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Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9

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Abstract

Introduction—Diabetic retinopathy remains one of the most feared complications of diabetes. Despite extensive research in the field, the molecular mechanism responsible for the development of this slow progressing disease remains unclear. In the pathogenesis of diabetic retinopathy, mitochondria are damaged and inflammatory mediators are elevated before the histopathology associated with the disease can be observed. Matrix metalloproteinases (MMPs) regulate a variety of cellular functions including apoptosis and angiogenesis. Diabetic environment stimulates the secretion of several MMPs that are considered to participate in complications, including retinopathy, nephropathy and cardiomyopathy. Patients with diabetic retinopathy and also animal models have shown increased MMP-9 and MMP-2 in their retina and vitreous. Recent research has shown that MMPs have dual role in the development of diabetic retinopathy; in the early stages of the disease (pre-neovascularization), MMP-2 and MMP-9 facilitate the apoptosis of retinal capillary cells, possibly via damaging the mitochondria, and in the later phase, they help in neovascularization.

Areas covered—This article reviews the literature to evaluate the role of MMPs, especially MMP-9, in the development of diabetic retinopathy, and presents existing evidence that the inhibitors targeted toward MMP-9, depending on the duration of diabetes at the times their administration could have potential to prevent the progression of this blinding disease, and protect the vision loss.

Expert opinion—Inhibitors of MMPs could have dual role: in the early stages of the diseases, inhibit capillary cell apoptosis, and if the disease has progressed to the angiogenic stage, inhibit the growth of new vessels.

Keywords

diabetes; diabetic retinopathy; matrix metalloproteinases; retina

1. Diabetic retinopathy

Diabetic retinopathy is one of the most common eye disease faced by diabetic patients. It is a slow progressing complication that results from damage to the blood vessels of the retina. In the initial stages of diabetic retinopathy, the disease may remain asymptomatic, but eventually, if not treated, can result in blindness [1]. The development of retinopathy is directly related to the duration of diabetes, by 10 years of diabetes approximately 50% of the patients, and by 20 – 25 years, nearly 90% of the diabetic patients have some stage of

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retinopathy [2,3]. Sustained hyperglycemia is considered as the major initiator of the development of diabetic retinopathy, but the mechanism by which hyperglycemia results in the retinal pathology remains unclear. In the pathogenesis of diabetic retinopathy, retinal cells, including capillary cells, Müller cells and ganglion cells undergo accelerated apoptosis [4–7], and the apoptosis of capillary cells precedes the appearance of microvascular histopathology characteristic of diabetic retinopathy suggesting that accelerated apoptosis can account for the pericyte `drop-out' and formation of `ghosts' [1,8,9]. Diabetes Control and Complications Trial and the United Kingdom Prospective Diabetes Study have clearly suggested that the control of circulating blood sugar and blood pressure has potential to delay the progression of retinopathy in diabetic patients [10,11], but it is difficult, or at times not possible, to maintain normal blood glucose levels, and adjacent therapeutic treatments are required. Elucidation of therapeutic targets to prevent this disease based on the molecular mechanism of its development is needed.

The development of diabetic retinopathy is complex involving interplay from several molecular and biochemical mechanisms affecting molecular, cellular and physiological environment of the retinal vasculature. Leading laboratories are actively pursuing research to understand the pathogenesis of diabetic retinopathy. Diabetes increases oxidative stress via a number of different mechanisms including auto-oxidation of glucose, increased superoxide production/decreased scavenging, activation of polyol pathway and protein kinase C and increased formation of advanced glycation end products [12]. Retinal mitochondria become dysfunctional, and overexpression of manganese superoxide dismutase in mice prevents these mitochondrial abnormalities and the development of diabetic retinopathy, suggesting a major role of mitochondria in the development of this disease [13–22]. The exact etiology of this multifactorial disease, however, remains elusive.

