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**Matrix Metalloproteinases as Regulators of Vein Structure and Function.
Implications in Chronic Venous Disease**

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List of Non-standard Abbreviations:

15-PGDH	15-hydroxyprostaglandin dehydrogenase
15d-PGJ2	15-deoxy-delta-12,14-prostaglandin J2
AP-1	activator protein-1
BK _{Ca}	large conductance Ca ²⁺ -activated K ⁺ channel
CEAP	clinical-etiology-anatomy-pathophysiology
CVD	chronic venous disease
CVI	chronic venous insufficiency
EC	endothelial cell
ECM	extracellular matrix
EDHF	endothelium-derived hyperpolarizing factor
EMMPRIN	extracellular matrix metalloproteinase inducer
ERK	extracellular signal-regulated kinase
GPCR	G protein-coupled receptor
GPI	glycosyl phosphatidylinositol
GSV	great saphenous vein
HIF	hypoxia-inducible factor
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IVC	inferior vena cava
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
MMPI	MMP inhibitor
mPGES-1	membrane-associated prostaglandin E synthase-1
MT-MMP	membrane-type MMP

NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
PAR	protease activated receptor
PDGF	platelet-derived growth factor
PGI ₂	prostacyclin
PI ₃ K	phosphoinositide 3-kinase
ROS	reactive oxygen species
SDX	sulodexide
siRNA	small interfering RNA
TGF- β	transforming growth factor- β
TIMP	tissue inhibitor of MMP
TNF- α	tumor necrosis factor- α
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle
VVs	varicose veins
Zn ²⁺	zinc
ZBG	Zn ²⁺ binding globulin

Abstract

Lower extremity veins have efficient wall structure and function and competent valves that permit upward movement of deoxygenated blood towards the heart against hydrostatic venous pressure. Matrix metalloproteinases (MMPs) play an important role in maintaining vein wall structure and function. MMPs are zinc-binding endopeptidases secreted as inactive proMMPs by fibroblasts, vascular smooth muscle (VSM) and leukocytes. ProMMPs are activated by various activators including other MMPs and proteinases. MMPs cause degradation of extracellular matrix (ECM) proteins such as collagen and elastin, and could have additional effects on the endothelium, as well as VSM cell migration, proliferation, Ca^{2+} signaling and contraction. Increased lower extremity hydrostatic venous pressure is thought to induce hypoxia inducible factors and other MMP inducers/activators such as EMMPRIN, prostanoids, chymase, and hormones, leading to increased MMPs expression/activity, ECM degradation, VSM relaxation, and venous dilation. Leukocyte infiltration and inflammation of the vein wall cause further increases in MMPs, vein wall dilation, valve degradation and different clinical stages of chronic venous disease (CVD) including varicose veins (VVs). VVs are characterized by ECM imbalance, incompetent valves, venous reflux, wall dilation, and tortuosity. VVs often show increased MMP levels, but may show no change or decreased levels, depending on the VVs region (atrophic regions with little ECM vs. hypertrophic regions with abundant ECM) and MMP form (inactive proMMP vs. active MMP). Management of VVs includes compression stockings, venotonics, and surgical obliteration or removal. Because these approaches do not treat the causes of VVs, alternative methods are being developed. In addition to endogenous tissue inhibitors of MMPs (TIMPs), synthetic MMP inhibitors have been developed, and their effects in treatment of VVs need to be examined.

Introduction

Veins are a large network of vessels that transfer deoxygenated blood from different tissues to the heart. In the lower extremity, an intricate system of superficial and deep veins is responsible for the transfer of blood against hydrostatic venous pressure. Superficial veins include the small saphenous vein, which is located in the back of the leg and runs from the ankle until it meets the popliteal vein at the saphenopopliteal junction, and the great saphenous vein, which is located in the medial side of the leg and runs from the ankle until it meets the common femoral vein at the saphenofemoral junction. Deep veins include the tibial, popliteal, femoral, deep femoral and common femoral veins (Recek, 2006). In all parts of the lower extremity other than the foot, blood flows from the superficial veins, which carry blood from the skin and subcutaneous tissue, to the deep veins, which are embedded in the muscles and carry blood from all other parts of the leg (Recek, 2006; Lim and Davies, 2009) (**Fig. 1**). The movement of blood from the superficial veins to deep veins and towards the heart is guided by bicuspid valves that protrude from the inner wall and ensure blood movement in one direction. Muscle contractions in the calf, foot and thigh also help to drive the blood towards the heart and against gravity and the high hydrostatic venous pressure, which could reach 90-100 mmHg at the ankle in the standing position (Recek, 2006).

Veins are relatively thin compared to arteries, but the vein wall still has three histological layers. The innermost layer, the tunica intima, is made of endothelial cells (ECs) which are in direct contact with blood flow. The tunica media contains a few layers of vascular smooth muscle (VSM), and is separated from the intima by the internal elastic lamina. The outermost layer, the adventitia, contains fibroblasts embedded in an extracellular matrix (ECM) of proteins such as collagen and elastin (Sansilvestri-Morel et al., 2007).

The ECM and other components of the vein wall are modulated by different ions, molecules, and enzymes. Matrix metalloproteinases (MMPs) are endopeptidases that are often recognized for their ability to degrade ECM components and therefore play a major role in venous tissue remodeling. MMPs may also affect bioactive molecules on the cell surface

and regulate the cell environment through G-protein coupled receptors (GPCRs). MMPs may promote cell proliferation, migration, and differentiation and could play a role in cell apoptosis, immune response, tissue healing, and angiogenesis. Alterations in MMPs expression and activity occur in normal biological processes such as pregnancy, but have also been implicated in vascular diseases such as atherosclerosis and aneurysms. MMPs also play a significant role in the pathogenesis of chronic venous disease (CVD) and varicose veins (VVs). VVs are a common health problem characterized by unsightly twisted and dilated veins in the lower extremities. If untreated, VVs may cause many complications including thrombophlebitis, deep venous thrombosis and venous leg ulcer, making it important to carefully examine the pathogenetic mechanisms involved in order to develop better treatment options.

In this review, we will use data reported in PubMed and other scientific databases as well as data from our laboratory to provide an overview of MMPs structure, substrates, and tissue distribution. We will then discuss CVD, the structural and functional abnormalities in VVs, the changes in MMPs levels, and the potential factors that could change MMPs expression/activity in VVs including venous hydrostatic pressure, inflammation, hypoxia, and other MMPs activators and inducers. We will also discuss the mechanisms of MMPs actions and how they could cause ECM abnormalities, and EC and VSM dysfunction leading to venous dilation and VVs formation. We will end the review by describing some of the current approaches used for treatment of VVs, and explore the potential benefits of endogenous TIMPs and synthetic MMP inhibitors as new tools for the management of VVs.

MMPs Structure

MMPs were discovered in the early 1960s as a collagen proteolytic activity during the ECM protein degradation associated with resorption of the tadpole tail (Gross and Lapiere, 1962). MMPs are endopeptidases, also called matrixins, and belong to the metzincins superfamily of proteases. MMPs are highly homologous, multidomain, zinc (Zn^{2+}) dependent metalloproteinases that degrade various components of ECM. Generally, MMPs have a

propeptide sequence, a catalytic metalloproteinase domain, a hinge region (linker peptide), and a hemopexin domain (**Fig. 2**). Additionally, all MMPs have: 1) Homology to collagenase-1 (MMP-1), 2) A prodomain cysteine switch motif PRCGXPD whose cysteine sulfhydryl group chelates the active site Zn^{2+} , keeping MMPs in the proMMP zymogen form, and 3) A Zn^{2+} -binding motif to which Zn^{2+} is bound by 3 histidines from the conserved sequence HEXXHXXGXXH, a conserved glutamate, and a conserved methionine from the sequence XBMX (Met-turn) that is located 8-residues downstream from the Zn^{2+} binding motif and supports the structure surrounding the catalytic Zn^{2+} (Bode et al., 1999; Visse and Nagase, 2003) (**Fig. 3**).

Since its discovery, the MMP family has grown to 28 members in vertebrates, at least 23 in humans, and 14 in blood vessels (Visse and Nagase, 2003) (**Table 1**). Based on their substrates and the organization of their different domains, MMPs are classified into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs, and other MMPs. Different classes of MMPs may have specific structural features that may vary from the prototypical MMP structure (Pei et al., 2000; English et al., 2001; Kucukguven and Khalil, 2013) (**Fig. 2**).

Pro- and Active MMPs

Matrixins are synthesized as pre-proMMPs, and the signal peptide is removed during translation to generate proMMPs. In proMMPs, the cysteine from the PRCGXPD 'cysteine switch' motif coordinates with the catalytic Zn^{2+} to keep proMMP inactive (Nagase 2006). In order to activate proMMPs, the cysteine switch is cleaved and the hemopexin domain is detached, often by other proteinases or MMPs (Nagase et al., 2006). Furin-containing MMPs such as MMP-11, -21, and -28 and MT-MMPs have a furin-like pro-protein convertase recognition sequence at the C-terminus of the propeptide and are activated intracellularly by the endopeptidase furin (Pei and Weiss, 1995) (**Fig. 2**). Following their intracellular activation by furin, MT-MMPs proceed to activate other proMMPs on the cell surface (English et al.,

2001). For example, activation of proMMP-2 occurs on the cell surface, where it first binds via its carboxyl terminus to an integrin or MMP-14-TIMP-2 complex thus allowing the catalytic site of MMP-2 to be exposed, then is cleaved and activated by MT-MMPs. Due to its cell surface activation, MMP-2 may accumulate pericellularly and induce substantial localized collagenolytic activity (Visse and Nagase, 2003). Stromelysins MMP-3 and -10 are also secreted from cells as proMMPs and activated on the cell surface. MMPs can also be activated by heat, low pH, thiol-modifying agents such as 4-aminophenylmercuric acetate, mercury chloride, N-ethylmaleimide, oxidized glutathione, sodium dodecyl sulfate, and chaotropic agents by interfering with the cysteine-Zn²⁺ coordination at the cysteine switch. Other specific MMP activators include plasmin for MMP-9, both MMP-3 and hypochlorous acid for MMP-7, and MMP-7 for MMP-1 (Kucukguven and Khalil, 2013).

MMP Substrates

MMPs play a major role in tissue remodeling by promoting turnover of ECM proteins. ECM is the extracellular component of mammalian tissue that provides support and anchorage for cells, segregates tissues from one another, regulates cell movement and intercellular communication, and provides a local depot for cellular growth factors. ECM consists mainly of fibers, proteoglycans and polysaccharides. Fibers are mostly glycoproteins and include collagen, the main extracellular protein, and elastin, which is exceptionally unglycosylated and provides flexibility for the skin, arteries and lungs. Proteoglycans contain more carbohydrates than proteins, attract water to keep the ECM hydrated, and bind and store growth factors. Syndecan-1 is a proteoglycan and integral transmembrane protein that binds chemotactic cytokines during the inflammatory process. Laminin is found in the basal lamina of epithelia, and fibronectin binds cells to ECM, modulates the cytoskeleton and facilitates cell movement. ECM contains other proteins and glycoproteins such as vitronectin, aggrecan, entactin, tenascin, and fibrin; polysaccharides such as hyaluronic acid; and proteolytic enzymes that continuously cause turnover of ECM proteins (Kucukguven and Khalil, 2013).

ECM and its protein components provide the structural scaffolding for blood vessel support, cell migration, differentiation and signaling, as well as epithelialization and wound repair. Collagen and elastin are essential for vein wall structural integrity and are important MMP substrates. Collagen type I, II, III, IV, V, VI, VII, VIII, IX, X, and XIV are broken down by MMPs with different efficacies. Other MMP substrates include the ECM proteins fibronectin, vitronectin, laminin, entactin, tenascin, aggrecan, and myelin basic protein (**Table 1**). Casein is not a physiological MMP substrate, but is digested by a wide range of proteinases and is, therefore, used to measure MMP activity (Kucukguven and Khalil, 2013).

