

Review

Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates

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Abstract

Intimal thickening occurs in blood vessels in response to injury or atherosclerosis. The balance of migration and proliferation of vascular smooth muscle cells (VSMC) over death by apoptosis has an important impact on the final size of intimal thickening and may also affect atherosclerotic plaque stability. All aspects of VSMC behaviour are under coordinated control by growth factors, cell–matrix and cell–cell interactions. We review the evidence that matrix-degrading metalloproteinases (MMPs) regulate migration, proliferation and survival of VSMC. Moreover, we discuss critically the underlying mechanisms, which include changing growth factor availability and remodelling cell–matrix and cell–cell contacts. We conclude that MMPs influence VSMC behaviour by cleaving both matrix and non-matrix substrates.

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1. Introduction

Physiological intimal thickening can occur in blood vessels, for example, spontaneously with age or in response to increased intraluminal pressure [1]. Intimal thickening can also be triggered in response to injury, for example, after balloon dilatation, stent implantation or during atherosclerosis formation [1,2]. All these forms of intimal thickening are associated with increases in numbers of vascular smooth muscle cells (VSMC) and the amount of VSMC-associated extracellular matrix (ECM). Most intimal VSMC probably originate by migration from the underlying media [3,4] even though transdifferentiation of endothelial cells or differentiation from circulating precursors [5] could add VSMC from the luminal side and immigration of fibroblasts [6] or ‘stem-cell’ precursors [7]

could add cells from the adventitia. We focus on factors that regulate migration, proliferation or death of VSMC because they together influence the final size of the intimal VSMC population.

Growth factors are the primary mediators of migration and proliferation of VSMC, as extensively reviewed previously [8,9]. Growth factors also act as survival factors for VSMC, thereby inhibiting death by apoptosis [9,10]. As discussed in this review, much evidence has accumulated recently that cell–matrix and cell–cell interactions are also important in regulating these three processes. Moreover, by remodelling these cell–matrix and cell–cell interactions, extracellular proteases, in particular matrix metalloproteinases (MMPs), play key regulatory roles. Previous reviews have focussed on the physiological and pathological roles of MMPs in blood vessels [11–13]. However, the mechanisms by which MMPs regulate growth factor availability, cell–matrix and cell–cell contacts and thereby promote migration, proliferation and survival of VSMC have not been thoroughly reviewed until now.

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2. Which MMP genes are expressed in VSMC and how are they regulated?

As reviewed in more detail elsewhere in this volume and previously [12,14], the MMPs are a family of more than 25 Zn²⁺-requiring proteases with overlapping activities against a variety of ECM components (Table 1). For simplicity, they are often divided into interstitial collagenases (e.g., MMPs-1, -8 and -13), basement membrane-degrading MMPs or gelatinases (MMPs-2 and -9), broad specificity stromelysins/matrilysins (e.g., MMPs-3 and -7), membrane-type MMPs (e.g., MMPs-14 to -17) and others including a metalloelastase (MMP-12). Each of the MMPs degrades at least one component of the ECM (Table 1) but also a number of potential non-matrix substrates (for detailed listings, see Refs. [12,14,15] and http://www.clip.uba.ca/mmp_timp_folder/mmp_substrates.shtml). Although many of these non-matrix substrates have been identified in cells other than VSMC, they suggest a wide spectrum of possible regulatory interactions that are discussed further below.

MMP activity can be either distributed into the ECM through secretion of freely soluble enzymes or confined to the pericellular region by direct or indirect attachment of MMPs to the cell surface (e.g., Fig. 1). Membrane-type MMPs are directly attached either by transmembrane domains or by glycosphosphatidyl inositol anchors, while indirect mechanisms confine other MMPs to the pericellular domain [15]. Examples include binding of MMP-1 to α_2 integrin subunits or EMMPRIN and binding of active MMP-2 to $\alpha v\beta 3$ integrins, pro-MMP-9 to $\alpha 2$ integrin subunits and active MMP-9 to the CD44 hyaluronan receptor (Fig. 1). Interestingly, MMP-14 can shed the CD44 receptor [16], which could alter the distribution of MMP-9 as well activating MMP-2 (Fig. 1). The activity of

MMPs is limited physiologically by binding to one of four endogenous tissue inhibitors of MMPs (TIMPs) [12,15]. Among these, TIMP-3 is unusual in having a significant affinity also for several adamalysin metalloproteinases (ADAMs) and for strong binding to the ECM, which may localise it to the pericellular region. Individual MMPs do not act alone but cooperate with other extracellular and cell surface proteases (see Fig. 1). For example, the serine proteinase, plasmin, can activate the pro-forms of many secreted MMPs [12,15], while activation of MMP-2 selectively involves membrane-type MMPs [15].