2. Matrix metalloproteinases

Matrix metalloproteinases (MMPs), a family of over 25 zinc-dependent proteinases which degrade at least one component of the extracellular matrix (ECM), regulate many normal and pathological processes [23,24]. They play a central role in organ development and subsequent tissue remodeling, and in inflammation and injury. MMPs have similar domain structures which consisting of a `pre' region to target for secretion, a `pro' region to maintain latency and an active catalytic region with a zinc-binding active site. They are synthesized and secreted as inactive pro-enzymes that subsequently become proteolytically cleaved and activated [25,26]. Activity of MMPs is modulated by endogenous tissue inhibitors of metalloproteinases (TIMPs), a four-member family of small proteins that bind to MMPs in a 1:1 stoichiometric ratio. TIMP-1 shows greater preference for MMP-9 than any other MMPs [27] and TIMP-2 for MMP-2. Normally, there is a tight balance between MMP and TIMP, but in pathological conditions this balance is impaired resulting in excess of activated MMP. Although both MMP-2 and MMP-9 are active in the degradation of type IV, MMP-2 is the most ubiquitous member and MMP-9 is the largest and the most complex member of the MMP family [28–31]. This review is mainly focused on the role of MMP-9 in the development of diabetic retinopathy, and has a brief discussion about the role of MMP-2.

MMPs are generally associated with the degradation of most ECM proteins, but recent studies have shown their presence within the cells in nuclear, mitochondrial and cytoplasmic compartments [26]. They have been shown to cleave other non-ECM proteins, including growth factors, cytokines and cell receptors [32]. Modification of the membrane-associated proteins by MMPs is crucial for communication between cells and the extracellular milieu, and determines cell fate and the integrity of tissues. Depending on the extracellular or subcellular localization, the same MMP may affect physiology and pathology in opposing manner. MMP-9 is secreted in the body in a latent form, but on activation it acts on many

inflammatory substrates and also on mitochondria [33]. The enzyme is under strict control at various levels: gene transcription, synthesis, secretion, activation, inhibition and glycosylation [34,35].

MMP-2 helps advancing front of the migrating column of endothelial cells to migrate through the basement membrane and ECM. Membrane type-1 matrix metalloproteinase (MT1-MMP) initiates the activation pathway by converting pro-MMP-2 into an activation intermediate that further undergoes autocatalytic conversion to generate the mature MMP-2. In contrast to MMPs, MT1-MMP is a cell membrane-bound proteinase with relatively short transmembrane domain and a cytoplasmic tail [36]. It helps associate these enzymes with discrete regions of the plasma membrane and the intracellular compartment. MT1-MMP also acts as a receptor for TIMP-2, and forms a tri-molecular complex with MMP-2 and TIMP-2 [28]. Although MT1-MMP has been identified as a key player during the angiogenic response, its regulation and participation in angiogenesis depends on the nature of the angiogenic stimulus [37].

3. Regulation of MMPs

Various regulators have been shown to regulate MMPs; a small molecular weight G-protein, H-Ras, regulates MMP-9, possibly via its downstream signaling pathway [38,39]. H-Ras-mediated activation of ERK1/2, followed by activation of nuclear transcription factor-kappa B (NF-kB), is shown to directly regulate the induction of MMP-9, suggesting a close relationship between H-Ras-ERK-NF-kB and MMP-9 activation [40,41].

The transcription of MMP-9 is mostly controlled by 670 base pairs of upstream sequence which includes AP-1, NF-kB, PEA3 and Sp1 binding sites. Human MMP-9 promoter contains cis-acting regulatory elements for binding of the transcription factors, including AP-1 (−533 bp, −79 bp), NF-kB (−600 bp) and Sp1 (−588 bp), which participate in the regulation of the MMP-9 gene and these NF-kB, AP-1, and Sp1 binding sites are considered indispensable [42–44]. Chromatin structure of the MMP-9 promoter is remodeled in coordination with the activation of MMP-9 gene transcription, and the composition of AP-1 and NF-kB on the MMP-9 promoter dynamically changes over the course of MMP-9 induction [45]. Inhibition of SIRT1, a member of the sirtuin family of proteins, has been reported to increase histone-4 acetylation at NF-kB binding sites (−594 to −604) at the MMP-9 promoter increasing the binding of NF-kB to the MMP-9 promoter [46], and these studies have suggested a role of epigenetic modifications in the regulation of MMP-9. In addition, the recent studies have shown that retinal MMP-9 promoter is epigenetically modified in diabetes, further contributing to its expression (Zhong & Kowluru, manuscript in preparation).