The hemopexin domain confers much of the substrate specificity for MMPs (Patterson et al., 2001; Suenaga et al., 2005) and is essential in the degradation of fibrillar collagen, while the catalytic domain is essential in degrading non-collagen substrates (Visse and Nagase, 2003). MMPs catalytic activity generally requires Zn^{2+} and a water molecule flanked by three conserved histidine residues and a conserved glutamate, with a conserved methionine acting as a hydrophobic base to support the structure surrounding the catalytic Zn^{2+} in the MMP molecule. In the initial transition states of the MMP-substrate interaction, Zn^{2+} is penta-coordinated with a substrate's carbonyl oxygen atom, one oxygen atom from the MMP glutamate-bound water (H_2O), and the three MMP conserved histidines. The Zn^{2+} -bound water then performs a nucleophilic attack on the substrate, resulting in the release of a water molecule and breakdown of the substrate (**Fig. 3**) (Bode et al., 1999; Pelmeshnikov and Siegbahn, 2002; Park et al., 2003; Jacobsen et al., 2010). Other alternative transition states during MMP-substrate interaction have been hypothesized whereby Zn^{2+} is penta-coordinated with a substrate's carbonyl oxygen atom, two oxygen atoms from the MMP conserved glutamate, and two of the three conserved histidines. One oxygen from glutamate then performs a nucleophilic attack on the substrate, causing substrate degradation (Manzetti et al., 2003). Peptide catalysis also depends on specific subsites or pockets (S) within the MMP molecule that interact with corresponding substituents (P) in the substrates. The MMP S1' pocket is the most important for substrate specificity and binding, is extremely variable, and

may be shallow, intermediate, or deep (Bode et al., 1999; Park et al., 2003; Jacobsen et al., 2010). MMP-1 and -7 have shallow S1' pockets, MMP-2, -9, and -13 have intermediate S1' pockets, and MMP-3, -8, and -12 have deep S1' pockets (Park et al., 2003). S2' and S3' are shallower and, therefore, more solvent exposed than S1' (Jacobsen et al., 2010). The S3 pocket is also important for substrate specificity, second to S1' (Nagase et al., 2006).

Degradation of particular substrates is carried out by specific classes of MMPs. Stromelysin-1 and -2 (MMP-3 and -10) do not cleave interstitial collagen but digest several ECM components and participate in proMMP activation. MMP-3 has similar substrate specificity but higher proteolytic efficiency than MMP-10. While stromelysin-3 (MMP-11) does not cleave interstitial collagen, it has very weak activity toward ECM molecules, and is distantly related to MMP-3 and -10 (Pei and Weiss, 1995). Also, certain ECM proteins may require the cooperative effects of several MMPs for complete degradation. For example, collagenases MMP-1, -13, and -18 unwind triple helical collagen and hydrolyze the peptide bonds of fibrillar collagen type I, II and III into $\frac{3}{4}$ and $\frac{1}{4}$ fragments (Chung et al., 2004; Nagase et al., 2006) and the resulting single α -chain gelatins are then further degraded into oligopeptides by gelatinases MMP-2 and -9 (Patterson et al., 2001). Gelatinases have three type-II fibronectin repeats in the catalytic domain that bind gelatin, collagen, and laminin (**Fig. 2**). While MMP-2 is a gelatinase, it can also function as a collagenase, acting much like MMP-1 but in a weaker manner (Nagase et al., 2006). Thus, MMP-2 degrades collagen in two stages; interstitial collagenase-like degradation followed by gelatinolysis promoted by the fibronectin-like domain (Aimes and Quigley, 1995). Similarly, MMP-9 is both a collagenase and gelatinase. As a collagenase, MMP-9 binds $\alpha 2$ chains of collagen IV with high affinity even when it is inactive (Olson et al., 1998), making the substrate rapidly available.

Tissue Distribution of MMPs

MMPs are produced by different tissues and cells (**Table 1**). Dermal fibroblasts and leukocytes are major sources of MMPs, especially MMP-2 (Saito et al., 2001), and platelets

are important sources of MMP-1, -2, -3, and -14 (Seizer and May, 2013). In general, MMPs are either secreted from cells or anchored to the plasma membrane by heparan sulfate glycosaminoglycans (Visse and Nagase, 2003). MMPs, including MMP-1, -2, -3, -7, -8, -9, -12, -13, and MT1-MMP and MT3-MMP, are expressed in vascular cells and tissues (Chen et al., 2013). In the rat inferior vena cava (IVC), MMP-2 and -9 are localized in different layers of the venous wall, suggesting interaction with ECs, VSM, and ECM (Raffetto et al., 2008). Other studies showed MMP-1, -2, -3, and -7 in ECs and VSMCs, MMP-2 in the adventitia (Sansilvestri-Morel et al., 2007), MMP-9 in ECs, medial VSMC, and adventitial microvessels, and MMP-12 in VSMCs and fibroblasts of human great saphenous vein (Woodside et al., 2003). Changes in MMPs expression, distribution, or activity could alter vein wall structure and function, and persistent changes in MMPs could contribute to the pathogenesis of CVD.

Chronic Venous Disease (CVD)

CVD is a common disease of the lower extremity with significant social and economic implications. According to CEAP (clinical-etiology-anatomy-pathophysiology) classification, CVD has seven clinical stages, C0-6, with C0 indicating no visible signs of CVD, C1 telangiectasies (spider veins), C2 VVs, C3 edema, C4a skin pigmentation or eczema, C4b lipodermatosclerosis or atrophie blanche, C5 healed ulcer, and C6 active ulcer (**Fig. 4**). The term chronic venous insufficiency (CVI) is often used for the advanced stages of CVD, C4-6 (Eklof et al., 2004).

VVs are a common venous disorder affecting over 25 million adults in the United States (Beebe-Dimmer et al., 2005). VVs are abnormally distended and twisted superficial lower extremity veins that often show incompetent valves, venous reflux, and wall dilation (**Fig. 1**). VVs pathology, however, may not be confined to the lower extremities and could affect other tissues in a generalized fashion. In patients with VVs, arm veins also show increased distensibility, suggesting a systemic disease of the vein wall (Zsoter and Cronin, 1966). In

addition to their unsightly cosmetic appearance, VVs can cause complications such as thrombophlebitis, deep venous thrombosis, and venous leg ulcers (Eklof et al., 2004).

Risk factors for VVs include age, gender, pregnancy, estrogen therapy, obesity, phlebitis, and prior leg injury. Females may have enhanced estrogen receptor-mediated venous relaxation and more distended veins as compared to men (Raffetto et al., 2010b). Pregnancy-associated increases in uterine size and body weight along with hemodynamic changes could contribute to increased venous pressure in the lower extremities and the development of VVs. Other environmental and behavioral factors such as prolonged standing (Mekky et al., 1969) and a sedentary lifestyle could increase the risk for CVD (Jawien, 2003; Lacroix et al., 2003).

Family history and genetic and hereditary factors could also contribute to the risk for VVs (Lee et al., 2005). Primary lymphedema-distichiasis, a rare syndrome characterized by a mutation in the FOXC2 region of chromosome 16, is associated with VVs at an early age (Ng et al., 2005). VVs have been linked to the D16S520 marker on chromosome 16q24 in nine families, and the localization of the marker near FOXC2 supports linkage between VVs and FOXC2, and provides evidence that CVD could be inherited in an autosomal dominant mode with incomplete penetrance (Serra et al., 2012). Also, patients with Klippel-Trenaunay Syndrome often have VVs, supporting heritability of VVs (Delis et al., 2007). Other evidence for a genetic component of VVs include: a heterozygous mutation in the Notch3 gene identified in the CASADIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) pedigree with VVs (Saiki et al., 2006); a microarray analysis of 3,063 human cDNAs from VVs showing upregulation of 82 genes, particularly those regulating ECM, cytoskeletal proteins, and myofibroblasts production (Lee et al., 2005); individuals with Ehlers-Danlos syndrome type-IV being prone to vascular pathology and VVs (Badauy et al., 2007); identification of single nucleotide polymorphisms in the promoter region of MMP-9 gene among Chinese patients with VVs, with a 1562 C to T substitution associated with increased MMP-9 promoter activity and plasma levels (Xu et al., 2011); and the reduction of the elasticity of the lower limb vein wall in children of VVs patients (Reagan and Folse, 1971).

Structural and Functional Abnormalities in VVs

Although VVs often appear as engorged, dilated, and twisted veins, structurally, they may have both atrophic and hypertrophic regions (**Fig. 4**). Atrophic regions have a decrease in ECM and possible increase in inflammatory cells, while hypertrophic regions may show abnormal VSMC proliferation and even ECM accumulation (Mannello et al., 2013). Histologically, VVs lack clear boundaries between the intima, media, and adventitia. In VVs, VSMCs are disorganized, jammed into the intima and media, and surrounded by abundant unstructured material. Elastic fibers are thickened and fragmented in the intima and adventitia, and collagen is disorganized making it difficult to distinguish between the media and adventitia (Sansilvestri-Morel et al., 2007).

VVs and adjacent veins show imbalance in ECM protein content mainly due to changes in collagen and/or elastin. Measurements of collagen content in VVs walls have shown variable results ranging from an increase (Gandhi et al., 1993), to a decrease (Haviarova et al., 1999), or no change (Kockx et al., 1998). Both cultured dermal fibroblasts from patients with CVD and cultured VSMCs from VVs show increased synthesis of type I collagen but decreased synthesis of type III collagen, despite normal gene transcription, suggesting that VVs patients may have a systemic abnormality in collagen production and post-translational inhibition of type III collagen synthesis in various tissues. Because type III collagen is important for blood vessels elasticity and distensibility, alterations in collagen synthesis and the collagen type I to type III ratio may affect vein wall architecture and lead to structural weakness, venous dilation, and VVs formation (Sansilvestri-Morel et al., 2007). Decreased elastin content has also been observed in VVs and may play a role in the pathogenesis of the disease, as decreased elasticity could lead to vein wall dilation (Venturi et al., 1996). However, some studies showed increased elastic networks in VVs (Sansilvestri-Morel et al., 2007).

VVs also show incompetent valves, but whether valve dysfunction precedes or follows the changes in VVs wall is currently debated. One view suggests that valve dysfunction leads to

higher venous pressure, which causes vein wall damage and dilation. The vein wall dilation would then further distort the valves leading to further increases in venous pressure and wall dilation. In support of this view, VVs show valve hypertrophy, increased width of the valvular annulus (Corcos et al., 2000), decreased collagen content and reduced viscoelasticity (Psaila and Melhuish, 1989), as well as increased monocyte and macrophage infiltration and inflammation in the valvular sinuses compared to distal VVs walls (Ono et al., 1998). However, venous dilation and VVs are often seen below competent valves (Naoum et al., 2007). Also, increased collagen and decreased elastin have been demonstrated in both VVs and competent saphenous vein segments in proximity to varicosities, suggesting that ECM imbalance may occur in the vein wall prior to valve insufficiency (Gandhi et al., 1993). These observations led to the suggestion that primary vein wall dilation may cause the valves to become distorted and dysfunctional, leading to venous reflux and higher venous pressure, ultimately causing more venous dilation and VVs (Raffetto and Khalil, 2008b). In support of this view, VVs do not always develop in a descending direction (from thigh, calf, to ankle), and antegrade VVs progression (normal direction of venous flow) may be caused by changes in the vein wall that lead to valve dysfunction (Naoum et al., 2007). Regardless of which pathological event occurs first, both wall dilation and valve dysfunction would lead to vein dysfunction and abnormal blood flow, with blood moving away from the heart during reflux (backflow) that typically lasts longer than 0.5s in superficial VVs (Raffetto and Khalil, 2008b).

