblast Growth Factor (FGF) has found to have an inhibitory effect. It is very interesting to note that collagen degradation and synthesis takes place simultaneously within the same plaque but are independent of each other. Matrix Metalloproteinases (MMPs), a class of proteinases/proteases are responsible for the degradation of the collagen in the fibrous cap.

Matrix Metalloproteinases

Proteolytic enzymes and organelle are mainly involved in the recognition and subsequent degradation of unused proteins. Even they can recognise and eliminate the unassembled and misfolded proteins too. Apart from these regular processes, there are additional roles for regulated proteolysis in various important biological processes necessary for cellular functions. Among these, the developmental significance of the modulation of the extra cellular microenvironment by metalloproteinases has become very evident [7].

Matrix metalloproteinases (MMPs) are the extrinsic regulators of each of the functions during the normal development of a cell [8]. The MMP family currently consists of more than 24 members characterized in humans, rodents and amphibians [9]. They were initially classified as the zinc dependent proteinases and are capable of digesting the various structural components of the extra cellular matrix (ECM), their specific proteolytic targets include other extra cellular proteins and components. These substrates include a series of other protein degrading enzymes, their inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors and cell-cell and cell-matrix adhesion molecules [10]. These MMPs are usually inactive zymogens and will be under the control of tissue inhibitors of MMPs (TIMPs).

Table-1: Classification of MMPs with their specific substrates

Subgroup	MMP	Name	Substrate
Collagenases	MMP-1	Collagenase-1	Col I,II,III, VII, VIII, X, Gelatin
	MMP-8	Collagenase-2	Col I,II,III, VII, VIII, X, Aggrecan, Gelatin
	MMP-13	Collagenase-3	Col I,II,III, IV, VIII, IX, X, XIV, Gelatin
Gelatinases	MMP_2	Gelatinase A	Gelatin, Col I,II,III, IV, VII, X
	MMP-9	Gelatinase B	Gelatin, Col IV, V
Stromelysins	MMP-3	Stromelysin -1	Col II, IV, IX, X, XI, Gelatin
	MMP-10	Stromelysin –2	Col IV, Laminin, Fibronectin, Elastin
	MMP-11	Stromelysin -3	Col IV, Fibronectin, Laminin, Aggrecan
Matrilysins	MMP-7	Matrilysin -1	Fibronectin, Laminin, Col IV, Gelatin
	MMP-26	Matrilysin - 2	Fibrinogen, Fibronectin, Gelatin
MT-MMP	MMP-14	MT1-MMP	Gelatin, Fibronectin, Laminin
	MMP-15	MT2-MMP	Gelatin, Fibronectin, Laminin
	MMP-16	MMT3-MMP	Gelatin, Fibronectin, Laminin
	MMP-17	MT4-MMP	Fibrinogen, Fibrin
	MMP-24	MT5-MMP	Gelatin, Fibronectin, Laminin
	MMP-25	MT6-MMP	Gelatin
Others	MMP-12	Macrophage	Elastin, Fibronectin, Col IV
	MMP-19	metalloelastase	
	MMP-20	Enamelysin	Aggrecan, Elastin, Fibrillin, Col IV, Gelatin
	MMP-21	XMMP	Aggrecan
	MMP-23		Aggrecan
	MMP-27	CMMP	Gelatin, Casein, Fibronectin
	MMP-28	Epilysin	Unknown
			Unknown

The MMP family

The MMPs are a large family of proteases known as matrixins and are having one or the other ECM component as their substrates (Table-1). Often they may name with respect to their substrate also. There are two closely related metalloproteinase families: matrix metalloproteinases (MMPs) and metalloproteinase-disintegrins (ADAMs) [11] belongs

to the Metzincins superfamily. Since they are metalloenzymes, MMPs require Zn^{2+} binding for their proteolytic activity. ADAMs are the transmembrane proteins that contain disintegrin and metalloproteinase domains, indicative of cell adhesion and proteinase activities [12] and they also require zinc for activity. Since the distribution of the substrates are varying in the tissues the MMPs are also located in different tissues.