MMPs are highly sensitive to oxidative stress, and they are induced by increase in reactive oxygen species (ROS) [47], possibly via direct oxidation of crucial cysteine residues contained within the DNA-binding domain [48]. MMPs are also the prime nitric oxide targets, and peroxynitrite, formed between ROS and nitric oxide can activate pro-MMPs via interacting with cytosolic glutathione [49,50]. Mitochondrial oxidative stress is shown to regulate MMP-9 activation [51], and also, the induction of MMP-9 is considered as a negative regulator of mitochondrial function. MMPs also have potential to impair mitochondrial function via damaging mitochondrial membrane potential by disrupting mitochondrial connexin-43 protein [17,52,53].

4. MMPs and apoptosis

Activation of MMPs is shown to accelerate the apoptosis process; increased MMPs activate apoptosis possibly by disrupting mitochondrial connexin-43 protein and impairing

mitochondrial membrane potential [17,20,52]. Inhibition of MMP-2 in myocytes is shown to decrease β-adrenergic receptor-stimulated apoptosis, and JNK-dependent mitochondrial death is considered as the major pathway [54]. Activation of MMP-2 cleaves the nuclear poly(ADP-ribose)polymerase (PARP) and results in apoptosis via mitochondrial pathway releasing apoptosis-inducing factor from the mitochondria [55–57].

5. MMPs and diabetic retinopathy

Diabetic environment stimulates the secretion of several MMPs that are considered to participate in many diabetic complications, including retinopathy, nephropathy and cardiomyopathy [58–65]. Patients with diabetic retinopathy and also animal models have shown increased MMP-9 and MMP-2 in their retina and vitreous [66–68]. Retinal mRNA levels of MMP-2, MMP-9 and MT1-MMP are elevated in diabetes [20,60,61,69], and the pro-forms of MMP-2 and MMP-9 are significantly elevated in the neovascular retinal membranes [62,70]. Although the exact mechanism operating in the retina in diabetes is not clear, MMPs could contribute in the disease process via number of different pathways. MMPs have an important role in maintaining the integrity of the blood–retinal barrier (BRB), and in the development of diabetic retinopathy, BRB damage is an early event [71]. Increased retinal MMPs in diabetes facilitate the increase in vascular permeability via proteolytic degradation of the tight junction protein occludin and disruption of the overall tight junction complex [67,71]. Pro-MMP-2 is efficiently activated in the fibrovascular tissues from patients with proliferative diabetic retinopathy, and interactions with MT1-MMP and TIMP-2 are suggested to play important role in this. Increased expression of MT1-MMP and MMP-2 are observed in retinal pericytes incubated in high glucose and in the retina in diabetes, and increased MMP-2 activity is considered to compromise retinal pericyte survival [72]. Heavily oxidized and glycated low-density lipoprotein (LDL), which is elevated in diabetes, increases MMP-2 in retinal pericytes [73]. MMP-9 is also up-regulated in retinal microvascular cells cultured under high glucose conditions [67,69,71]. Increased MMP-9 is also observed in the human retina showing active neovascularization [62,74]. The exact mechanism by which MMP-9 could contribute to the development of diabetic retinopathy is not clear.

5.1 MMPs and inflammation

MMPs act on pro-inflammatory mediators to regulate varied aspects of inflammation, and can act as switch in acute and chronic inflammation [75]. IL-1β is considered as a critical substrate for MMP-9, and MMP-9 activates the pro-forms of IL-1β increasing the cleaved forms of IL-1β in the dorsal root ganglion [76]. MMP-9 knockout mice, after permanent focal ischemia, have reduced inflammatory mediators, cell apoptosis and ischemic lesions, and these mice also have decreased immune complex-induced arthritis [77]. MMP-9 is also an important effector molecule in inflammatory cells; it can act as a switch in acute and chronic inflammation, and is postulated to be involved in both the initial phase of inflammation and the later phase of tissue remodeling [75,78]. In the development of diabetic retinopathy, subclinical inflammation has been considered to play a role in the vascular lesions associated with diabetic retinopathy [79,80]. The levels of cytokines, including IL-1β and TNF-α, are increased in the vitreous fluid of the patients with proliferative diabetic retinopathy and in the retina from diabetic rats and mice [81,82]. The capillaries become non-perfused and ischemic, and the number of platelet-fibrin thrombi increases; these pro-inflammatory changes and leukostasis constitute as some of the earliest changes observed in the retina of diabetic animals [83]. Intracellular adhesion molecule-1 and CD18 upregulation, leukocytes adherence to the retinal microvasculature, and endothelial cell damage are also reported early in the development of retinopathy in diabetic rats [83]. Intravitreal injection of IL-1β increases TUNEL-stained capillary cells in the retina of normal rats; the apoptosis process is mediated via activation of NF-kB [84]. Diabetes-