The different structures of the MMP gene proximal promoters (Fig. 2) imply different patterns of regulation for the various MMPs. Consistent with the absence of a TATA box, MMP-2 is secreted constitutively from VSMC [17–20]. Nevertheless, mechanical injury upregulates MMP-2 production [21–24] and so does stretch [25]. The relative importance of gene transcription, mRNA stability, translational control and protein stability in these effects has yet to be fully clarified. Recently the chemokine, CCL1, was reported to increase MMP-2 mRNA and protein in human VSMC but the mechanisms was not elucidated [26]. The presence of the constitutive Sp-1 element in the promoter of the MMP-14 gene may account for constitutive expression of this MMP in VSMC, while occupation of this site by alternate transcription factors allows for further upregulation [27,28].

Inflammatory cytokines, such as interleukin-1 (IL-1), IL-4 and tumour necrosis factor- α (TNF- α) coordinately induce a broad range of MMPs, including MMPs-1, -3 and -9 [19,29,30]. Cytokines act synergistically with growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2) [20,31–34]. Interestingly, upregulation of MMP-1 and -3 in particular show a

Table 1
Extracellular matrix substrates of MMPs

Classification	Trivial names	ECM substrates
MMP-1	Collagenase-1	Collagen types I, II, III, VII, VIII, X, and gelatin, aggrecan, casein, nidogen, serpins, versican, perlecan, proteoglycan link protein, and tenascin-C
MMP-2	Gelatinase-A	Collagen types I, IV, V, VII, X, XI, XIV, and gelatin, aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-3	Stromelysin-1	Collagen types II, IV, IX, X, and gelatin, aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, and versican
MMP-7	Matrilysin-1	Collagen types I, II, III, V, IV, and X, aggrecan, casein, elastin, enactin, laminin, and proteoglycan link protein
MMP-8	Collagenase-2, neutrophil collagenase	Collagen types I, II, III, V, VII, VIII, X, and gelatin, aggrecan, laminin, and nidogen
MMP-9	Gelatinase-B	Collagen types IV, V, VII, X, and XIV, fibronectin, laminin, nidogen, proteoglycan link protein, and versican
MMP-10	Stromelysin-2	Collagen types III, IV, V, and gelatin, fibronectin, laminin, and nidogen
MMP-11	Stromelysin-3	Laminin
MMP-12	Macrophage metalloelastase	Elastin
MMP-13	Collagenase-3	Collagen types I, II, III, IV, V, IX, X, XI, and gelatin, aggrecan, fibronectin, laminin, perlecan, and tenascin
MMP-14	MT1-MMP	Collagen types I, II, III, and gelatin, aggrecan, dermatan sulphate proteoglycan, fibrin, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin
MMP-15	MT2-MMP	Collagen types I, II, III, and gelatin, aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, and vitronectin
MMP-16	MT3-MMP	Collagen types I, III, and gelatin, aggrecan, casein, fibronectin, laminin, perlecan, and vitronectin
MMP-17	MT4-MMP	Gelatin, fibrin, fibronectin

Adapted from Ref. [14].