Structure of MMPs

These proteinases are of having characteristic structures. MMPs contain a signal sequence, a prodomain, a catalytic domain, and usually hemopexin – like domain, although this is absent in MMPs-7 and -26 and replaced with an immunoglobulin –like domain in MMP-23 [2] (Figure-1). Most MMPs are expressed as inactive, latent proforms, although MMP-11, -21, -23 and -27 and the membrane –type MMPs (MT-MMPs) are activated by removal of the prodomain by furins in the endosomal pathway [2]. Except in MMP-23, latency is maintained because a conserved cysteine residue in the prodomain sequence PRCGXPD displaces the catalytic water molecule from the active site zinc ion [13].

A typical zinc binding sequence HExGHxxGxxHS is seen in the catalytic domain of MMPs and is said to be very unique. There may be slight variations in structure among different MMPs like in the gelatinases, MMP-2 and -9, the catalytic domain is interrupted by three repeats of a fibronectin type II – like sequence [2]. The COOH-terminal hemopexin domain in MMP-1 helps in the recognition of large matrix molecule substrates and is important in dimerization and in interaction of other pro-MMPs with TIMPs during activation or degradation of substrates [14].

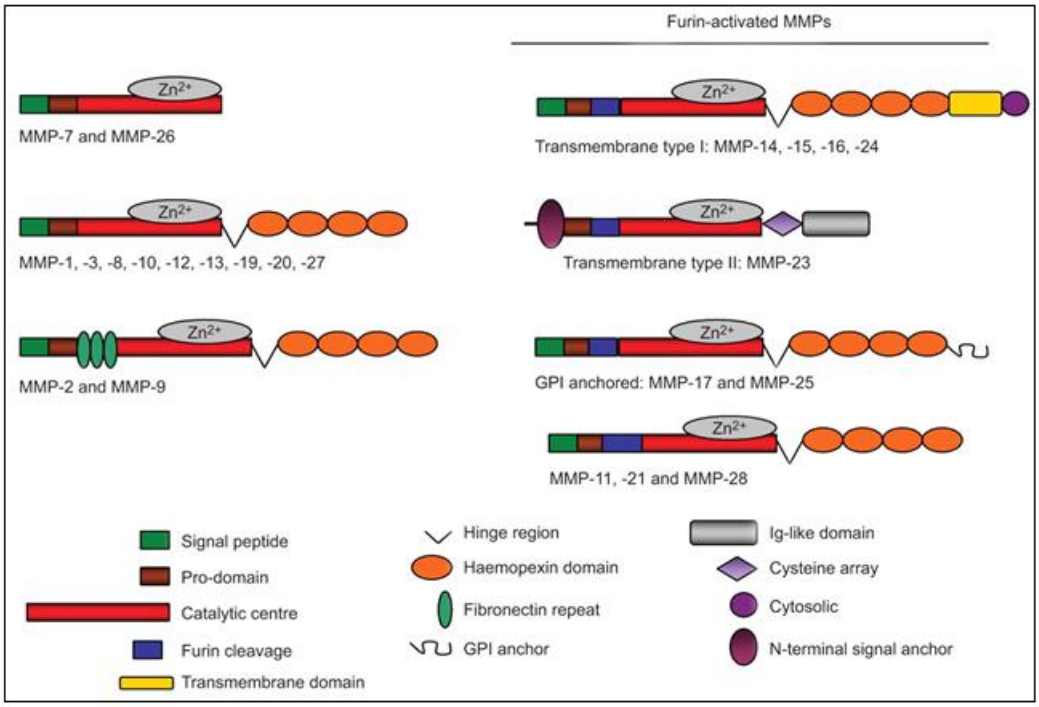

Fig-1: Structure of different types of MMPs

Regulation of MMP activities

The MMPs are synthesized as inactive zymogens (pro-MMPs). They are kept inactive by an interaction between a cysteine-sulph-hydril group in the propeptide domain and the zinc ion bound to the catalytic domain: activation requires proteolytic removal of the propeptide domain [15]. Generally MMPs activated by already activated MMPs via zymogen activation or may be by other proteases which have serine residues in their active site. However, MMP11, MMP28 and the MT-MMPs can also be activated by intracellular furin-like serine proteinases before they reach the cell surface [15].