MMP Levels in VVs

Several studies have shown increased MMP levels in VVs. One study showed increased MMP-1, -2, -3, and -7 levels in VVs, with a particular increase in MMP-2 activity (Sansilvestri-Morel et al., 2007). Another study showed increased MMP-1 protein levels in the great saphenous vein (GSV), along with increased MMP-1 and -13 in proximal vs. distal VVs segments, with no change in MMPs mRNA levels, suggesting that the increased MMPs protein levels may be caused by changes in MMP post-transcriptional modification and protein

degradation (Gillespie et al., 2002). MMP levels also vary in different layers of the VVs wall. Immunohistochemical studies in VVs showed localization of MMP-1 to fibroblasts, VSMCs, and ECs, MMP-9 to ECs and adventitial microvessels with increased levels in medial VSMCs, and MMP-12 to VSMCs and fibroblasts (Woodside et al., 2003). MMPs localization to the adventitia and fibroblasts is consistent with MMPs role in ECM degradation. Other studies showed increased MMP-2 levels in all layers, and MMP-1, -3 and -7 in the intima and media of VVs (Sansilvestri-Morel et al., 2007).

Despite studies showing increased MMPs in VVs, some studies showed no change or even a decrease in specific MMPs. In one study, active MMP-1 and total MMP-2 decreased, while total MMP-1 did not change in VVs (Gomez et al., 2014). Interestingly, measurements of collagen content in VVs wall also showed variable results ranging from an increase (Gandhi et al., 1993), no change (Kockx et al., 1998), to decrease (Haviarova et al., 1999). The variability in MMPs levels may be caused by examining VVs segments from different regions i.e. atrophic versus hypertrophic regions, or veins from patients at different stages of CVD, or failure to distinguish between pro- and active forms of MMPs.

MMP activity can be modulated by multiple factors *in vivo* and *ex vivo*. Some of these factors include increased venous hydrostatic pressure, inflammation, hypoxia, and oxidative stress. These factors may promote MMPs activity in VVs.

Venous Hydrostatic Pressure and MMPs in VVs

Increased venous hydrostatic pressure is one of the factors that could increase MMPs levels/activity in VVs (**Fig. 4**). Increased mechanical stretch or pressure in human tissues is associated with increased expression of specific MMPs in fibroblasts, ECs and VSMCs (Asanuma et al., 2003). Also, in isolated rat IVC, prolonged increases in vein wall tension are associated with decreased vein contraction and increased levels of MMP-2 and -9 in the intima and MMP-9 in the media. The decreased contraction was prevented in IVC pretreated with MMP inhibitors, supporting a role of MMPs as a link between increased venous pressure/wall

tension, decreased vein contraction, and increased venous dilation (Raffetto et al., 2008). Increased venous pressure may increase venous MMP expression by inducing intermediary factors such as inflammation and hypoxia inducible factors (HIFs).

Inflammation and MMPs in VVs

Increased venous pressure may induce EC permeability and injury, leukocyte attachment and infiltration, and inflammation (Schmid-Schonbein et al., 2001). In a rat model of femoral arteriovenous fistula, the resulting pressurized saphenous vein showed increased leukocyte infiltration and inflammation with increased expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1) (Takase et al., 2004). Leukocytes are a major source of MMPs (Saito et al., 2001). Increased adhesion molecules could further facilitate leukocyte adhesion and infiltration, causing further inflammation and increased MMP levels in the vein wall. MMPs in turn cause ECM degradation, vein wall weakening, and wall/valve fibrosis, leading to vein wall dilation, valve dysfunction, further increases in venous pressure, and CVD (Kucukguven and Khalil, 2013) (**Fig. 4**). The pressure/inflammation/MMPs pathway may occur mostly in atrophic regions of VVs, as indicated by the increased ECM degradation in these regions.

Studies have shown increased monocyte/macrophage infiltration in the walls and valves of saphenous vein specimens from patients with CVD (Ono et al., 1998; Sayer and Smith, 2004). VVs show increased infiltration of inflammatory cells and expression of vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1 in ECs (Aunapuu and Arend, 2005). Also, the plasma levels of endothelial and leukocyte activation markers including ICAM-1, VCAM-1, angiotensin converting enzyme, and L-selectin are increased in patients with VVs, and are associated with increased plasma proMMP-9, suggesting polymorphonuclear activation and granule release in response to postural blood stasis in VVs (Jacob et al., 2002).

Pressure-induced leukocyte infiltration and inflammation could increase MMPs by increasing the release of cytokines. Urokinase may be involved in the inflammatory reaction and could upregulate tumor necrosis factor- α (TNF- α) expression in damaged vessels. TNF- α

increases MMP-9 promoter activity in plasmids, likely through activator protein-1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) or specificity protein-1 (Sp-1) (Sato et al., 1993). Likewise, interleukins such as IL-17 and -18 induce MMP-9 expression via NF- κ B and AP-1 pathways (Reddy et al., 2010).

Cytokines may also augment MMPs expression by increasing reactive oxygen species (ROS). MMPs expression in fibroblasts depends on NADPH oxidase-1 (Nox-1) expression (Arbiser et al., 2002). Also, urokinase may regulate MMP-9 expression by increasing ROS generation in fibroblasts (Zubkova et al., 2014). In addition to producing MMPs, leukocytes generate oxidants that can both activate (via oxidation of the prodomain thiol, followed by autolytic cleavage) and inactivate MMPs (via modification of amino acids critical for catalytic activity), providing a mechanism to control bursts of proteolytic activity (Fu et al., 2004).

Hypoxia and MMPs in VVs

Increased venous pressure may augment MMP levels and reduce venous contraction via a pathway involving HIFs (**Fig. 5**). HIFs are nuclear transcriptional factors that regulate genes involved in oxygen homeostasis. The expression and activity of HIFs may also be regulated by mechanical stretch. For instance, HIF-1 α and -2 α mRNA and protein levels are increased in rat capillary ECs of skeletal muscle fibers exposed to prolonged stretch (Milkiewicz et al., 2007), and HIF-1 α protein increases in response to mechanical stress in the rat ventricular wall (Kim et al., 2002). Also, our laboratory has shown that prolonged increases in rat IVC wall tension are associated with increased HIF-1 α and -2 α mRNA, increased MMP-2 and -9 mRNA expression, and reduced contraction that is reversed by the HIF inhibitors U0126 and echinomycin and enhanced with the HIF stabilizer dimethylxaloylglycine (DMOG) (Lim et al., 2011) (**Fig. 5**). The mechanism by which mechanical stretch regulates HIFs may involve phosphoinositide 3-kinase (PI₃K) and mitogen-activated protein kinase (MAPK). Mechanical stretch may stimulate PI₃K by activating Ca²⁺ influx through transient receptor potential ion channels such as TRPV4 (Thodeti et al., 2009). Mechanical stretch may also be transduced by

integrins to activate signaling cascades that ultimately cause MAPK activation. MAPK could also be activated via stretch-induced stimulation of receptor tyrosine kinases or GPCRs or generation of ROS in response to biomechanical stress. The role of MAPK is supported by the observation that the increase in HIF mRNA expression and the reduction in IVC contraction associated with prolonged vein wall stretch are reversed in veins treated with MAPK inhibitors (Lim et al., 2011). Additional evidence supporting the role of HIFs as factors linking increased venous pressure and MMP expression in VVs is that HIF-1 α and -2 α transcription factors and HIF target genes are upregulated in VVs (Lim et al., 2012) and that the expression and activity of MMP-2 and -9 can be regulated by HIF-1 α in hemodialysis grafts and arteriovenous fistulas (Misra et al., 2008). In addition to mechanical stretch, low oxygen tension, hormones, cytokines, heat, and low pH may also induce HIF expression and, in turn, increase MMP levels.

Other Potential MMP Activators in VVs

Other MMP activators have been identified in veins and may promote MMP activity in VVs. Extracellular MMP inducer (EMMPRIN, CD147, Basigin) is a widely expressed membrane protein of the immunoglobulin superfamily that has been implicated in tissue remodeling and various pathological conditions including cancer, rheumatoid arthritis, heart failure, atherosclerosis and aneurysm. Interestingly, high volume mechanical ventilation causes acute lung injury and upregulation of MMP-2, MMP-9, MT1-MMP and EMMPRIN mRNA (Foda et al., 2001). Also, EMMPRIN, MMP-2, MT1-MMP and MT2-MMP are overexpressed in dermal structures of venous leg ulcers, which would favor unrestrained MMP activation and ECM turnover (Norgauer et al., 2002).

Prostanoids (PG) are bioactive lipids produced by most blood and vascular cells and may play a role in VVs. Prostaglandin E2 (PGE2) controls vascular tone, inflammation and vascular wall remodeling through activation of EP1-4 receptor subtypes (Majed and Khalil, 2012). PGE2 stimulation of EP2/EP4-receptors causes MMP activation in human endometriotic epithelial

and stromal cells (Lee et al., 2011). PGE₂ synthesis may be decreased in VVs due to an increase in anti-inflammatory 15-deoxy-delta-12,14-PGJ₂ (15d-PGJ₂), a decrease in the synthesizing enzyme membrane-associated prostaglandin E synthase-1 (mPGES-1), and an increase in the degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH), with an overall decrease in both PGE₂ and its receptor EP₄, leading to decreased MMP-1 and -2 activity and collagen accumulation that may account for the intimal hyperplasia observed in some regions of VVs (Gomez et al., 2014) (**Fig. 4**).

Other MMP activators include chymase, a chymotrypsin-like serine protease purified from mast cell granules and cardiovascular tissues in mammals, that may be involved in increasing MMP-9 activity and monocyte/macrophage accumulation in the aorta of stroke-prone spontaneously hypertensive rats (Takai et al., 2014). Also, hormones such as estrogen and progesterone increase the levels and activity of MMP-2 and -9 in uterine and aortic tissues (Yin et al., 2012; Dang et al., 2013). Some MMP modulators could enhance MMP activity by preventing MMP degradation. For instance, neutrophil gelatinase-associated lipocalin (NGAL) forms a complex with MMP-9 and thereby protects it from proteolytic degradation and increases its levels (Serra et al., 2014). The list of MMP activators is growing and whether these activators are increased in VVs needs to be examined.

Mechanisms of MMP Actions in VVs

While MMPs are generally recognized for their proteolytic effects on ECM proteins, MMPs could also contribute to the pathogenesis of VVs by affecting different components of the vein wall and different cellular and molecular pathways in VSMCs and the endothelium.

MMPs and ECM Abnormalities in VVs

Changes in MMP activity alter the composition of ECM and, in turn, contribute to structural and functional abnormalities in VVs. While it is generally believed that MMP levels increase in VVs, decreased MMP levels have also been observed (Gomez et al., 2014), and this may

partially explain the different pathologies in the atrophic vs. hypertrophic regions of VVs. An increase in MMP activity is predicted to degrade and decrease ECM in atrophic regions of VVs (Mannello et al., 2013). On the other hand, a decrease in MMP activity may lead to ECM accumulation in the media of hypertrophic regions of VVs, which could interfere with the contractile function of VSMCs, thus hindering venous contraction and leading to venous dilation and VVs (Badier-Commander et al., 2000) (**Fig. 4**).