induced TNF-α up-regulation is shown to contribute to increased apoptosis of retinal capillary cells [85]. How MMP-9 increases inflammatory mediators is not clear, but by degrading a major component of the basement membrane, it has potential to enable invasiveness of immune system cells to the injured tissue (suggesting MMP-9 as a byproduct) or to the tissue that is about to be injured, acting as a direct etiological factor [34]. MMPs also facilitate tissue availability of bound VEGF [86], and VEGF is considered one of the major growth factors in the development of diabetic retinopathy [87]. Activated MMP increases vascular permeability in the retina in diabetes by proteolytic degrading occludin and disrupting the overall tight junction complex, and breakdown of the BRB is considered an early event in the pathogenesis of diabetic retinopathy [67,71]. Thus, there appears a strong relationship between MMP-9 and inflammation in the development of diabetic retinopathy.

5.2 MMPs and mitochondrial dysfunction

In the pathogenesis of diabetic retinopathy, Ras-Raf-MEK-ERK cascade is responsible for the activation of MMP-9 in the retina resulting in the apoptosis of its capillary cells [61]. As stated above, MMPs are regulated by mitochondrial oxidative stress, and, by contrast, the induction of MMPs serves as a negative regulator of mitochondrial function, suggesting a vicious cycle of mitochondria damage and activation of MMPs [17,52]. The recent studies have shown increased mitochondrial localization of MMP-2 and MMP-9 in the pathogenesis of diabetic retinopathy, and have demonstrated a pro-apoptotic role for these MMPs in the pre-angiogenic stages of diabetic retinopathy [17,20,60]. Furthermore, there appears to be a direct role of MMP-9 in the development of diabetic retinopathy; the retinal vasculature of diabetic mice with MMP-9 gene abrogated is protected from accelerated apoptosis, and from the histopathology characteristic of diabetic retinopathy. These MMP-9 gene knockout mice are protected from diabetes-induced mitochondria damage suggesting a direct role of activated MMP-9 in mitochondria damage and in membrane permeability. Damaged mitochondria allow Bax to move into the mitochondria, and apoptotic machinery is activated. The molecular mechanism via which MMP-9 is increased in the retinal mitochondria appears to be via the regulation of chaperons, and Hsp70 and Hsp60 appear to be important in chaperoning MMP-9 to the mitochondria [17,20,60,69].

Similarly, diabetes also activates MMP-2 in the retinal mitochondria damaging their integrity, and the process is mediated via the regulation of Hsp60 and connexin-43; mitochondrial MMP-2 damages retinal mitochondria by modulation of Hsp60 and connexin-43, allowing cytochrome c to leak out and activate the apoptotic machinery. Thus, the damage of mitochondria appears to be one of the pathways via which increased MMPs can contribute to the development of diabetic retinopathy [20,60,69].

5.3 MMPs and neovascularization

During the advanced stages of the development of diabetic retinopathy, subsequent to capillary basement membrane thickening and loss of pericytes and endothelial cells, neovascularization begins and collateral vessels start to appear. These new retinal vessels are fragile, and are prone to bleeding resulting in vitreous hemorrhage, and if not treated, lead to the retinal detachment [1]. MMPs, in particular, MMP-2 and MMP-9, assist in angiogenesis, and multiple mechanisms are implicated. MMPs degrade the capillary basement membrane, which is a requirement for the penetration of endothelial cells in the subendothelial matrix and for the formation of a new lumen. MMPs are active players in the VEGF-mediated cell proliferation and the development of new vasculature [88–90]. MMPs also facilitate tissue availability of bound VEGF [86], and VEGF is considered one of the major growth factors in the development of diabetic retinopathy [87,91]. Alternatively, MMP-9 could also act as an angiogenesis antagonist; it is shown to activate the angiostatin an angiogenesis inhibitor

factor [92]. The activity of both, MMP-9 and MMP-2, are increased in epiretinal neovasculature membrane of patients with proliferative diabetic retinopathy [67,74,93]. Further, patients with proliferative diabetic retinopathy present activated pro-MMP-2, and the activation of pro-MMP-2 in the fibrovascular tissues is postulated to be mediated via its interaction with MT1-MMP and TIMP-2 [72].