Regulation of MMP function occurs at multiple levels. MMP mRNA expression is under tight, cell type-dependent control, with expression of individual MMPs associated with specific inflammatory, connective tissue or epithelial cell types [16]. MMP transcripts are generally expressed at low levels, but these levels rise rapidly when tissues undergo remodeling, such as in inflammation, wound healing, cardiovascular diseases and cancer [17]. MMPs are synthesized as proenzymes or latent enzymes and can be stored in inflammatory cell granules. However they are secreted and found to be attached to the cell surface or can join with other proteins on the cell surface or even with the ECM. Active MMPs are formed when these get loosened from TIMPs (Figure-

2). Latent MMPs are proteolytically activated in multiple steps resulting in the release of propeptide domains [18]. Once active, MMPs are subject to inhibition by a family of endogenous tissue inhibitors as well as by α_2-macroglobulin, a plasma inhibitor [19].

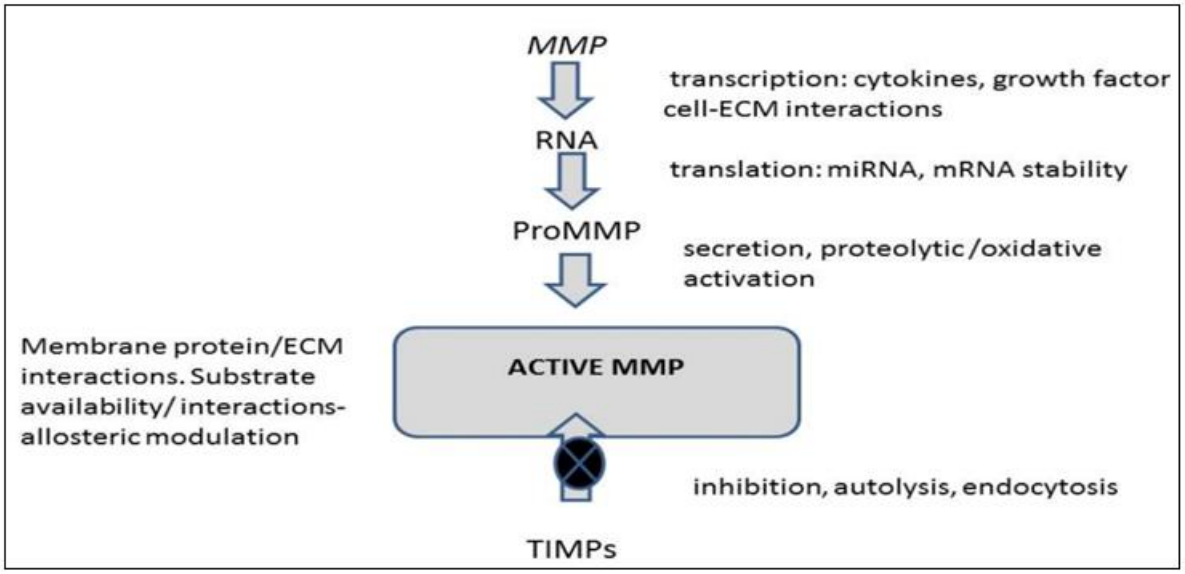

Fig-2: Transcription of MMPs at genetic level. It requires other factors like cytokines and growth factors

Inhibitors of MMP

A variety of protein inhibitors of MMP have been described, among which the TIMPs (Tissue Inhibitors of Metalloproteinases) (Figure-3) are by far the most potent and selective [20]. Tissue inhibitors of metalloproteinases (TIMPs) are a family of secreted proteins that selectively, but reversibly, inhibit metalloproteinases [15]. There are at least 4 members of the TIMP family that form tight inhibitory 1:1 complexes with MMPs. TIMP-1 is a much weaker inhibitor of Membrane type MMPs (MT-MMPs) than other TIMPs [21]. TIMPs -1 to 4 are each made up of two domains containing three disulphide bonds [11]. The NH2 – terminal cysteine is particularly important in inhibition, since its free alpha amino group and carbonyl function displace the catalytic water molecule from the essential zinc ion in the MMP active site [22].