Changes in ECM content in VVs often involve MMP-induced degradation of major ECM proteins such as collagen and elastin. Collagen type III and fibronectin are decreased in cultured VSMCs from VVs likely due to post-transcriptional degradation by MMP-3 (Sansilvestri-Morel et al., 2005). Elastin levels are also decreased in VVs possibly due to increased elastolytic activity of MMPs or other elastases produced by macrophages, monocytes, platelets, and fibroblasts (Venturi et al., 1996). The changes in collagen and elastin content in VVs are dynamic processes that may depend on the stage of CVD. For instance, increased collagen content may compensate for decreased elastin levels at early stages of VVs, while collagen levels may decrease at later stages of the disease. This may explain the divergent reports regarding collagen content in VVs, where some studies showed a decrease (Haviarova et al., 1999), while other studies showed hardly any change (Venturi et al., 1996; Kockx et al., 1998) or even an increase (Gandhi et al., 1993).

MMPs and VSM Dysfunction in VVs

In addition to changes in ECM, VVs may show alterations in VSMC growth, proliferation, migration, and contractile function (**Fig. 5**). When compared with SMCs from normal veins, SMCs from VVs are more dedifferentiated and show increased migration, proliferation, and MMP-2 production, which may contribute to vein wall remodeling and weakening against increased hydrostatic pressure (Xiao et al., 2009). MMP-1 and -9 levels have also been shown to increase human aortic SMC migration (Jin et al., 2008; Shi et al., 2010). MMP-induced ECM proteolysis may modulate cell-matrix adhesion either by removal of sites of adhesion or by

exposing a binding site and in turn facilitate VSMC migration. MMPs can also facilitate VSMC proliferation by promoting permissive interactions between VSMCs and components of ECM, possibly via integrin-mediated pathways (Morla and Mogford, 2000). MT1-MMP may stimulate the release of transforming growth factor- β (TGF- β) and promote the maturation of osteoblasts (Karsdal et al., 2002). Also, upregulation of MMP-2 increases the expression of VEGFa, while downregulation of MMP-2 decreases VEGFa expression in human gastric cancer cell line SNU-5 (Mao et al., 2014). MMP-induced release of growth factors may involve cleaving the growth factor-binding proteins or matrix molecules, and may partly explain the VSMC proliferation observed in hypertrophic regions of VVs (Zhang et al., 2004).

While MMPs stimulate growth factor release, MMPs may be regulated by growth factors (Hollborn et al., 2007). For example, overexpression of VEGFa in SNU-5 cells increases MMP-2 expression, while downregulation of VEGFa decreases MMP-2 expression (Mao et al., 2014). Also, platelet derived growth factor-BB (PDGF-BB) increases MMP-2 expression in rat VSMCs, possibly via Rho-associated protein kinase, extracellular signal-regulated kinases, and p38 MAPK phosphorylation (Cui et al., 2014). Also, in a study on carotid plaques, EGF upregulated MMP-9 activity and increased MMP-1, -9, and EGFR mRNA transcripts in VSMCs (Rao et al., 2014). In contrast, TGF- β 1 may downregulate MMPs via a TGF- β 1 inhibitory element in the MMP promoter. Interestingly, MMP-2 does not have this element, and TGF- β 1 may upregulate MMP-2.

Because proliferating and synthetic VSMCs do not contract, the MMP and growth factor-mediated VSMC proliferation is expected to decrease venous contraction and promote dilation (**Fig. 5**). MMPs may also affect VSM contraction mechanisms. VSM contraction is triggered by increases in Ca^{2+} release from intracellular stores and Ca^{2+} entry from the extracellular space. MMPs do not inhibit phenylephrine-induced aortic contraction in Ca^{2+} -free solution, suggesting that they do not inhibit the Ca^{2+} release mechanism (Chew et al., 2004). On the other hand, MMP-2 and -9 cause aortic relaxation by inhibiting Ca^{2+} influx (Chew et al., 2004), and MMP-2 inhibits Ca^{2+} -dependent contraction in rat IVC (Raffetto et al., 2010a). It has been proposed

that during substrate degradation MMPs may produce Arg-Gly-Asp (RGD)-containing peptides, which could bind to $\alpha_v\beta_3$ integrin receptors and inhibit Ca^{2+} entry into VSM (Waitkus-Edwards et al., 2002). This is unlikely as RGD peptides do not affect IVC contraction (Raffetto et al., 2010a). The mechanism by which MMPs inhibit Ca^{2+} entry could involve direct effects on Ca^{2+} or K^+ channels. In rat IVC, MMP-2 induced relaxation is abolished in high KCl depolarizing solution, which prevents K^+ ion from moving out of the cell via K^+ channels. Importantly, blockade of large conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) by iberiotoxin inhibited MMP-2 induced IVC relaxation, suggesting that MMP-2 actions may involve hyperpolarization and activation of BK_{Ca} , which in turn lead to decreased Ca^{2+} influx through voltage-gated Ca^{2+} channels (Raffetto et al., 2007) (**Fig. 5**). The MMP-induced inhibition of venous tissue Ca^{2+} influx and contraction may lead to venous dilation and VVs.

MMPs and Endothelium-Dependent Relaxation

The endothelium controls vascular tone by releasing relaxing factors including nitric oxide (NO) and prostacyclin (PGI_2) and through hyperpolarization of the underlying VSMCs by endothelial-derived hyperpolarizing factor (EDHF) (Feletou and Vanhoutte, 2006). MMPs may stimulate protease activated receptors (PARs), which are GPCRs that may play a role in venous dilation in VVs (**Fig. 5**). PARs 1-4 have been identified in humans, and MMP-1 has been shown to activate PAR-1 (Boire et al., 2005). PAR-1 is expressed in VSMCs (McNamara et al., 1993), ECs, and platelets (Coughlin, 2000) and is coupled to increased NO production (Garcia et al., 1993), which could contribute to venous dilation and VVs formation.

EDHF-mediated relaxation may involve the opening of small and intermediate conductance Ca^{2+} -activated K^+ channels and hyperpolarization of ECs. EC hyperpolarization may spread via myoendothelial gap junctions causing relaxation of VSMCs. EDHF could also cause hyperpolarization through opening of BK_{Ca} in VSM (Feletou and Vanhoutte, 2006). MMP-2 may increase EDHF release and enhance K^+ efflux via BK_{Ca} , leading to venous tissue hyperpolarization and relaxation (Raffetto et al., 2007). However, MMP-3 upregulation may

impair endothelium-dependent vasodilation (Lee et al., 2008), making it important to further examine the effects of MMPs on EDHF.

Management of VVs

The first line of management of VVs includes graduated compression stockings, which help relieve edema and pain and control progression to the more severe forms of CVI with skin changes and ulceration (**Fig. 4**). Compression stockings could also prevent venous thromboembolism following surgery, although they may not improve deep vein hemodynamics. Pharmacological treatment of VVs using venotonic drugs may improve venous tone, capillary permeability, and leukocyte infiltration. Venotonic drugs include naturally occurring plant extracts and glycosides such as α -benzopyrones (coumarins), γ -benzopyrones (flavonoids), saponosides (escin, horse chestnut seed extract, ruscus extract, *Centella asiatica*), and plant extracts (blueberry and grape seed, *Ginkgo biloba*, ergots) (Kucukguven and Khalil, 2013). Benzopyrones include esclatin, umbelliferone, dicoumarols, diosmin (Daflon-500), quercetin, oxerutin, troxerutin, rutosides, hesperitin, hesperidine, catechin (Green tea), flavonoic acid, and venoruton (Raffetto and Khalil, 2008a). Diosmin is a flavonoid and an active ingredient in Daflon-500 that may improve venous tone, microvascular permeability, lymphatic activity, and microcirculatory nutritive flow (Le Devehat et al., 1997). Rutosides enhance endothelial function in patients with CVI (Cesarone et al., 2006). Flavonoids may affect the endothelium and leukocytes and reduce edema and inflammation. Saponosides, such as escin (horse chestnut seed extract), may limit venous wall distensibility and morphologic changes by causing Ca^{2+} dependent venous contraction (Raffetto and Khalil, 2011). Other compounds tested in advanced CVI include pentoxifylline functioning through unknown mechanisms, and red vine leaves (AS 195) and PGE1, which may increase microcirculatory blood flow and transcutaneous oxygen tension and alleviate edema (Kucukguven and Khalil, 2013).

Sclerotherapy under duplex ultrasound guidance is another approach to obliterate dilated VVs and improve venous hemodynamics. Concentrated sclerosing agents such as hypertonic

saline, sodium morrhuate, and ethanolamine oleate have side effects and are not frequently used. Sodium tetradecyl sulfate (STS), which is a liquid or foam detergent, sodium morrhuate, and polidocanol are FDA-approved sclerosing agents for treatment of VVs (Mann, 2011).

Surgical approaches for treatment of VVs include endovenous ablation with a radiofrequency or infrared laser typically at wavelengths 810 to 1320 nm, but as high as 1470 and 1550 nm. The high intensity endoluminal thermal heat causes endothelial protein denaturation and vein occlusion (Proebstle et al., 2002). Laser treatment has relatively good outcome and occlusion rates with only a 2% recanalization rate 4 years after radiofrequency therapy (Merchant et al., 2005) and 3-7% VVs recurrence rate 2-3 years after infrared laser therapy (Min et al., 2003). Other approaches include surgical stripping of the saphenous vein with high ligation of the saphenofemoral junction. Large VVs clusters that communicate with the incompetent saphenous vein can also be avulsed by “stab phlebectomy”. Transilluminated power phlebectomy is an alternative to open phlebectomy in removing clusters of varicosities, as it uses fewer incisions and less operation time (Aremu et al., 2004).

While there are some treatment options for VVs, these treatments focus on the symptoms rather than the causes of the venous disorder. The identification of the role of MMPs in the pathogenesis of CVD has prompted the search for inhibitors of MMP expression or activity. MMP inhibitors (MMPi) can be used to prevent MMPs from binding with their substrates and degrading the ECM, and thereby prevent the development or recurrence of VVs. MMPi include endogenous inhibitors such as TIMPs and α 2-Macroglobulin, as well as synthetic Zn²⁺-dependent and Zn²⁺-independent inhibitors.

TIMPs and TIMP/MMP Ratio

TIMPs are endogenous, naturally occurring MMPi that bind MMPs in a 1:1 stoichiometry (Bode et al., 1999; Nagase et al., 2006) (**Fig. 6**). Four homologous TIMPs, TIMP-1, -2, -3 and -4, have been identified. TIMP-1 and -3 are glycoproteins, while TIMP-2 and -4 do not contain carbohydrates. TIMPs have poor specificity for a given MMP, and each TIMP can inhibit

multiple MMPs with different efficacy. For example, while TIMP-2 and -3 can inhibit MT1-MMP and MT2-MMP, TIMP-1 is a poor inhibitor of MT1-MMP, MT3-MMP, MT5-MMP and MMP-19 (Baker et al., 2002). Also, the binding of TIMP-1 and -2 to MMP-10 (stromelysin-2) is 10-fold weaker than their binding to MMP-3 (stromelysin-1) (Batra et al., 2012). TIMP-1 has a threonine-2 (Thr2) residue that interacts with the specific P1' substituent of MMP-3, and substitutions at Thr2 affect the stability of the TIMP-MMP complex and the specificity for different MMPs. For example, a substitution of alanine for Thr2 causes a 17-fold decrease in binding of TIMP-1 to MMP-1 relative to MMP-3 (Meng et al., 1999).

