Role of matrix metalloproteinase-2 and -9 in the development of diabetic retinopathy

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Abstract Diabetic retinopathy represents the most common causes of vision loss in patients affected by diabetes mellitus. The cause of vision loss in diabetic retinopathy is complex and remains incompletely understood. One of the earliest changes in the development of retinopathy is the accelerated apoptosis of retinal microvascular cells and the formation of acellular capillaries by unknown mechanism. Results of a recent research suggest an important role of matrix metalloproteinases (MMPs) in the development of diabetic retinopathy. MMPs are a large family of proteinases that remodel extracellular matrix components, and under pathological condition, its induction is considered as a negative regulator of cell survival; and in diabetes, latent MMPs are activated in the retina and its capillary cells, and activation of MMP-2 and -9 induces apoptosis of retinal capillary cells. This review will focus on the MMP-2 and MMP-9 in the diabetic retina with special reference to oxidative stress, mitochondria dysfunction, inflammation and angiogenesis, as well as summarizing the current information linking these proteins to pathogenesis of diabetic retinopathy.

Keywords Diabetic retinopathy · MMP-2 · MMP-9 · Apoptosis · Oxidative stress · Inflammation · Angiogenesis

Diabetes is a complex and severe disease that may develop at any time during a person's life. The incidence of diabetes is increasing rapidly and has become one of the main challenges to current health care. Diabetic retinopathy (DR), a slow-

progressing, multifactorial microvascular complication, is the most common cause of vision loss in patients with diabetes [1]. The early (i.e., nonproliferative diabetic retinopathy) histological characteristic features of diabetic retinopathy include thickening of basement membrane and the loss of pericytes and endothelial cells, which results in vascular leakage [2–4]. At advanced stages (i.e., proliferative diabetic retinopathy), inflammation, ischemia-induced angiogenesis, and expansion of extracellular matrix (ECM) in association with the outgrowth of fibrovascular membranes at the vitreoretinal interface, results in neovascularization and retinal detachment, leading to blindness [5, 6]. No recent national population-based estimate of the prevalence and severity of diabetic retinopathy exists. The cause of vision loss in diabetic retinopathy is complex and remains incompletely understood. It is believed that the sustained high level of blood glucose in diabetes induces acellular capillaries and pericyte ghosts in retinal microvasculature by unknown mechanisms. Development of DR is a multifarious process where proteases, growth factors, cytokines, and chemokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, stromal cell-derived factor-1 α (SDF-1 α), cyclooxygenase-2 (COX-2) and prostaglandin E2 production, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and connective tissue growth factor are released from retinal cells under hyperglycemia and interact with each other as well as activate several signaling pathways to promote neovascularization and fibrosis in the retina [7–11]. Considerable experimental evidence, generated in both tissue culture and animal models, indicate that in diabetes, retinal cells undergoes accelerated apoptosis and induces acellular capillaries and pericyte ghosts in retinal vasculature by unknown mechanism [12–14]. Over the last several years, multiple mechanisms on the altered cell signaling pathways and its components, including oxidative stress and polyol pathway, protein kinase C (PKC), and mitogen-activated

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protein kinases (MAPK) in the microvasculature of the diabetic retina have been shown [15–23]. Understanding on such mechanistic pathways might help to develop future adjuvant therapies for diabetic retinopathy. Results of a recent research indicate that MMPs and tissue inhibitors of metalloproteinases (TIMPs) play a pivotal role in the pathogenesis of diabetic retinopathy [24–27].

MMPs are a large family of zinc-dependent endogenous proteolytic enzymes. Currently, there are more than 24 known MMPs, which are subdivided into collagenases, gelatinases, stromelysin, matrilysins, and membrane-type MMP. Structure analysis reveals that MMPs are multidomain enzymes, usually consisting of a prodomain, a catalytic domain, a hinge region, and in case of collagenases, gelatinases, and MT-MMPs, consist of an extra hemopexin domain. Most MMPs are secreted as proenzyme (inactive pro-MMPs) that can be activated by cleavage of the prodomain by proteinases such as plasmin and MT-MMPs or by oxidation of a reactive cysteine within the prodomain [28]. TIMPs are endogenous inhibitors that bind specifically and inhibit MMPs activation. Four types of TIMPs (TIMP1–4) have been identified; these proteins share an analogous structure which fits into the active site of the MMP catalytic domain [29]. Thus, the balance disturbances of MMP and TIMP lead to tissue remodeling. Various studies have also suggested that extracellular matrix and cell interactions influence the gene regulation, cytoskeleton structure, differentiation, and many aspects of cells growth; therefore, tight balance between degradation and regeneration of the extracellular matrix are important for cell to cell communication and its survival [30]. Altered activity of MMPs is reported under many pathological conditions, including diabetes. Diabetes is believed to stimulate the secretion of several MMPs which participate in both macrovascular and microvascular abnormality-associated diseases such as coronary artery disease, peripheral arterial disease, stroke, nephropathy, neuropathy, and retinopathy [31–33]. Extensive experimental data generated in both tissue culture and animal models as well as in human patients signify that in diabetes among all MMPs, type IV collagenases, MMP-2 (72 kDa), and gelatinase-B, MMP-9 (92 kDa) are most likely to be involved in the development of diabetic retinopathy. Results of recent studies advocate that due to diabetes, the over expression of MMP-2 and -9 in retina inhibit cell proliferation, differentiation, and accelerate apoptosis, a phenomenon that precedes the development of histopathology characteristic of diabetic retinopathy [14, 34, 35]. We, along with others, have shown that retinal cells incubated in high glucose upregulate the MMP-2 and -9 expression/activation, and inhibition of MMP-2 and -9 is able to prevent high glucose-induced retinal capillary cell apoptosis. Furthermore, we have also shown that high glucose translocate MMP-2 and -9 into the mitochondria and induce mitochondria dysfunction, and inhibition of MMP-2 and -9 ameliorate the high glucose-induced