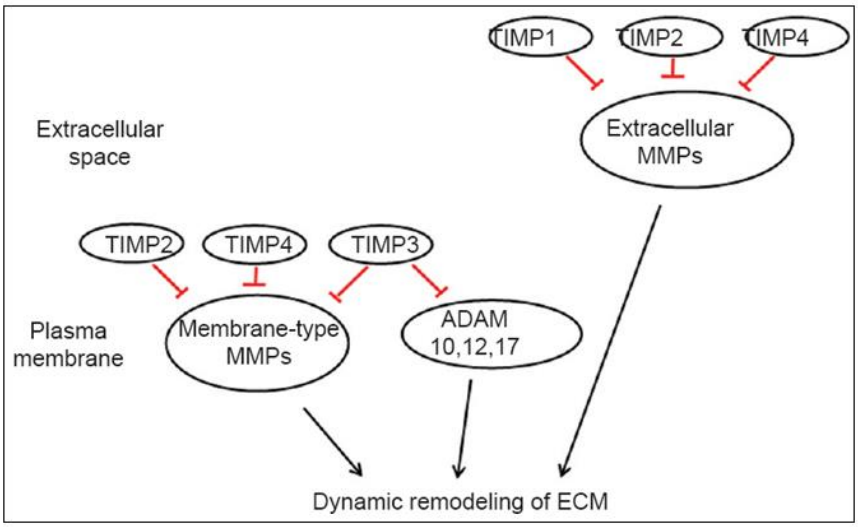

Fig-3: Tissue inhibitors of MMPs (TIMPs), which are selective inhibitors of MMPs

MMPs in CAD and in AMI

Developing from unstable to atherosclerotic plaque, the formation of a thrombus is an important pathogenic mechanism that leads to ischemic myocardial infarction and cerebral stroke [23]. MMPs have been linked with the pathophysiology of CADs. It has been reported that a gene mediated increased proteolysis in the arterial wall may act as a susceptibility factor for the development of CAD in most of the patients [24]. The complications of CAD including acute myocardial infarction is thought to be caused by the increased proteolysis of the fibrous cap of

the plaque by MMPs. Earlier studies have proved that the MMP2 and MMP9 have been linked with plaque rupture, the most complicated event in many forms of the acute coronary syndromes including acute myocardial infarction [24, 25]. The enhanced activities of MMP2 and MMP9 in the fibrous cap make the collagenous material to disintegrate and release the contents of the plaque into the surroundings. Through the blood stream these contents will move and block the branches of the small arteries, thus causing an infarction. Sometimes these infarctions will be very severe leading to the sudden death of the patient. Other forms of the CAD such as coronary aneurism (CA) as well as unstable angina (UA) have also been linked with other MMPs such as MMP3 and MMP12 along with MMP2 and MMP9 [26]. Further studies have shown that oxidative stress and other risk factors like smoking can also increase the MMP activities leading to further precipitation of AMI [27] (Figure-4).