TIMPs localize to specific regions within the veins. In one study, TIMP-2 and -3 localized predominately to VSM of the tunica media, and while TIMP-2 was not identifiable in the connective tissue of the endothelium or tunica media, TIMP-3 was identified in the elastic tissue of the vein wall (Aravind et al., 2010). Other studies showed TIMP-1, -2, and -3 in the intima and TIMP-1 and -2 in the media of control veins, as compared to TIMP-1 and -3 in the intima and TIMP-1, -2, and -3 in the media of VVs (Sansilvestri-Morel et al., 2007).

A change in either TIMP or MMP levels could alter the TIMP/MMP ratio and cause a net change in specific MMP activity. In one study, MMP-1 and -7, and TIMP-1, -2 and -3 levels were only slightly modified, while MMP-2 and -3 levels were increased in skin from patients with VVs compared with control subjects, thereby decreasing the TIMP/MMP ratio, increasing MMP-2 and -3 activity, and promoting ECM degradation (Sansilvestri-Morel et al., 2007), particularly in atrophic regions of VVs. Other studies have shown an increase in TIMP/MMP ratios and a net decrease in certain MMPs activities in specific regions of VVs. For instance, one study showed a 3-fold increase in TIMP-1/MMP-2 ratio in pulverized VVs and suggested that the resulting decrease in MMP-2 proteolytic activity could be the cause of the accumulation of ECM often observed in hypertrophic regions of VVs (Badier-Commander et al., 2001). These observations highlight the importance of further examining the levels of TIMPs as related to specific MMPs in different regions of VVs.

α 2-Macroglobulin, Monoclonal Antibodies, and Other Endogenous MMP Inhibitors

Human α 2-Macroglobulin is a glycoprotein consisting of four identical subunits and is found in blood and tissue fluids. α 2-Macroglobulin is a general proteinase inhibitor that inhibits most endopeptidases, including MMPs, by entrapping the peptidase within the macroglobulin. The complex is then rapidly cleared by endocytosis via low density lipoprotein receptor-related protein-1 (Strickland et al., 1990). Monoclonal antibodies are MMPis that have high specificity and affinity for MMPs and can detect MMPs in the body fluids and tissues of patients (Raffetto and Khalil, 2008a). Monoclonal antibodies REGA-3G12 and REGA-2D9 are specific to MMP-9 and do not cross react with MMP-2. MMP inhibition by REGA-3G12 does not appear to involve the MMP Zn²⁺ binding group or the fibronectin region, but may involve the catalytic domain. REGA-1G8 is not as specific and cross reacts with serum albumin.

Other proteinase inhibitors inhibit certain MMPs. For example, a secreted form of β -amyloid precursor protein or a C-terminal fragment of procollagen C-proteinase enhancer protein can inhibit MMP-2. Reversion-inducing-cysteine-rich protein with kazal motifs (RECK) is a GPI-anchored glycoprotein expressed in many cells including VSMCs, and when it is expressed in transfected human fibrosarcoma-derived cell line HT1080 it causes inhibition of MMP-2, -9 and -14 activity (Oh et al., 2001). Also, tissue factor pathway inhibitor-2 is a serine proteinase inhibitor that inhibits MMP- 1 and -2 (Herman et al., 2001).

Synthetic MMP Inhibitors

Synthetic MMPis have been developed as potential therapies for degenerative and vascular diseases (**Table 2**). For more details and for the original references regarding the different categories of MMPis, their MMP specificity, IC₅₀ or K_i, and their use in experimental trials the reader is referred to previous reviews (Benjamin and Khalil, 2012; Kucukguven and Khalil, 2013).

MMP inhibition can be achieved via a Zn²⁺ binding group (e.g. hydroxamic acid, carboxylic acid, sulfhydryl group) or non-covalent interaction with groups on the MMP backbone such as

the S1', S2', S3', and S4' pockets to which the MMPI side chains bind in a fashion similar to that of the substrate P1', P2', P3', and P4' substituents. The efficacy and specificity of inhibition are determined by which pockets are blocked for a given MMP (Hu et al., 2007).

Zn²⁺ binding globulins (ZBGs) displace the Zn²⁺-bound water molecule on an MMP and inactivate the enzyme. A ZBG is also an anchor that keeps the MMPI in the MMP active site and allows the backbone of the MMPI to enter the MMP substrate-binding pockets (Jacobsen et al., 2010). ZBGs have the potential to be used clinically if their potency and selectivity against specific MMPs are enhanced and their targets are better-defined using site-specific delivery (Li et al., 2005). ZBGs such as hydroxamic acids include succinyl (Hu et al., 2007), sulfonamide (Scozzafava and Supuran, 2000), and phosphinamide hydroxamates (Pochetti et al., 2006). Batimastat (BB-94), marimastat (BB-2516), and ilomastat (GM6001) are examples of broad spectrum succinyl hydroxamates (Hu et al., 2007) with a collagen mimicking structure that inhibits MMPs by bidentate chelation of the active site Zn²⁺ (Wojtowicz-Praga et al., 1997). Other ZBGs include carboxylic acids, sulfonylhydrazides, thiols (Jacobsen et al., 2010), aminomethyl benzimidazole-containing ZBGs, phosphorous- and nitrogen-based ZBGs (Skiles et al., 2001; Jacobsen et al., 2010), and heterocyclic bidentate chelators (Puerta et al., 2004). Tetracyclines (Hu et al., 2007) and mechanism-based inhibitors also depend on Zn²⁺ ion for inhibition. SB-3CT (compound 40) is a mechanism-based MMPI that coordinates with MMP Zn²⁺, thus allowing conserved MMP Glu202 to perform a nucleophilic attack and form a covalent bond with the compound (Jacobsen et al., 2010). The covalent bond impedes MMPI dissociation as compared to the traditional chelating competitive MMPIs, and therefore reduces the rate of catalytic turnover and decreases the amount of MMPI needed to saturate the MMP active site (Bernardo et al., 2002). SB-3CT and its successors have shown clinical promise, and more selective MMPIs may be developed through covalent modifications in the active site (Jacobsen et al., 2010).

Some MMPIs such as compound 37 do not have ZBGs and do not bind to the highly conserved Zn²⁺ binding group (Johnson et al., 2007). Also, some MMPIs are specific to certain

MMPs. Small interfering RNA (siRNA) inhibits the transcriptional product of MMP-2 (Chetty et al., 2006). Glycosaminoglycan sulodexide (SDX) decreases MMP-9 secretion from white blood cells without MMP prodomain displacement (Mannello et al., 2013), and may specifically inhibit proteases with cysteine residues such as MMP-2 and -9 (Serra et al., 2014). Statins such as atorvastatin inhibit MMP-1, -2, and -9 expression in human retinal pigment epithelial cells (Dorecka et al., 2014), and MMP-1, -2, -3, and -9 secretion from rabbit macrophages and cultured rabbit aortic and human saphenous vein VSMCs (Luan et al., 2003). Also, in a rat model of heart failure, pravastatin suppressed an increase in myocardial MMP-2 and -9 activity (Ichihara et al., 2006). Deep sea water components such as Mg^{2+} , Cu^{2+} , and Mn^{2+} may inhibit proliferation and migration of cultured rat aortic VSMCs by inhibiting not only ERK1/2 and MAPK kinase (MEK) phosphorylation but also MMP-2 activity (Li et al., 2014), a mechanism that may involve interference with Zn^{2+} binding at the MMP catalytic active site. Despite the marked advances in the design of MMPIs, therapeutic inhibition of MMPs remains a challenge and the tetracycline antibiotic doxycycline is the only FDA-approved MMPI (Chen et al., 2013). Another limitation of MMPIs is that they may cause musculoskeletal side effects including joint stiffness, inflammation, pain, and tendonitis (Renkiewicz et al., 2003).

Venous vs. Arterial MMPs

Although this review focused on venous MMPs, this should not minimize the role of MMPs in other parts of the circulation. MMPs are expressed in the arterial system, and could have significant effects on the arterial structure and function as those discussed in the veins. For example, MMP-2 and -9 cause inhibition of Ca^{2+} entry-dependent mechanisms of contraction not only in rat IVC (Raffetto et al., 2010a), but also in rat aorta (Chew et al., 2004). However, veins differ from arteries in their structure and function, and the effects of MMPs on the veins should not always be generalized to the arteries. For instance, veins have few layers of VSMCs compared to several layers in the arteries. Also, venous and arterial VSMCs originate from distinct embryonic locations and are exposed to different pressures and hemodynamic

conditions in the circulation (Deng et al., 2006). Interestingly, cell culture studies have shown that while cell migration and MMP-2 and -9 levels could be similar in saphenous vein VSMCs and internal mammary artery VSMC, venous VSMCs exhibit more proliferation and invasion as compared to arterial VSMCs (Turner et al., 2007). Other studies have shown that MMP-2 expression is higher in cultured VSMCs from human saphenous veins than in those from human coronary artery. In contrast, MMP-3, -10, -20, and -26 expression is higher in coronary artery VSMCs than saphenous vein VSMCs (Deng et al., 2006). As with MMPs, TIMPs may show different expression levels in veins vs. arteries. For instance, the expression levels of TIMP-1, -2, and -3 are higher in cultured VSMCs from human saphenous veins than in human coronary artery VSMCs (Deng et al., 2006). These observations make it important to further study the differences in the expression and activity of MMPs and TIMPs in veins vs. arteries and in venous vs. arterial disease.

Perspectives

MMPs and TIMPs could be important biomarkers for the progression of CVD and potential targets for the management of VVs. One challenge to understanding the pathogenesis of VVs is that studies often focus on measuring certain MMPs or TIMPs, and it is important not to generalize the findings to other MMPs and TIMPs. Because vein remodeling is a dynamic process, an increase in one MMP in a certain region may be paralleled by a decrease of other MMPs in other regions. Also, because of the differences in the proteolytic activities of different MMPs towards different substrates, the activities of MMPs may vary during the course of CVD. This makes it important to examine different MMPs and TIMPs in various regions of VVs and at different stages of the disease. Another challenge is that TIMPs and other available MMPs have poor selectivity and many biologic actions, and therefore cause side effects (Hu et al., 2007). New synthetic MMPs are being developed, and their effectiveness in treatment of VVs needs to be examined. It is also important to develop specific approaches to modify the

expression or activity of MMPs locally in the vicinity of VVs while minimizing their systemic effects.

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Footnotes

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Legends For Figures

Fig. 1. The lower extremity venous system, and changes in VVs. The lower extremity has an intricate system of superficial and deep veins connected by perforator veins (A), and venous valves that allow blood flow in the antegrade direction toward the heart (B). Vein dysfunction may manifest as small spider veins and could progress to large dilated VVs with incompetent valves (C). VVs mainly show atrophic regions where an increase in MMPs increases ECM degradation, but could also show hypertrophic regions in which increased MMPs and ECM degradation would promote VSMC proliferation, leading to tortuosity, dilation, defective valves, and venous reflux (C).

Fig. 2. Major subtypes and structures of MMPs. A typical MMP consists of a propeptide, a catalytic metalloproteinase domain, a linker peptide (hinge region), and a hemopexin domain. The propeptide has a cysteine switch PRCGXPD whose cysteine sulfhydryl (–SH) group chelates the active site Zn^{2+} , keeping the MMP in the latent proMMP zymogen form. The catalytic domain contains the Zn^{2+} binding motif HEXXHXXGXXH, two Zn^{2+} ions (one catalytic and one structural), specific S1, S2,...Sn and S1', S2',...Sn' pockets, which confer specificity, and two or three Ca^{2+} ions for stabilization. Some MMPs show exceptions in their structures. Gelatinases have 3 type-II fibronectin repeats in the catalytic domain. Matrilysins have neither a hinge region nor a hemopexin domain. Furin-containing MMPs such as MMP-11, 21 and 28 have a furin-like pro-protein convertase recognition sequence in the propeptide C-terminus. MMP-28 has a slightly different cysteine switch motif PRCGVTD. Membrane-type MMPs (MT-MMPs) typically have a transmembrane domain and a cytosolic domain. MMP-17 and -25 have a glycosylphosphatidylinositol (GPI) anchor. MMP-23 lacks the consensus PRCGXPD motif, has a cysteine residue located in a different sequence ALCLLPA, may remain in the latent inactive proform through its type-II signal anchor, and has a cysteine-rich region and an immunoglobulin-like proline-rich region.