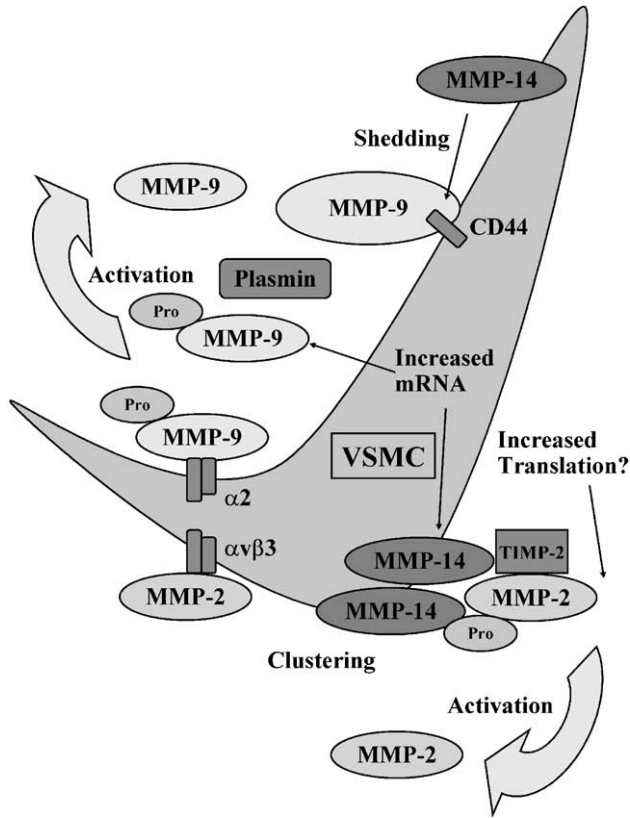


Fig. 1. Activation mechanisms and pericellular location of MMP-2 and MMP-9. MMP-14 is an intrinsic membrane protein that activates pro-MMP-2 at the cell surface by a complex that also involves TIMP-2. Active MMP-2 can be retained at the cell surface by binding to $\alpha v \beta 3$ integrins. Pro-MMP-9 either free or cell bound to $\alpha 2$ integrins can be activated by plasmin (PL). Active MMP can be retained at the cell surface by CD44, which can be shed by MMP-14. Transcription, translation and clustering mechanisms all contribute to regulating the system.

requirement for ECM contacts either through integrins [35,36] or discoidin domain receptors [37]. Cell contact with T-lymphocyte membranes and addition of recombinant CD40 ligand induce MMPs-1, -3, -8 and -9 in VSMC [38–40]. Many of the MMPs genes contain a proximal AP-1 together with another AP-1 complex further upstream (see Fig. 2) both of which appear necessary for cytokine responsiveness [41]. In the case of MMP-9, this complex also contains an NF- κ B binding site, which is also essential [34]. Surprisingly, NF- κ B is also essential for MMP-1 and -3 induction by cytokines, despite the absence of clearly recognised binding sites in the proximal promoter [34].

The regulation of TIMPs in VSMC shows a distinctly different pattern from MMPs. TIMP-1 is either constitutive [19,32] or upregulated by the fibrogenic cytokines, PDGF and transforming growth factor- β (TGF- β) [42]. TIMP-2 secretion is constitutive [19,32], while TIMP-3 expression is upregulated by a combination PDGF and TGF- β [32]. Hence, stretch, injury, inflammation and immune activation progressively move the MMP/TIMP balance in VSMC towards proteolysis, while fibrogenic stimuli tend to reverse this.

MMPs-2 and -9 are upregulated and activated in VSMC during intima formation in organ cultures and animal models, including angioplasty restenosis and vein grafting. Induction of MMP-9 and activation of MMP-2 occur rapidly in organ cultures of human saphenous vein [24], in pig vein grafts [43] and after balloon injury to rat [22,44], pig [23,45], baboon [46], rabbit [47] and mouse [48,49] arteries. Upregulation of MMP-14 and MMP-16 [50] and uPA [51] accompanies MMP-2 and MMP-9 activation, consistent with the interactions proposed in Fig. 1. Increased expression of MMP-1 and MMP-3 were much less evident in human saphenous vein [52], but levels of MMP-1, -3, -12 and -13 were also increased after femoral artery injury in mice [48]. TIMP-1 and TIMP-2 expression is either not changed or increased after balloon injury [53,54], while levels of TIMP-3 and TIMP-4 are increased [55].

Levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-11, MMP-12, MMP-13, MMP-14 (MT1-MMP) and MMP-16 are all elevated in human atherosclerotic plaques, especially at the macrophage-rich shoulder regions; a broad spectrum of MMPs is also expressed in aneurysms (reviewed in Ref. [13]). MMP expression is prominent in macrophages but also present in VSMC, lymphocytes and endothelial cells [56–61]. TIMP-1 levels are unchanged [56] or elevated [62] in atherosclerotic plaques. TIMP-2 is also abundant in plaques [60] and TIMP-3 is clearly increased, prominently in macrophages but also in VSMC [42]. Irrespective of their cellular source, secreted MMPs and TIMPs could affect the behaviour of VSMCs in atherosclerotic plaques or aneurysms. MMP-1, MMP-2, MMP-3 and MMP-9 levels are increased also during atherosclerosis in rabbits [63,64]. VSMC at the base of rabbit plaques overexpress TIMP-1 and TIMP-2, which may prevent medial destruction [65]. MMP-3, MMP-11,