mitochondria dysfunction [23, 24, 26, 35, 36]. Hyperglycemia can lead to the activation of MMP-2 and -9 by oxidative stress, MAP kinase pathways [23, 26, 37].

MMPs and oxidative stress

One of the most important contributors in complication associated with diabetes is oxidative stress; reactive oxygen species (ROS) generated by high glucose are consider as major causal links between elevated glucose and the other metabolic abnormalities. In the development of diabetic retinopathy, there is evidence of increased lipid peroxidation, superoxide production, and of decreased activity of the antioxidant enzymes such as glutathione peroxidase, super oxide dismutase, and catalase in the retina of human subjects or hyperglycemic animal models [38, 39]. Together, these studies strongly suggest the involvement of oxidative stress in the pathogenesis of diabetic retinopathy. One of the important molecular consequences of oxidative stress is the activation of MMPs; MMPs are regulated by reactive oxygen species [40, 41]. It was reported that the levels of H_2O_2 , which is a specific quantitative biochemical marker for oxidative stress, is increased with the functional severity of hyperglycemia in the retina of diabetic animals. However, supplement of antioxidant agents such as Aminoguanidine and alpha-lipoic acid was shown to improve retinal function in diabetic animals [42, 43]. MMP-2 and -9, induced in response to hyperglycemia are associated with increased ROS (such as hydroxyl radicals and superoxide) production and decreased ROS scavengers (i.e., antioxidants) in the retina-derived endothelial cells in diabetes [26, 37]. Apparently, hyperglycemia increases the generation of ROS, activates aldose reductase, induces advanced glycation end-product (AGE) formation, and activates the nuclear transcription factor NF κ B. Thus, production of ROS may represent one common underlying mechanism for several pathways leading to microvascular damage in diabetic retinopathy as well as other microvascular diabetic complications. ROS can impact on MMP-2 and -9 inductions indirectly through the modulation of signaling networks that contribute to its transcription or through direct oxidative activation of the enzymes. Several lines of evidence suggest that H_2O_2 generated by mitochondrial superoxide dismutase is important to enhance expression of MMP-2 and -9 [26, 44]. Studies have also demonstrated that retinal endothelial cell exposed to high glucose caused a profound increase in the expression of MMP-2 and -9 [45]. This is further supported by our previously published work from transgenic mice that are overexpressing the antioxidant enzyme superoxide dismutase (an antioxidant responsible for superoxide elimination) able to prevent the upregulation of MMP-2 in diabetic retina [36]. Transgenic MMP-9 Knockout mice showed enhanced retinal protection against oxidative stress and reduced

apoptosis after diabetes. Supporting this, high glucose-incubated retinal endothelial cells treated with MMP-2 and -9 inhibitors or knock down by siRNA showed reduced levels of apoptosis; such reduction in total cell death may contribute in prevention of pathogenesis of diabetic retinopathy [24].