Fig. 3. MMP-substrate interaction. For simplicity, MMP-3 is used as a prototype, and only the catalytic domain is shown, with the rest of the MMP structure interrupted by squiggles. A) In preparation for substrate binding, an incoming H₂O molecule is polarized between the free MMP basic glutamate-202 (Glu202) and acidic Zn²⁺. Zn²⁺ is localized in the MMP HEXXHXXGXXH-motif by binding imidazole rings from 3 histidines (His201, 205, 211), while methionine-219 (Met219) in the conserved XBMX Met-turn serves as a hydrophobic base to support the structure surrounding the catalytic Zn²⁺. B) Using H⁺ from free H₂O, the substrate carbonyl binds to Zn²⁺, forming a Michaelis complex. MMP S1, S2, S3, . . . Sn pockets on the left side of Zn²⁺ and primed S1', S2', S3', . . . Sn' pockets on the right side of Zn²⁺ confer binding specificity to substrate P1, P2, P3, . . . Pn and P1', P2', P3', . . . Pn' substituents, respectively. S1 and S3 are located away from the catalytic center, while S2 is close to Zn²⁺. C) Substrate-bound H₂O is freed. Zn²⁺-bound O from Glu-bound H₂O performs a nucleophilic attack on substrate carbon, and Glu202 abstracts a proton from Glu-bound H₂O to form an N-H bond with substrate N, thus forming a tetrahedral intermediate. D) Free H₂O is taken up, and the second proton from Glu-bound H₂O is transferred to the substrate, forming another N-H bond. This allows the substrate scissile C-N bond to break, releasing the N portion of the substrate and forming an MMP-carboxylate complex. Another free H₂O is taken up, releasing the remaining carboxylate portion of the substrate and the free MMP (A). The positions of conserved His and Glu vary in different MMPs.

Fig. 4. Pathophysiology and management of CVD. Certain genetic, behavioral, and environmental risk factors cause an increase in hydrostatic pressure in the lower extremity saphenous and femoral veins leading to venous reflux and valve dysfunction. Increased hydrostatic pressure also increases vein wall tension leading to increases in MMPs, and could also cause EC injury, increased permeability, leukocyte infiltration, and increased levels of adhesion molecules, inflammatory cytokines and reactive oxygen species (ROS) leading to further increases in MMPs. Increased MMPs may cause VSM hyperpolarization and relaxation

as well as ECM degradation leading to vein wall dilation, valve dysfunction and progressive increases in venous hydrostatic pressure (vicious cycle). Increased MMPs generally promote ECM degradation particularly in atrophic regions. Other theories (indicated by interrupted arrows) suggest a compensatory anti-inflammatory pathway involving prostaglandins and their receptors that leads to decreased MMPs and thereby ECM accumulation, particularly in hypertrophic regions of VVs. Persistent valve dysfunction, and progressive vein wall dilation and tortuosity lead to different stages of CVD and CVI. Current therapies of CVD and CVI (presented in shaded arrows) include physical, pharmacological and surgical approaches. Inhibitors of the activity or action of MMPs (also presented in shaded arrows) may provide potential tools for the management of CVD/CVI. 15d-PGJ2, 15-deoxy-delta-12,14-prostaglandin J2; mPGES-1, membrane-associated prostaglandin E synthase-1; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; PGE2, prostaglandin E2.

Fig. 5. Mechanisms linking increased venous pressure to increased MMP expression and VVs. Increased venous pressure causes vein wall stretch, which increases HIF mRNA and protein levels, and in turn MMP levels. Increased wall stretch may also increase other MMP inducers such as EMMPRIN, NGAL, chymase and hormones. Increased MMPs may activate PARs in ECs leading to NO production and venous dilation. MMPs may also stimulate ECs to release EDHF which in turn opens BK_{Ca} channels in VSM, leading to hyperpolarization, decreased Ca²⁺ influx, and decreased vein contraction. Loss of contractile function in VSM could cause a phenotypic switch to synthetic VSMCs. MMPs may also increase the release of growth factors, leading to VSMC proliferation. MMPs also cause ECM degradation leading to VSMC migration, proliferation, further decreases in vein contraction and increases in venous dilation, and VVs. MMP-induced ECM degradation may also cause valve degeneration leading to further increases in venous pressure. As indicated in shaded arrows, inhibitors of MMP synthesis (U-0126, HIF siRNA, 17-DMAG, Echinomycin, MMP siRNA), activity (MMPIs) or actions (Iberiotoxin) may provide new tools for management of VVs. BK_{Ca}, large conductance

Ca²⁺-activated K⁺ channels; DMOG, dimethylxaloylglycine, is an experimental inhibitor of HIF-prolyl hydroxylase; FGF, fibroblast growth factor; HIF, hypoxia-inducible factor; Hsp90, heat shock protein 90; IGF, insulin-like growth factor; MAPK, mitogen-activated protein kinase; NGAL, neutrophil gelatinase-associated lipocalin; PARs, protease activated receptors; TGF- β , transforming growth factor β ; VEGF, vascular endothelial growth factor.

Fig. 6. TIMP-MMP interaction. TIMP-1 and MMP-3 are used as prototypes. A) TIMP is a ~190 aa protein, with an N-terminal domain (loops L1, 2, and 3) and C-terminal domain (loops L4, 5 and 6), which fold independently as a result of 6 disulfide bonds between 12 specific Cys residues. The N-terminal Cys1-Thr-Cys-Val4 and Glu67-Ser-Val-Cys70 are connected via a disulfide bond between Cys1 and Cys70 and are essential for MMP inhibition, as they enter the MMP active site and bidentately chelate the MMP Zn²⁺. The carbonyl oxygen and α -amino nitrogen in the TIMP Cys1 coordinate with the MMP Zn²⁺, which is localized in the MMP molecule via the 3 histidines in the HEXXHXXGXXH motif. The TIMP α -amino group then expels Zn²⁺-bound H₂O by binding the MMP H₂O binding site and forming a H bond with carboxylate oxygen from conserved MMP Glu202 (E in the HEXXHXXGXXH sequence). B) TIMP Thr2 side chains then enter the MMP S1' pocket in a manner similar to that of a substrate P1' substituent, largely determining the affinity to MMP. Thr2 -OH group could also interact with Glu202, further contributing to expelling Zn²⁺-bound H₂O and preventing substrate degradation. Additionally, the TIMP Cys3, Val4 and Pro5 interact with MMP S2', S3', and S4' pockets in a P2', P3', and P4'-like manner, further preventing substrate binding or degradation. The amino acids involved in Zn²⁺- and pocket-binding vary in different TIMPs.

Table 1. Members of the MMP family, their distribution in vascular tissues and other representative tissues, and examples of their substrates.

MMP (Other Name) Chromosome	MW KDa Pro/ Active	Distribution	Collagen Substrates	Non-Collagen ECM Substrates	Non-Structural ECM Components Substrate
Collagenases MMP-1 (Collagenase-1) 11q22.3	55/45	Platelets, endothelium, intima, SMCs, fibroblasts, vascular adventitia, VVs (interstitial/fibroblast collagenase)	I, II, III, VII, VIII, X, gelatin	Aggrecan, casein, nidogen, perlecan, proteoglycan link protein, serpins, tenascin-C, versican	α 1-antichymotrypsin, α 1-antitrypsin, α 1-proteinase inhibitor, IGF-BP-3, -5, IL-1 L-selectin, ovostatin, pro-TNF- α peptide, SDF-1
MMP-8 (Collagenase-2) 11q22.3	75/58	Macrophages, neutrophils (PMNL collagenase)	I, II, III, V, VII, VIII, X, gelatin	Aggrecan, elastin, fibronectin, laminin, nidogen	α 2-antiplasmin, proMMP-8
MMP-13 (Collagenase-3) 11q22.3	60/48	SMCs, macrophages, VVs, preeclampsia, breast cancer	I, II, III, IV, gelatin	Aggrecan, casein, fibronectin, laminin, perlecan, tenascin	Plasminogen activator 2, proMMP-9, -13, SDF-1
MMP-18 (Collagenase-4) 12q14	70/53	<i>Xenopus</i> (amphibian, <i>Xenopus</i> collagenase) human heart, lung, colon	I, II, III, gelatin		α 1-antitrypsin
Gelatinases MMP-2 (Gelatinase-A, Type IV Collagenase) 16q13-q21	72/66	Platelets, endothelium, VSM, adventitia, aortic aneurysm, VVs	I, II, III, IV, V, VII, X, XI, gelatin	Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, versican	Active MMP-9, -13, FGF-R IGF-BP-3, -5, IL-1 β , pro-TN α , TGF- β
MMP-9 (Gelatinase-B, Type IV Collagenase) 20q11.2-q13.1	92/86	Endothelium, VSM, adventitial microvessels, macrophages, aortic aneurysm, VVs	IV, V, VII, X, XIV, gelatin	Aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, versican	CXCL5, IL-1 β , IL2-R, plasminogen, pro-TNF- α , SDF-1, TGF- β
Stromelysins MMP-3 (Stromelysin-1) 11q22.3	57/45	Platelets, endothelium, intima, VSM, coronary artery disease, hypertension, VVs, synovial fibroblasts, tumor invasion	II, III, IV, IX, X, XI, gelatin	Aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein, versican	α 1-antichymotrypsin, α 1-proteinase inhibitor, antithrombin III, E-cadherin fibrinogen, IGF-BP-3, L-selectin, ovostatin, pro-HB-EGF, pro-IL-1 β , proMMP-1 8, -9, pro-TNF- α , SDF-1
MMP-10 (Stromelysin-2) 11q22.3	57/44	Atherosclerosis, uterine, preeclampsia, arthritis, carcinoma cells	III, IV, V, gelatin	Aggrecan, casein, elastin, fibronectin, laminin, nidogen	ProMMP-1, -8, -10
MMP-11 (Stromelysin-3) 22q11.23	51/44	Uterine, brain, angiogenesis	Does not cleave	Aggrecan, fibronectin, laminin	α 1-antitrypsin, α 1-proteinase inhibitor, IGF-BP-1
Matrilysins MMP-7 (Matrilysin-1) 11q21-q22	29/20	Endothelium, intima, VSM, uterine, VVs	IV, X, gelatin	Aggrecan, casein, elastin, enactin, fibronectin, laminin, proteoglycan link protein	α 2-antiplasmin, β 4 integrin, decorin, defensin, E-cadhe Fas-ligand, plasminogen, proMMP-2, -7, -8, pro-TNF syndecan, transferrin
MMP-26 (Matrilysin-2, Endometase) 11p15	28/19	Breast cancer cells, human endometrial tumor	IV, gelatin	Casein, fibrinogen, fibronectin	β 1-proteinase inhibitor, fibri fibronectin, proMMP-2