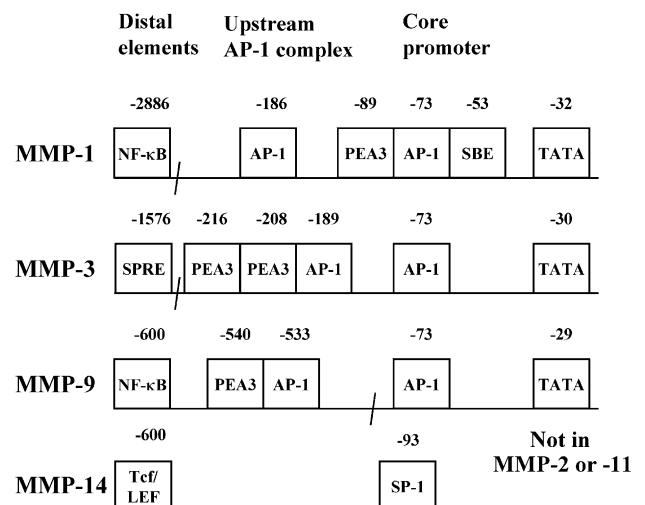


Fig. 2. Promoter regions of some MMPs. Abbreviations: AP-1, activator protein-1; NF- κ B, nuclear factor κ B; PEA-3, polyomavirus enhancer-A-binding protein-3; SBE, Stat binding element; Sp-1, Stimulatory protein-1; SPRE stromelysin PDGF response element; Tcf/LEF, T-cell factor/lymphoid enhancer factor.

MMP-12 and MMP-13 are upregulated also in mouse plaques [38,66,67].

3. Role of MMPs in migration of VSMC

Synthetic MMP inhibitors or gene transfer of TIMPs has been shown to reduce VSMC migration in vitro and inhibit or delay intimal thickening in vivo. For example, synthetic MMP inhibitors inhibited emigration of VSMC from rabbit aortic and baboon saphenous artery explant cultures [17,46]. TIMP-1, TIMP-2 and TIMP-3 gene transfer inhibited migration of isolated VSMC in vitro [68] and reduced neointima formation in organ cultures of human saphenous vein [69–71]. Synthetic MMP inhibitors also inhibited early VSMC migration in the rat carotid balloon injury model [22,72–75]. Seeding of cells stably overexpressing TIMP-1 reduced neointima formation after balloon injury [76], as did adenovirus-mediated gene transfer of TIMP-1 or TIMP-2 [77,78]. When seeking to identify the individual MMPs responsible, most experiments have concerned the gelatinases, MMP-2 and MMP-9. Pauly et al. showed that MMP-2 mediated migration of isolated VSMC through a synthetic basement membrane (BM) [18]. Chemokine-induced chemotaxis of human VSMC monolayers also depended on MMP-2 activity [26]. In vivo, knockout of MMP-2 decreased VSMC migration and intima formation in the mouse carotid ligation model [79,80]. In relation to MMP-9, addition or gene transfer promoted invasion of collagenase-isolated VSMC through a collagen matrix in vitro [81]. Knockout of MMP-9 impaired VSMC migration and intima formation after filament loop injury [82] or arterial occlusion [83] in mice in vivo. These results do not exclude a role for other MMPs (see Note added in proof).

Remodelling of ECM and non-matrix substrates by MMPs could influence VSMC migration in a multitude of ways. Cells in the media or adventitia would need to break physical barriers resulting from existing contacts, for example, between BM components and cell surface integrins (Fig. 3). Consistent with this, we found that 95% of medial VSMCs in human saphenous veins are normally surrounded by BMs [84]. Medial VSMC lost BMs during organ culture and all VSMCs that migrated into the neointima lacked BMs [84]. Furthermore, blocking MMPs by TIMP gene transfer preserved medial BMs and inhibited VSMC migration to the intima.