Thus, MMPs appear to have dual role in the development of diabetic retinopathy: in the earlier stages of diabetic retinopathy (pre-neovascularization), they facilitate accelerated apoptosis of retinal capillary cells, making retinal microvasculature low in endothelial cells and pericytes, and in later stages, assisting in the new vessel formation (Figure 1).

6. MMPs and other ocular diseases

Upregulation of MMPs is also observed in many other ocular diseases, including age-related macular degeneration, proliferative vitreoretinopathy, secondary cataract and conjunctivochalasis [94]. In age-related macular degeneration, several MMPs, including MMP-2 and MMP-9 are elevated in the Bruch's membrane; especially high levels are observed in the areas of choroidal new vessel, and are associated with the choroidal neovascularization [95]. In glaucomatous eyes, alterations in MMPs are considered to contribute to the increased outflow resistance [96], and increased activity of MMP-9 has an important role in the corneal stroma pathology observed in the dysfunctional tear state [97]. Furthermore, high MMP-9 activity is observed in the eyes with cortical cataract, and the activity increases with age in the lens epithelial cells of patients with age-related cataract [98]. Thus, there is a great deal of evidence that MMPs play important roles in the pathogenesis of other ocular diseases.

7. Inhibitors of MMPs

As reviewed above, MMPs are involved in many diseases, including diabetic retinopathy making them viable drug targets in the therapy of these diseases. There are several classes of pharmacological MMP inhibitors with a majority of them based on the binding to the zinc site of the MMP to block its activity. Doxycycline is considered to be the most potent MMP inhibitor with a broad-spectrum range and inhibits MMP-1, -2, -7, -8, -9, -12 and -13 [99]. Other synthetic MMP inhibitor, bisphosphonates, has potent MMP-inhibitory properties, probably through cation-chelation of zinc [100]. Because of a close relationship between MMP-9 and inflammation, non-steroidal anti-inflammatory drugs, such as indomethacin, are shown to reduce PGE2 synthesis and consequently MMP-9 production [101]. In the development of diabetic retinopathy, pharmacological inhibition of MMPs is shown to prevent retinal and choroidal neovascularization [102], and inhibit MMP-9-mediated retinal vascular permeability and inflammation [71,103]. Furthermore, administration of a synthetic MMP inhibitor prevents the induction of proliferative vitreoretinopathy [104]. MMP-2 and MMP-9 inhibition in presence of COX inhibitor is shown to prevent retinal abnormalities [105]. However, there have been no clinical trials to use the inhibitors of MMPs to treat diabetic retinopathy patients. The recent studies using mice genetically modified for MMP-9 have shown that these mice, when made diabetic, are protected from the accelerated loss of retinal capillary cells, and also the development of early signs of diabetic retinopathy [17], and this strengthens the possible use of therapies to target MMPs to ameliorate the development of diabetic retinopathy in diabetic patients.

Since the late 70s, more than 70 inhibitors of MMPs have been pursued as clinical candidates for cancer, arthritis, cardiovascular diseases and many others, but the results have been less than satisfactory. This can be attributed to either poor selectivity of the inhibitors, or target validation. Lessons from previous failures, and recent research showing an association between mitochondrial damage and MMPs, novel non-matrix-related intra- and

extracellular targets of MMPs and their regulation by epigenetic modifications, are opening channels to develop new inhibitors targeting MMPs. It is believed that the understanding of the molecular mechanism(s) regulating the induction and repression of MMPs should help provide valuable insights for developing therapeutic agents.