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Membrane-Type MMP-14 (MT1-MMP) 14q11-q12	66/56	Platelets, VSM, human fibroblasts, uterine, brain, angiogenesis	I, II, III, gelatin	Aggrecan, elastin, fibrin, fibronectin, laminin, nidogen, perlecan, proteoglycan, tenascin, vitronectin	$\alpha_v\beta_3$ integrin, CD44, proMMP-2, -13, pro-TNF- α SDF-1, tissue transglutaminase
MMP-15 (MT2-MMP) 16q13	72/50	Human fibroblasts, leukocytes, preeclampsia	I, gelatin	Aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, vitronectin	ProMMP-2, -13, tissue transglutaminase
MMP-16 (MT3-MMP) 8q21.3	64/52	Human leukocytes, angiogenesis	I	Aggrecan, casein, fibronectin, laminin, perlecan, vitronectin	ProMMP-2, -13
MMP-17 (MT4-MMP) 12q24.3	57/53	Brain, breast cancer	Gelatin	Fibrin	
MMP-24 (MT5-MMP) 20q11.2	57/53	Leukocytes, cerebellum, astrocytoma, glioblastoma	Gelatin	Chondroitin sulfate, dermatin sulfate, fibrin, fibronectin	ProMMP-2, -13
MMP-25 (MT6-MMP) 16p13.3	34/28	Leukocytes (Leukolysin), anaplastic astrocytomas, glioblastomas	IV, gelatin		Fibrin, fibronectin, proMMP
Other MMPs MMP-12 (Metalloelastase) 11q22.3	54/45 - 22	SMCs, fibroblasts, macrophages	IV, gelatin	Casein, elastin, fibronectin, laminin	Plasminogen
MMP-19 (RASI-1) 12q14	54/45	Liver	I, IV, gelatin	Aggrecan, casein, fibronectin, laminin, nidogen, tenascin	
MMP-20 (Enamelysin) 11q22.3	54/22	Tooth enamel	V	Aggrecan, cartilage oligomeric protein, amelogenin	
MMP-21 (Xenopus-MMP) 10q26.13	62/49	Fibroblasts, macrophages, human placenta			α_1 -antitrypsin
MMP-22 (Chicken-MMP) 1p36.3	51	Chicken fibroblasts	Gelatin		
MMP-23 (CA-MMP) 1p36.3	28/19	Ovary, testis, prostate Other (type II) MT-MMP	Gelatin		
MMP-27 (Human MMP-22 homolog) 11q24		Leukocytes, macrophages, heart, kidney, endometrium, menstruation, bone, osteoarthritis, breast cancer			
MMP-28 (Epilysin) 17q21.1	56/45	Skin keratinocytes		Casein	

CA-MMP, cysteine array MMP; CXCL5, chemokine (C-X-C motif) ligand 5; FGF-R1, fibroblast growth factor receptor 1; IGF-BP, insulin-like growth factor binding protein; IL, interleukin; MW, molecular mass; PMNL, polymorphonuclear leukocytes; pro-HB-EGF, pro-heparin-binding epidermal growth factor-like growth factor; RASI-1, rheumatoid arthritis synovium inflamed-1; SDF-1, stromal cell-derived factor-1

Table 2. MMP inhibitors, their specificity to MMPs (K_i or $IC_{50} < 1$ nM to 10 μ M), and their experimental trials.

MMPs Main Categories, Number, Specific Chemistry, Other Names		MMP Specificity, IC_{50} or K_i					Experimental Trials
		<1 nM	1-10 nM	11-100 nM	0.1-1 μ M	1-10 μ M	
1	ZBGs, Hydroxamic Acids Succinyl Hydroxamate Batimastat (BB-94) Marimastat (BB-2516) Ilomastat (GM6001, Galardin)	MMP-1, 2, 8, 9, 26	MMP-1, 2, 8, 9 MMP-1, 2, 9, 14 MMP-7, 12	MMP-3 MMP-7 MMP-3, 14			Glioblastoma, lung, breast, ovarian, prostate cancer
2	Sulfonamide Hydroxamate, AG3340, Prinomastat	MMP-2, 3, 9, 13, 14	MMP-1	MMP-7			Neovascularization, lung cancer, uveal melanoma, gliomas, prostate cancer
3	Succinyl Hydroxamate		MMP-1				
4	RS-104966	MMP-13		MMP-1			
7	Sulfonamide Hydroxamate	MMP-2	MMP-8, 9, 14		MMP-1, 3	MMP-7	Decrease tumor invasion
8	Sulfonamide Hydroxamate		MMP-3		MMP-2	MMP-9	Chronic non-healing wounds
10				MMP-2, 3		MMP-1	
12						MMP-3	
5	Carboxylic Acids		MMP-13	MMP-3, 8	MMP-2	MMP-7, 9, 14	Osteoarthritis
6			MMP-11		MMP-3, 12	MMP-1, 9, 14	Chronic obstructive pulmonary disease
9	Sulfonylhydrazides		MMP-2, 9	MMP-1	MMP-7	MMP-3	
16	Thiol- & Cyclic Mercaptosulfides	MMP-9	MMP-2	MMP-1, 7, 14	MMP-3		
19	Aminomethyl Benzimidazoles				MMP-11	MMP-2, 9, 13	
17	Phosphorous-Based Sulfonamide Phosphonate	MMP-8	MMP-2	MMP-3			
18	Sulfonamide Phosphonate		MMP-8	MMP-2, 9, 13, 14	MMP-1, 3	MMP-7	Liver disease, multiple sclerosis, breast cancer
20	Carbamoyl Phosphonate			MMP-2			Melanoma
21	Carbamoyl Phosphonate					MMP-2	Melanoma, prostate cancer

22	Nitrogen-Based Oxazoline					MMP-11	
23	Dionethiones and Pyrimidine-2,4,6 triones Ro-28-2653		MMP-2, 14	MMP-8, 9		MMP-3	Anti-angiogenic and anti-invasive in tumor models
24	Dionethiones and Pyrimidine-2,4,6 triones	MMP-13			MMP-2, 9, 12		Osteoarthritis
25	Heterocyclic Bidentate Chelators Terphenyl Backbone, AM-6			MMP-3			
26	Biphenyl Backbone, 1,2 -HOPO-2			MMP-8, 12	MMP-2, 3	MMP-13	Heart ischemia and reperfusion
27	Diphenyl Ether Backbone		MMP-2, 9, 13	MMP-3		MMP-1	Brain edema following ischemia–reperfusion
28	Biphenyl Backbone, Pyrone-based			MMP-3, 9, 12	MMP-8	MMP-2, 13	
29	Biphenyl Backbone, Hydroxypyridinone Derivative					MMP-8, 12	
30	Biphenyl Backbone, AM-2			MMP-8, 12	MMP-3	MMP-2	
31	Biphenyl Backbone					MMP-2, 8, 12, 13	
34	Non-ZBGs			MMP-13			Osteoarthritis
35				MMP-12	MMP-2, 8, 13	MMP-3, 9	
36						MMP-2, 8, 13	
37		MMP-13					Osteoarthritis
38	Pyrimidine Dicarboxamide					MMP-13	
39	Pyrimidine Dicarboxamide			MMP-13			
40	Mechanism-Based Diphenyl Ether Backbone, SB-3CT			MMP-2	MMP-9, 14	MMP-3	Inhibits liver metastasis in T-cell lymphoma and bone metastasis in prostate cancer
42	Diphenyl Ether Backbone, Thiol-Containing			MMP-2, 9	MMP-14	MMP-3	
43	Diphenyl Ether Backbone		MMP-2	MMP-14	MMP-9	MMP-3	
45	Diphenyl Ether Backbone		MMP-9	MMP-2	MMP-3, 14		

For details and original references see (Benjamin and Khalil, 2012; Kucukguven and Khalil, 2013).

Fig. 1

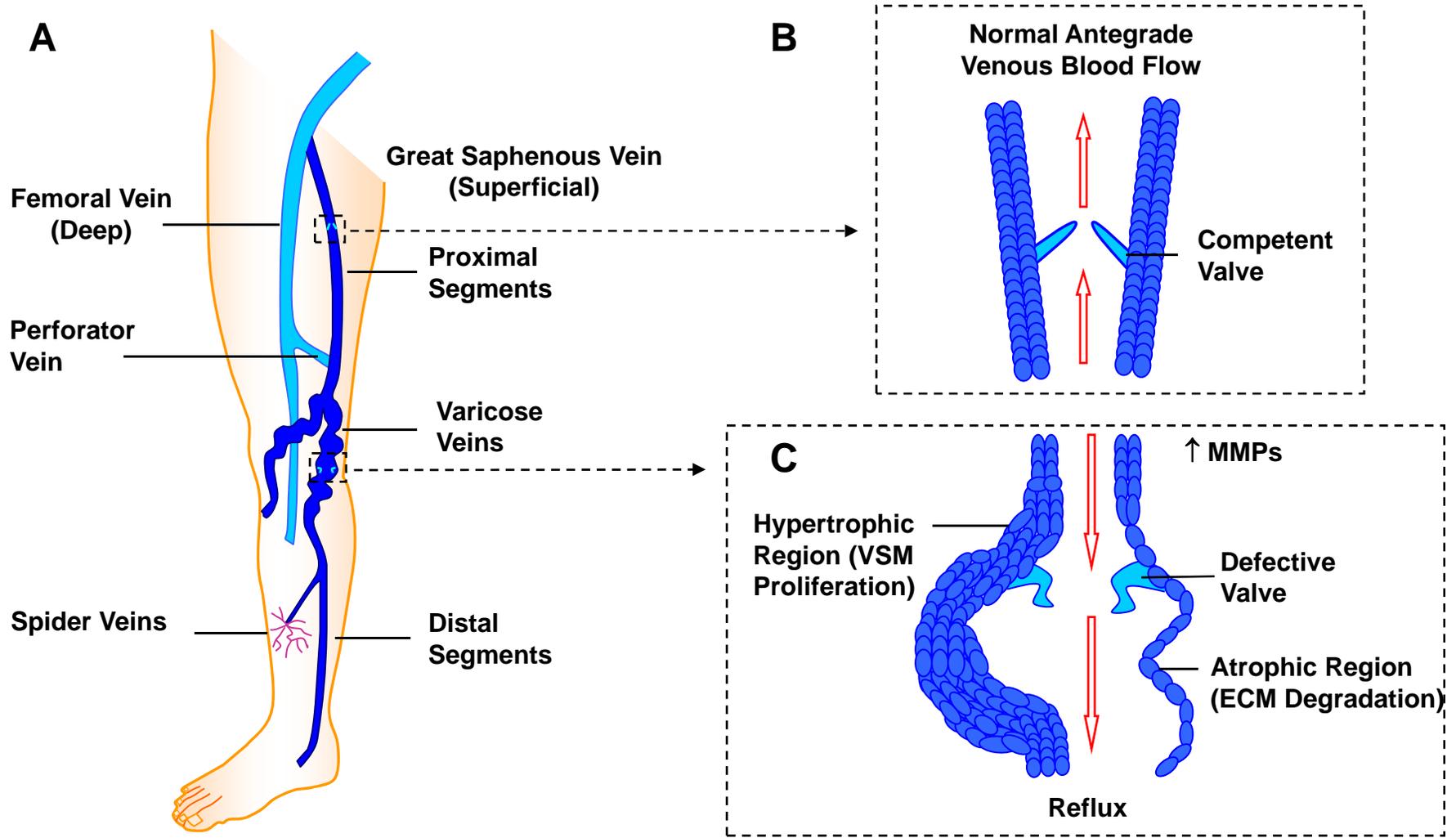


Fig. 2

- Collagenases
MMP-1, 8, 13, 18
- Stromelysins
MMP-3, 10
- Other MMPs
MMP-12, 19, 20, 22, 27

- Gelatinases
MMP-2, 9

- Matrilysins
MMP-7, 26

- Furin-Containing MMPs
Secreted
MMP-11, 21, 28

- Type-I MT-MMPs
(MT-1, 2, 3, 5-MMP)
(MMP-14, 15, 16, 24)