For VSMC to move, several signal transduction pathways must be activated to reorganise the cytoskeleton and to form reversible contacts with existing or newly expressed cell surface and ECM components. Signalling pathways originating from growth factor receptors, cadherins and integrins appear to be of cardinal importance [85–87]. ECM–integrin interactions, in particular, promote focal adhesion formation and activation of focal adhesion kinase (FAK) (Fig. 3). Loss of contact with BM components, type IV collagen and laminin, are likely to be responsible at least in part for the

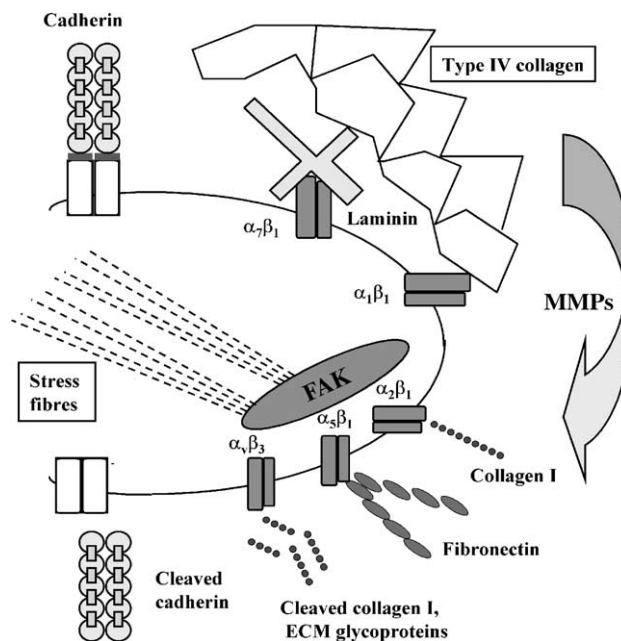


Fig. 3. MMP influences on VSMC migration. MMPs can remodel basement membrane components, including laminin and type IV collagen and free cells to migrate. Loss of basement membranes promotes phenotypic modulation of VSMC. This leads to synthesis, amongst other things, of new integrin subunits and new matrix components that include glycoprotein ligands for these integrins, for example, vitronectin, osteopontin and tenascin. MMPs also fragment existing membrane components such as type I collagen and this can create new integrin-binding sites. By acting through integrins and focal adhesion kinase (FAK) ECM components influence intracellular pathways that regulate the cytoskeletal changes necessary for motion. MMPs could shed cadherins and could thereby relieve constraints on movement caused by adherens junctions.

profound changes in gene expression that occur in VSMC during intima formation. These include changes in the spectrum of cell surface integrins [88] and upregulation of interstitial matrix components, including type I collagen, elastin, and the multiadhesive glycoproteins fibronectin, vitronectin, osteopontin and tenascin [89–91]. Hence, MMPs by degrading BMs can indirectly facilitate a host of new ECM–integrin interactions leading to activation of FAK and increased migration (Fig. 3). Cleavage of existing ECM proteins may also reveal cryptic integrin binding sites. For example, PDGF stimulated VSMC migrate more rapidly on collagen I cleavage into 1/4, 3/4 length fragments than on intact collagen I [92]. Moreover, adhesion and migration on cleaved collagen was mediated by newly induced $\alpha_v\beta_3$ integrins, while migration on native collagen I was mediated by constitutive $\alpha_2\beta_1$ integrins (Fig. 3).

All these findings imply that correctly modulated MMP activation can increase VSMC migration. On the other hand, in one study [93], growth of VSMC on degraded collagen led to calpain-mediated cleavage of FAK, cytoskeletal rearrangement and decreased migration of VSMC. Overexpression of the membrane type MMPs, MMPs-14 and MMP-16 also caused FAK cleavage in another study [94], implying that deregulated MMP activity could actually impair migration of VSMC.

Intimal thickening is also associated with increased hyaluronic acid production [95,96]. Hyaluronan binds the cell surface through CD44, and shedding of CD44 by MMP-14 modulates motility, at least in tumour cell lines [16]. Another factor may be the need to regulate cell–cell contacts through cadherins (Fig. 3). Recent evidence suggests that E-cadherin in epithelial cells, VE cadherin in endothelial cells and N-cadherin in VSMC can be cleaved MMPs [97–99]; this dissolves adherence junctions and presumably frees the cells to move. These two last examples illustrate how MMPs could modify migration of VSMC through non-matrix substrates.