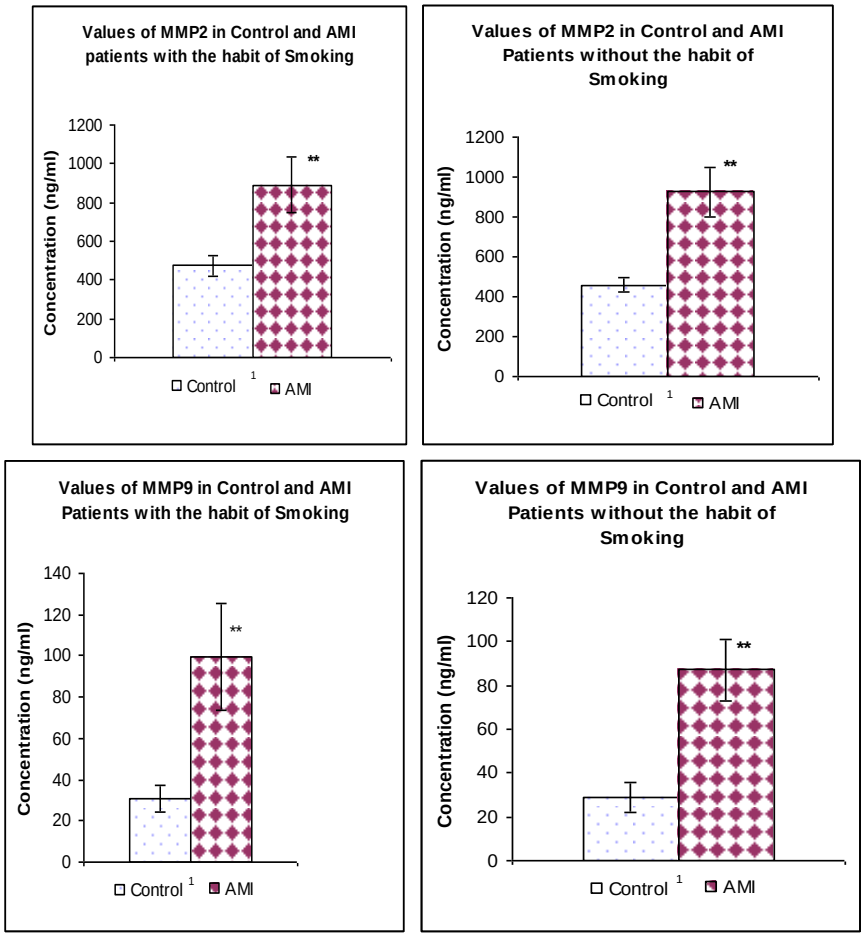

Fig-4: Values of MMPs in control subjects and in AMI patients with and without the habit of smoking

Expression of MMP System Components in Atherosclerosis

A potential role for increased proteolysis in atherosclerosis is supported by the enhanced expression of plasminogen activator (PA) and several MMPs in plaques [28]. Several MMP system components (MMP-1, -2, -3 and -9) are expressed in atherosclerotic tissue [29], in their active form they may contribute to vascular remodeling and plaque disruption. Stromelysin – 3 (MMP11), an unusual MMP that does not degrade any of the major ECM components, is also expressed in human atherosclerotic lesions and is regulated via CD40-CD40 ligand signalling [30]. Expression of MT-1 MMP in human atherosclerotic plaque is up regulated by proinflammatory mediators [31]. Co-expression of cyclooxygenase-2 with MMP-9 and MT1- MMP by macrophages and SMC in atherosclerotic lesions was also reported in some studies [32]. SMCs and macrophages in human atherosclerotic plaques also express MT3-MMP; its increased expression by macrophages induced by inflammatory molecules may promote ECM degradation and contribute to plaque destabilization [33]. Expression of MMP9 is up regulated and that of TIMP1 is down regulated in human monocyte derived macrophages by oxidized low density lipoprotein, suggesting that these may contribute to matrix degradation in the atherosclerotic plaque, predisposing to plaque rupture and/or vascular remodeling [34].

Vascular Remodeling and MMPs

Matrix metalloproteinases are actively involved in vascular remodeling by regulating the degradation of

extra cellular matrix and its components. Recent reports show that 72 kDa and 92 kDa type collagenases [matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9) respectively] act specifically on the basement membrane and partially degraded collagen play a pathogenic role in the development of atherosclerotic plaques and later in plaque rupture [35, 36] by various stimuli. It has been shown that MMP-2 is constitutively expressed in vascular smooth muscle cells and infiltrating macrophages. Mast cells in rupture prone areas of human coronary atheromas contain tumour necrosis factor-α (TNF- α), a powerful pro-inflammatory cytokine that is able to stimulate the production of MMP-9 by macrophages. Degradation of fibrous cap may result from further production and release of metalloproteinases such as collagenases, elastases and stromelysins [26]. Activated T-lymphocytes may stimulate metalloproteinase production by macrophages in the lesions, which promotes plaque instability and further implicates an immune response. Matrix metalloproteinases may thus, play a viatl role in plaque rupture [37] (Figure-5).