- GPI Anchored MT-MMPs
(MT-4, 6-MMP)
(MMP-17, 25)

- Other MT-MMPs
Type-II MT-MMPs
(MMP-23)

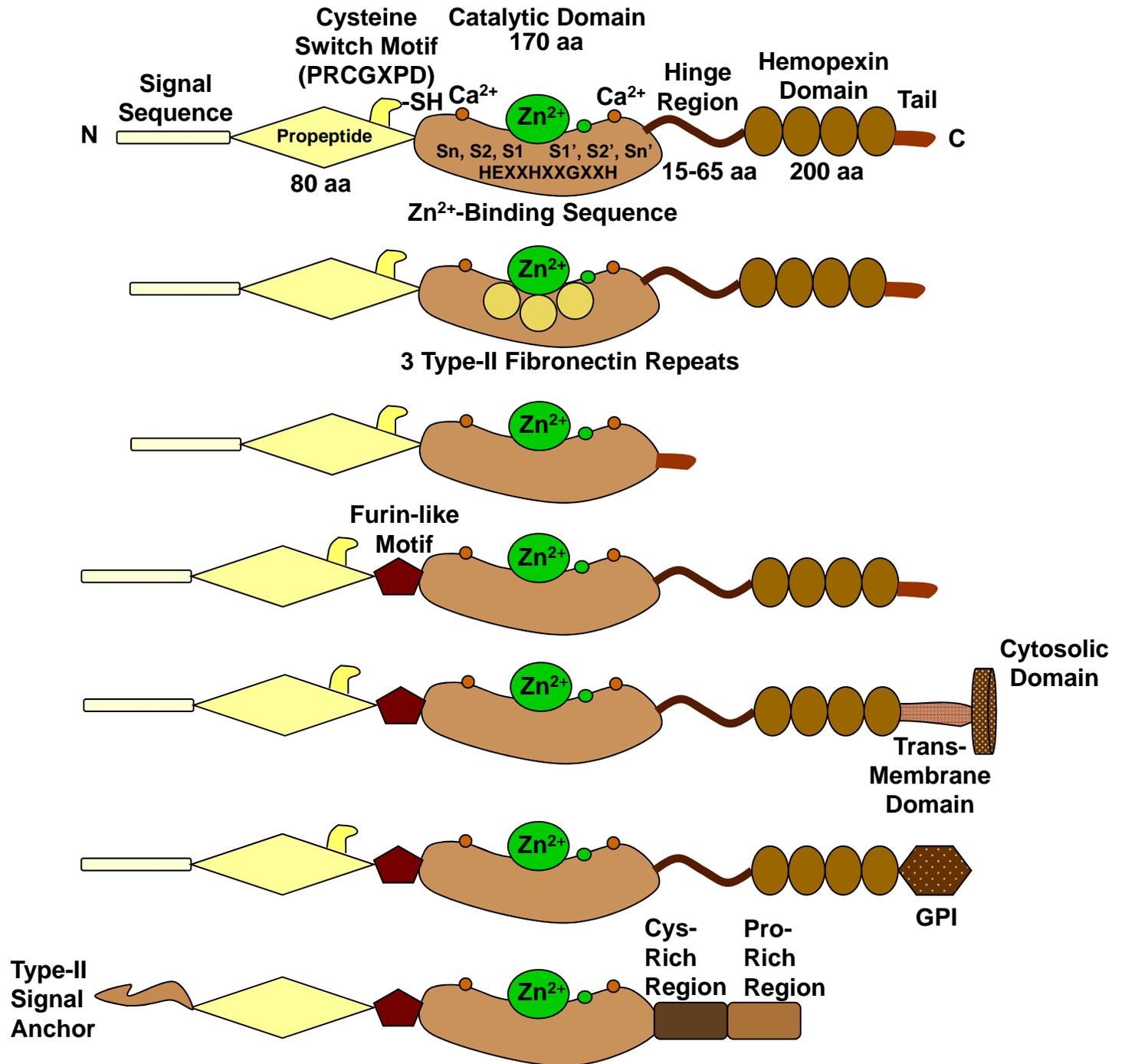
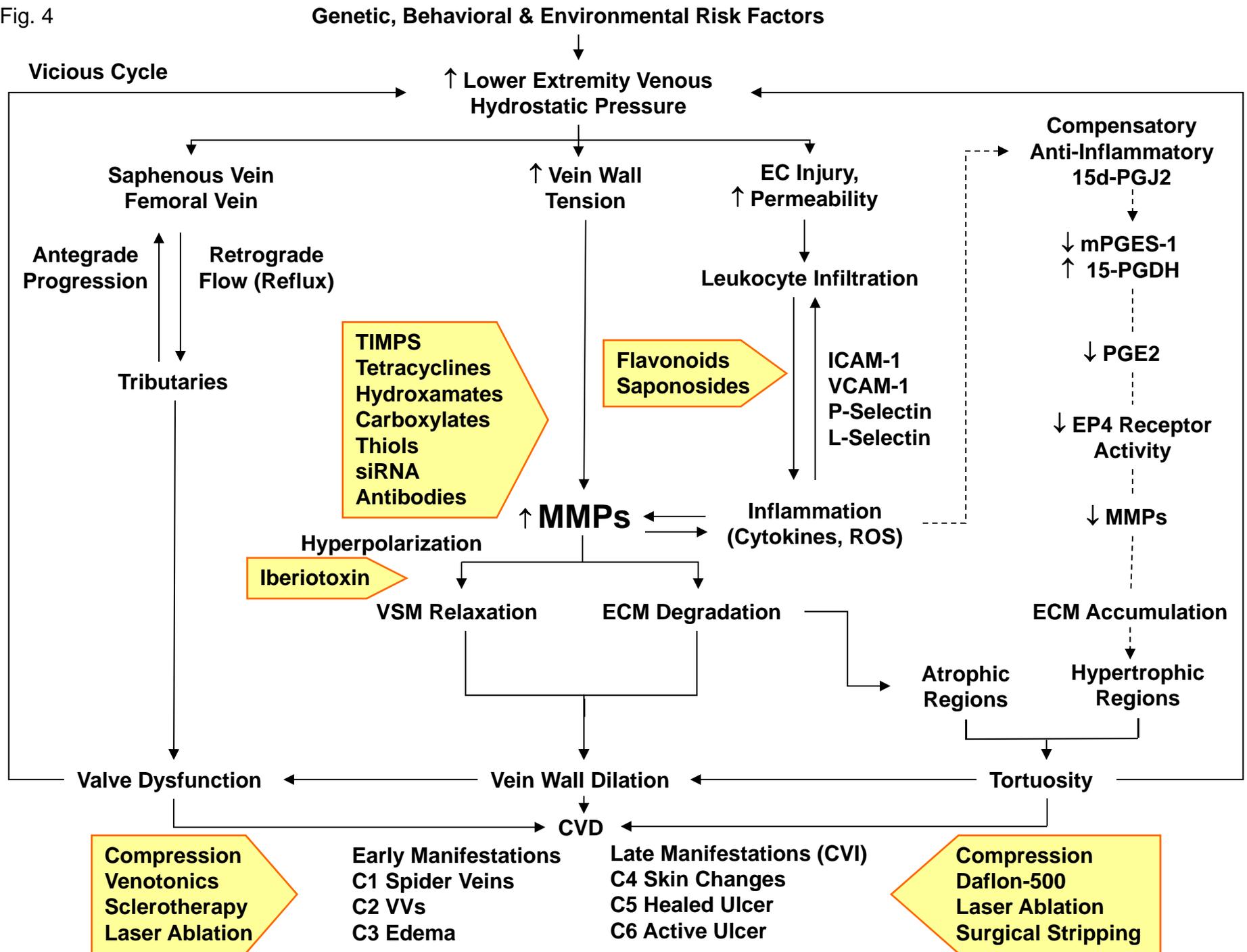


Fig. 4



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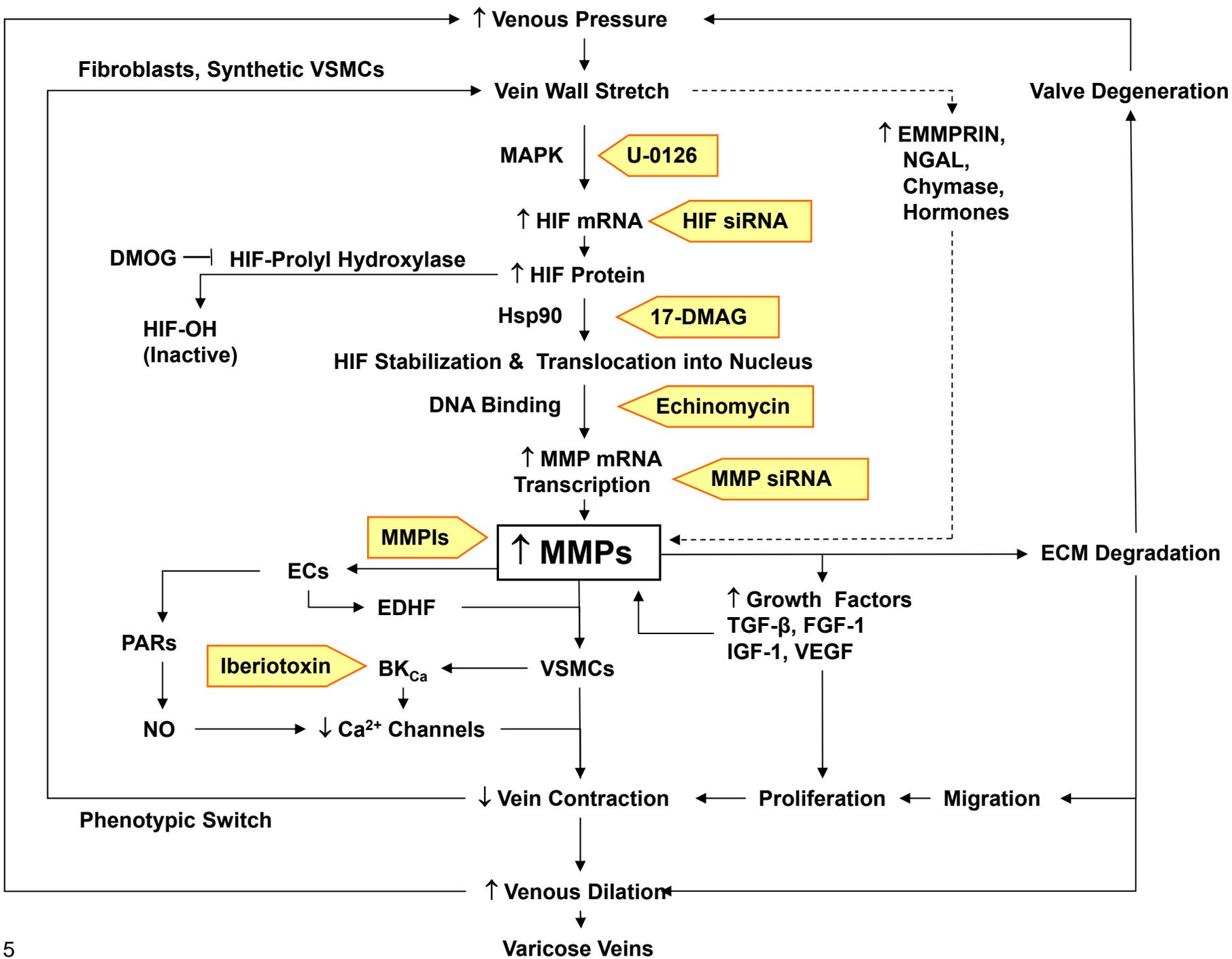


Fig. 5

Fig. 6