4. Role of MMPs in VSMC proliferation

Several studies of structurally unrelated, broad-spectrum, synthetic MMP inhibitors demonstrated inhibition of VSMC proliferation in vitro [17,99,100] but others did not [73]. Furthermore, only two of four studies found inhibition of medial VSMC proliferation 2 days after balloon injury to the rat carotid artery [73,75]. Two studies using hydroxamate-based inhibitors showed a subsequent excess of proliferation in the neointima after 7–10 days that led to catch-up growth in neointimal size [72,73]. In contrast, two studies using tetracycline-based inhibitors detected inhibition of intimal VSMC proliferation and a reduction of neointimal size [74,75]. Knockout of MMP-9 also gave inconsistent results; it caused delayed inhibition of VSMC proliferation after filament loop injury [82] but not after tying off the carotid artery [83]. A possible explanation for these inconsistent effects is the compensatory activation of other proteases, for example, the serine proteases plasmin and plasminogen activators, in some models [46]. However, this idea is speculative and needs to be tested by inhibiting both MMPs and serine proteases simultaneously. None of the studies with TIMP gene transfer in organ culture or in vivo showed any effect on VSMC proliferation [69–71,77,78]. However, the level of TIMP expression is hard to measure after gene transfer, especially in vivo. Hence, the different results could be explained by less complete inhibition of MMPs with TIMP gene transfer than with synthetic inhibitors. Alternatively synthetic inhibitors might have had additional actions unrelated to inhibition of MMPs.

A requirement to remodel cell–cell and cell–ECM contacts is less obviously needed for proliferation since cells can proliferate without migrating. Nevertheless, VSMC proliferation in response to serum is 10,000-fold less in intact rat aorta compared to VSMCs isolated by collagenase digestion [101]. Similarly, serum-induced VSMC proliferation in the medial layer of intact rabbit aorta [102] or uninjured human saphenous vein [103] is undetectable despite robust intimal VSMC proliferation [103–106]. Platelet adhesion [107], or infusion of platelet derived growth factor [108], does not cause VSMC

proliferation in the media of gently denuded rat carotid arteries, although FGF-2 apparently does [109]. The different response to PDGF and serum of VSMC in the media of intact vessels compared to cells isolated by collagenase suggests that regulatory interactions with the ECM could indeed be remodelled by endogenous collagenases (Fig. 4).

One possibility is that degradation of collagen type IV and core proteins of several heparan sulphate proteoglycans by MMPs-2 and -9 (Table 1) could disrupt inhibitory interactions with BM components [110,111]. However, although 60–70% of proliferating VSMCs in human saphenous vein organ cultures had lost their BM [84] the remaining 30–40% continued to proliferate, despite the presence of a BM. It therefore appears unlikely that BM components completely constrain VSMC proliferation.

MMPs could also promote permissive interactions between VSMC and components of the remodelling ECM. Consistent with this, integrin-mediated pathways appear essential as well as growth factors to stimulate VSMC proliferation (Fig. 4) [112–114]. Components of remodelling matrices such as newly synthesised collagen [115] and

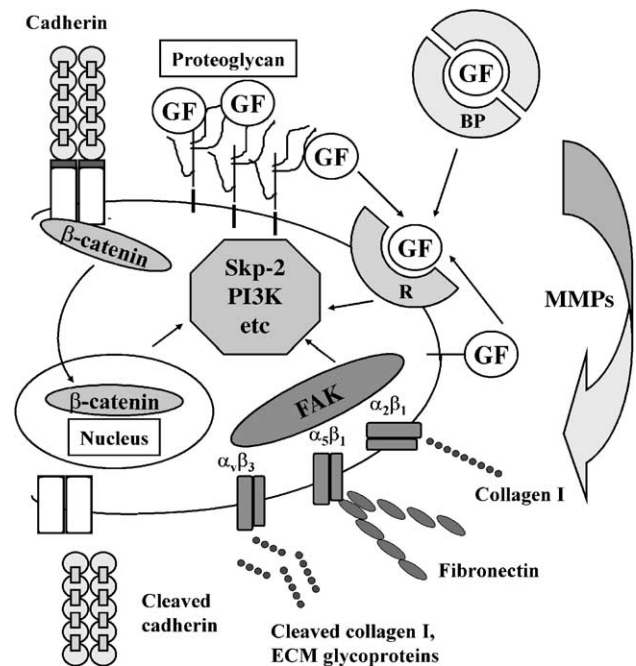


Fig. 4. MMP influences on proliferation and survival of VSMC. MMPs can free growth factors (GF) from attachment to matrix components or the cell surface from where they can act on receptors (R). MMPs can also liberate active IGF-1 by degrading binding proteins. Together with signals from focal adhesion kinase (FAK), this upregulates and/or stabilises many key regulators of the cell cycle. One proximal pathway uses the ubiquitin ligase subunit Skp-2. MMP-induced cadherin shedding promotes dissolution of adherens junctions and promotes translocation of β -catenin to the nucleus where it can act as a transcription factor to further promote proliferation. Many growth factors (GF) also act as survival factors for VSMC. GFs and matrix signals partly from focal adhesion kinase (FAK) share the ability to upregulate PI3 kinase that activates one survival pathway through the protein kinase Akt. Recent data show that survival of VSMC is also regulated by cadherin contacts that could be influenced by MMP-mediated shedding.