The delivery of a drug to the retina, however, presents additional challenges for the treatment of diabetic retinopathy. The BRB impedes delivery of many compounds which fail (or poorly) to pass this barrier, and targeted delivery (via intravitreal injections) though now in practice for anti-VEGF treatment, comes with some complications, including the possibility of undesired cataracts and retinal infections. The other major issue is the safety of the drug for this lifelong disease. Furthermore, since the duration of diabetes is an important contributor in the development of this slowly progressing disease, the time of initiation of a therapy has major role in the outcome of the therapy, and initiation of a therapy during early stages has better potential for a positive outcome than at later stages of the disease. But lack of screening of patients leaves many high-risk patients without any treatment for longer durations. Recent ongoing research is coming up with novel means to deliver the drug, for example, the use of nanoparticles, should help provide clinicians a more feasible means for drug delivery to the retina.

Furthermore, the authors recognize that the treatment strategies that could enhance the degree of inhibition of MMPs represent one possible direction for clinical research, but it is unlikely that the development of retinopathy could be completely prevented by just one drug. It is believed that inhibitors of MMPs, however, could become an important part of the complex approach to inhibit the pathogenesis of diabetic retinopathy.

8. Expert opinion

One of the most devastating complications of diabetes is the retinopathy. This progressive disease, which remains asymptomatic in the early stages, if not treated, can rob patient's vision. The pathogenesis of diabetic retinopathy is complex, and involves several molecular and biochemical mechanisms affecting cellular metabolism of the retina. But, despite extensive cutting-edge research in the field, the mechanism remains unclear.

MMPs have been shown to play an important role in many chronic diseases including cancer and arthritis. Although higher levels of MMPs were observed in the vitreous of patients with proliferative retinopathy over two decades ago, their role in the development of this blinding disease has remained unclear. In the pathogenesis of diabetic retinopathy, MMPs in addition to being pro-angiogenic during the neovascularization phase, could act as pro-apoptotic in the early stages, and the accelerated apoptosis of retinal capillary cells is considered to predict the development of retinopathy in diabetes.

With the data available implicating the role MMPs in diabetic complications, it is believed that the inhibitors of MMPs could have dual role: in the early stages of the diseases, inhibit capillary cell apoptosis, and if the disease has progressed to the angiogenic stage, inhibit the growth of new vessels. This will help patients save their vision from deteriorating, and prevent them becoming blind.

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Article highlights

- Retinal MMPs are activated in diabetes.
- Epigenetic modifications regulate retinal MMP-9 in diabetes.
- In the early stage of diabetic retinopathy, MMPs, especially MMP9 and MMP2, damage mitochondria and accelerate the apoptosis of retinal capillary cells, and in the advanced stage of the disease, these MMPs assist in angiogenesis.
- Inhibitors of MMPs may have potential to halt the development of diabetic retinopathy via inhibiting the early events, and also the formation of neovesculature.

This box summarizes key points contained in the article.

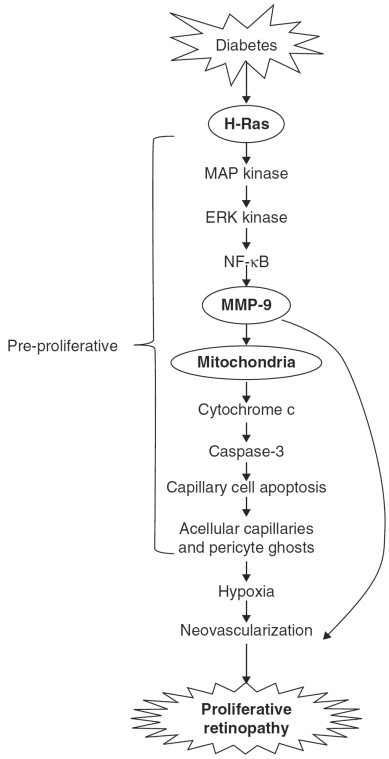

Figure 1. In diabetes, activation of H-Ras in retinal capillary cells activates MMP-9 via Raf-1-MAP kinase-ERK kinase-NF-kB cascade

In the early stages of diabetic retinopathy, activated MMP-9 damages the mitochondria, and cytochrome c is released from the mitochondria. This accelerates capillary cell apoptosis, ultimately resulting in acellular capillaries and pericyte ghosts (the pathology characteristic of early stages of diabetic retinopathy). With time, the capillaries become hypoxic ultimately leading to neovascularization. Furthermore, MMP-9, via degrading the capillary basement membrane and assisting the penetration of endothelial cells in the subendothelial matrix, can also result in neovascularization.