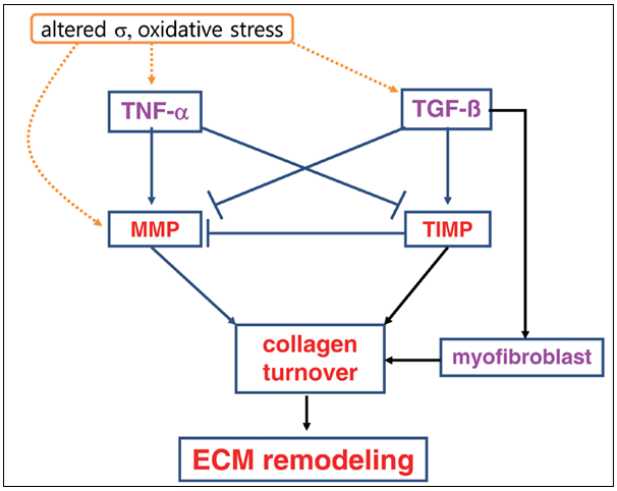

Fig-5: ECM remodeling by MMPs by mechanical stress (σ) and oxidative stress, which alter the morphology of ECM through pro-inflammatory markers. The control of TIMPs on MMPs will be lost resulting in increased degradation of collagen matrix

MMPs and Plaque rupture

Macrophages exposed to inflammatory cytokines also stimulate the production of matrix-degrading enzymes [38]. Coronary atherectomy specimens from patients with acute coronary syndromes have been shown to contain a 92-kDa gelatinase that is produced predominantly by macrophages and smooth muscle cells [39]. Plaque rupture, which accounts for more than 80% of fatal CADs in men, occurs in regions of high tangential stress and where collagen is depleted [40]. The implication is that matrix destruction weakens the plaque to the point where it can no longer resist the cyclical strain caused by the cardiac cycle [41].Within the atherosclerotic plaques, the highest stress regions have a two-fold greater matrix metalloproteinase (MMP-1) expression than the lowest stress regions. Over expression of MMP-1 in vulnerable plaques is associated with a substantial increase in circumferential stress. Degradation and weakening of the collagenous extra cellular matrix at critical points of high shear stress may play an important role in the pathogenesis of plaque rupture. A study conducted in India also revealed the increased activities of MMP2 and MMP 9 in AMI patients suggestive of increased collagen degradation in AMI [42].

Fibrous cap thickness can be maintained by smooth muscle cell mediated collagen synthesis (local repair); however, interferon -ϒ (IFN -ϒ), an inflammatory cytokine found within atherosclerotic plaques, decreases the ability of smooth muscle cells to express the collagen gene. Because only T-lymphocytes can elaborate IFN – ϒ [43], it has been suggested that chronic immune stimulation within atherosclerotic plaques leads to the production of IFN-ϒ from T- cells that subsequently inhibits collagen synthesis in vulnerable regions of the fibrous cap. IFN – ϒ can also contribute to apoptosis and, therefore, may be a key biochemical determinant of plaque vulnerability. The recent observation that mast cells may be involved with macrophage / foam cell development has raised questions concerning their potential involvement in plaque rupture. Human mast cells contain proteoglycans, proteolytic enzymes including chymase and tryptase. In normal coronary arteries, mast cells amount to 0.1 % of all nucleated cells; however, within the fibrous cap, lipid core, and shoulder regions of atheromatous lesions, there are 5-, 5- and 10 fold-increased densities, respectively [44]. Electron and light microscopic studies of mast cells in the plaque shoulder region have revealed evidence of degradation, a sign of activation that may contribute to matrix degradation and plaque rupture in acute coronary syndromes [45].

CONCLUSION

In short, several MMPs with complimentary substrates are needed to mediate the critical events leading to plaque erosion and rupture in cardiovascular diseases. Descriptive and correlative studies consistently proved the overexpression of MMPs and matrix destruction at the macrophage rich regions of the atherosclerotic plaques which are very viable and prone to rupture leading to acute myocardial infarction. On the other hand early fatty streaks in the atherosclerotic vasculature also show upregulation of MMPs, which could therefore be involved in plaque formation by their increased expression in the intima showing the involvement of ECM remodeling in plaque formation too. Thus the MMPs play a pivotal role in coronary artery diseases especially in AMI via matrix remodeling and collagen disintegration.

Conflict of Interest

The author claims that there is no conflict of interest.

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