fibronectin [112] are implicated, probably initially by activating FAK (Fig. 4). Consistent with this, inhibiting FAK by forcing VSMC into suspension or overexpressing dominant-negative FAK potently inhibits VSMC proliferation in vitro [113,116] and in vivo [116]. Cell cycle progression in VSMC as in other cells requires the correct assembly of cyclin-dependent kinases and the degradation late in G1 of cyclin-dependent kinase inhibitors (CDKIs). Nonproliferative VSMC in intact arteries fails to down-regulate CDKIs, including p27^{Kip1} and p16^{INK4} [101] as is also found in nonadherent VSMC or cells expressing dominant negative versions of FAK [117]. To explain this data, we showed recently that FAK activation is required to upregulate Skp-2, a component of the SCF^{Skp2} ubiquitin-ligase, which is essential for proteosomal degradation of p27^{Kip1} and, hence, VSMC proliferation [117] (Fig. 4).

An important role for MMPs in regulating VSMC proliferation through the non-matrix substrate, N-cadherin, has also recently emerged (Fig. 4). In cultures of isolated human saphenous vein VSMCs cell surface N-cadherin was shed into the medium during proliferation [99], and this was accompanied by translocation of β -catenin to the nucleus, where it can act as a transcription factor [87]. Moreover, overexpression of dominant negative N-cadherin, which decreased cell surface levels, significantly increased β -catenin nuclear translocation and proliferation [99]. Inhibition of MMPs with a synthetic inhibitor or by overexpressing TIMPs inhibited N-cadherin shedding elevated cell membrane N-cadherin levels, blocked β -catenin nuclear translocation and decreased proliferation in cultured human VSMC [99]. Dismantling of cadherin:catenin complexes also occurs in balloon-injured rat carotid arteries in vivo and is associated with VSMC proliferation [118].

MMP-mediated mobilisation of growth factors from the ECM could also influence VSMC proliferation (Fig. 4). Heparin-binding growth factors, in particular FGF-1 and FGF-2, are potent mitogens for VSMC and could be released by action of MMPs on proteoglycan core proteins [12]. Although ADAMs are more often implicated, MMPs could also be responsible for releasing cell-surface HB-EGF [119,120]. Another recent study showed that MMP-9 released stem-cell factor from the surface of human smooth muscle cells, and that c-kit receptors were present to complete an autocrine loop [121]. On the other hand, extracellular proteases including MMPs are capable of activating TGF- β , by cleaving off the latency-associated peptide [122]. Since TGF- β most likely acts as an inhibitor of VSMC proliferation [123], it is hard to predict the net effect of these actions of MMPs.

5. Role of MMPs in VSMC apoptosis

Apoptosis of VSMC probably plays an important part in limiting intimal thickening and, by destabilising atherosclerotic plaques, could also precipitate plaque rupture and

myocardial infarction [9,10]. Apoptosis is a form of cell death that involves activation of the intracellular cysteine proteases, caspases. Death signals originating from outside the cell and processes within the cell, such as DNA damage, cell cycle status and levels of the tumour suppressor p53 promote apoptosis [10]. However, several survival signals can maintain VSMC viability even in the face of a pro-apoptotic environment. Survival pathways are closely linked to those triggering proliferation and therefore could be influenced by MMPs in similar ways (Fig. 4). For example, survival factors such as PDGF, HB-EGF and IGF-1 act via intrinsic tyrosine kinase receptors to stimulate the PI3-kinase/Akt pathway (Fig. 4). MMP-2, MMP-7 and MMP-9 can cleave cell surface pro-HB-EGF and liberate the soluble active growth factor [119,120]. Moreover, MMP-1, MMP-2, MMP-8 and MMP-9 all degrade members of the IGF binding protein family [12,14] and could therefore increase the bioavailability of IGF-1, although direct evidence is lacking for an effect on viability of VSMC. Cell–matrix contacts also promote VSMC survival since their disruption leads to apoptosis in a process originally called anoikis [124]. FAK activation triggered by ECM–integrin interactions can stimulate several survival signalling pathways, for example, by induction of p53 [125,126]. As discussed above, regulated MMP production appears to favour FAK activation and hence survival signalling. Conversely, excessive production of MMPs could degrade ECM proteins or integrins and promote anoikis for which there is direct evidence in endothelial cells [127].

A recent study of human VSMC in vitro found that cell–cell contacts through cadherins were as protective against VSMC apoptosis as cell–matrix contacts [128]. The evidence that MMPs mediate N-cadherin shedding in the same cells [99] suggests a further potential link between MMP activity towards this non-matrix substrate and apoptosis. Indeed studies in epithelial [98] and endothelial [97] cells showed a role for MMPs in shedding of E-cadherin and VE cadherin, respectively.

MMPs could also modulate apoptosis by cleaving death ligands (e.g., TNF- α and Fas ligand) and their receptors, which trigger apoptosis by acting in an autocrine or paracrine manner. For example, MMP-1, MMP-2, MMP-9, MMP-8, MMP-13 and the MT-MMPs, MMPs 14–17 can all cleave pro-TNF- α [12,14], although only ADAM-17 and with lesser specificity MMP-7 produce the correctly processed soluble form [129]. This would be expected to reduce the possibility of autocrine activation of apoptosis but release a source of pro-apoptotic signal to neighbouring cells. Similarly, MMP-7 sheds Fas-L (CD95) from the surface of several cell types [130,131]. Given that MMP-7, TNF- α , Fas and Fas-L are all expressed in human atherosclerotic plaques [9], these regulatory interactions could well be operating, and direct studies in VSMC are therefore warranted. Further interest in the area was sparked by the observation that TIMP-3 is an effective stimulator of apoptosis in many cells, including VSMC [68]. Apoptosis in

response to TIMP-3 appears to require MMP inhibitory activity but the effect is not shared by TIMP-1 or TIMP-2 [68,132]. This suggests that an ADAM rather than an MMP is the target. The death ligand involved in VSMC apoptosis in response to TIMP-3 is not known [133]. Intriguing recent report suggests that TIMP-4 may also stimulate VSMC apoptosis [134] but the mechanism is again unknown.

One characteristic step in apoptosis is caspase-mediated cleavage of the DNA repair enzyme poly-ADP ribose-polymerase. A recent study in isolated cardiac myocytes [135] showing that nuclear localised MMP-2 can also carry out this cleavage suggests an entirely unexpected non-matrix, pro-apoptotic action of MMPs. However, the role of this mechanism in vivo and its implications for VSMC apoptosis have not yet been addressed.

6. Conclusions

In summary, VSMC produce a spectrum of MMPs, some spontaneously and others in response to stretch, injury, growth factors and inflammatory mediators. Together with MMPs from endothelial and inflammatory cells, they have the capacity to remodel a wide range of ECM and non-matrix substrates. MMPs clearly mediate migration of VSMC in vitro and in vivo and this can be explained by relieving the physical constraints of BM components and by promoting expression of new ECM components and integrins that provoke necessary intracellular signals. Relevant non-matrix substrates include the CD44 hyaluronan receptor and cadherins. The role of MMPs in VSMC proliferation is less clear, possibly owing to redundant action with other protease classes. However, the ability of MMPs to promote permissive interactions between components of the remodelling ECM and integrins could contribute to upregulating Skp-2, a key step in downregulating p27 CDKI and thereby promoting cell cycle progression. Mobilisation of growth factors and cleavage of cadherins with translocation of β -catenin to the nucleus are relevant growth-promoting actions on non-matrix substrates. There is potential for both stimulatory and inhibitory actions of MMPs on VSMC apoptosis. Survival would be favoured by MMP-mediated remodelling ECM or growth factor liberation. On the other hand, death could be promoted by cleaving death ligands and cadherins. Many of these mechanisms remain incompletely described and there is therefore much left to achieve through future research.

7. Note added in proof

Fillipov and colleagues [136] recently demonstrated a role for MMP-14 (MT-1 MMP) in migration of VSMC through 3-dimensional collagen in vitro and in the mouse carotid artery in vivo. This adds to the established roles of MMP-2 and MMP-9 in other models discussed above.

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