MMPs and cell signaling

The MAPK pathway has emerged as a ubiquitous factor involved in the regulation of cell proliferation, differentiation, and apoptosis. MAPK family consists of extracellular signal-regulated kinase (ERK) and stress-activated components, namely c-jun N-terminal kinase and p38 [46]. MAP kinase pathway mediates transmission of extracellular signals into their intracellular targets by a network of interacting proteins by multiple extracellular signals. It is well accepted that two major signaling pathways, Ras/Rac/JNK and Ras/Raf/ERK, can be rapidly activated via a phosphorylation cascade in cells exposed to various stimuli and stressful conditions [47]. The Ras and Raf-1 play as a central intermediate in many signaling pathways by connecting upstream tyrosine kinases with downstream serine/threonine kinases, such as a MAPK and mitogen-activated protein kinase (MKK or MEK). Upon activation by ligand-stimulated receptors, activated Ras complexes promote the activation of the Raf-1 serine/threonine kinase. Raf-1 then activates MAP kinases (MEK1 and MEK2), which in turn activate p42 and p44 MAP kinases, also known as extracellular signal-regulated kinases (ERKs). ERK1/2 kinase phosphorylates a variety of downstream targets, which results in changes in gene expression and the catalytic activities of various enzymes [48]. The Ras-Raf pathway also regulates the activities of different transcription factors, such as c-Jun, AP1, and the NF κ B. The precise mechanism by which Ras triggers Raf-1 is still under investigation. Results of a recent research suggest that the pathways activated by Ras and many of its intermediates play a crucial role in the diabetes-associated complications including retinopathy [23, 49–51]. Glucose-induced biochemical alterations induce ROS, often activate Raf-1-MAPK/ERK kinase (MEK)-ERK cascade via Ras, and activate a cascade of signaling pathways leading to cellular dysfunction or death [52–55]. The PI3K/Akt and ERK1/2 pathways have been shown to regulate MMP-2 and -9 expressions in diabetes including diabetic retina [56, 57]. Diabetes activates a small molecular weight G-protein, H-Ras, in the retina, and its capillary cells is implicated in the apoptosis of retinal capillary cells [54, 57]. It was shown that H-ras regulate MMP-9 induction; hence, its activation is associated with increased vascular permeability in diabetes. Similarly, diabetes-activated PI3K/Akt/mTOR pathway is involved in the early development of diabetic retinopathy. Recently, it was shown that inhibitors of PI3K/Akt/mTOR pathway represent a

unique opportunity to prevent MMP-2 and -9 inductions in early retinal changes induced by diabetes [58, 59]. The other hyperglycemia-induced regulators of MMP-2 and -9 are protein kinase C (PKC), transforming growth factor- β , and the renin-angiotensin-aldosterone system, and AGE. These factors collectively regulate MMP-2 and -9 inductions in diabetes [32, 60–63].

MMPs and mitochondria dysfunction

An active role of mitochondria dysfunction in the induction of apoptosis is well known, and it is postulated to play a major role in the development of diabetic retinopathy [36, 64], but the mechanism which alters the mitochondria function and contributes in the development of retinopathy in diabetes is not clear. MMPs induction is considered as a negative regulator of cell survival in diabetes; latent MMPs are activated in the retina and its capillary cells, and activation of MMP-2 and -9 induces apoptosis of retinal capillary cells, a phenomenon that precedes the development of histopathology characteristic of retinopathy. Recent studies have shown that the damage of retinal mitochondria in diabetes is also mediated via MMPs, especially MMP-2 and -9, and over expression of the enzyme responsible for quenching mitochondrial superoxide, in addition to preventing diabetes-induced superoxide accumulation, inhibits MMPs activation and capillary cell apoptosis in retina of diabetic rodents [26, 36, 37]. Furthermore, under hyperglycemic condition, MMP-2 and -9 are activated in the mitochondria and elucidated as a causative link for mitochondria dysfunction [26, 37]. In general, mitochondrial targeting signals are recognized by outer membrane import receptors, translocase of the outer membrane (TOM), and the translocase of the inner membrane (TIM) import is further assisted by Hsp70 complex in pulling these proteins into the matrix [65]. In diabetes, MMP-9 translocates to mitochondria via TOM/TIM44 complex and induces modulation in the mitochondrial redox state, leading to mitochondria dysfunction [24]. The mechanisms responsible for mitochondria dysfunction by MMPs in diabetes might be that the mitochondria is a major source of superoxide generation and MMPs residing in the mitochondria are sensitive to superoxide. This can lead to activation of MMPs; once activated it induces proteolysis of mitochondria membrane, which results in changing mitochondria permeability and open transit pore, and starts leaking cytochrome c to cytosol, which leads to activation of mitochondria-mediated apoptotic pathway. However, the role of MMP-2 and -9 in mitochondria dysfunction in pathogenesis of diabetic retinopathy needs to be further investigated.

MMPs and inflammation

Inflammation is the second-line defense system of the body in which the innate immune system of the body protects itself after contact to a foreign pathogen or antigen. Inflammation typically has beneficial effects if it is acute, but it will have adverse effects if persists longer or frequently. The immune system of the body identified this foreign pathogen or antigen by specific-binding receptors, such as receptor for advanced glycation end-products and toll like receptors (TLRs). Activation of these receptors after binding with antigen induces production of cytokines (IL-1 β , IL-8, and TNF- α); this further helps in induction or expression of proinflammatory mediators [66, 67]. Various reports suggest a contributory role of inflammation in the development of diabetic retinopathy [68]. A variety of biochemical and physiological alterations that are consistent with inflammation have been found to increase in the retina or vitreous of diabetic animal or patients; gene profiling analysis pattern of diabetic retina from rodents share similarity with inflammatory response [69, 70]. Analysis of inflammatory molecules in vitreous, serum, and retina from diabetic patients or experimental animals indicates that DR is associated with significant increase in proinflammatory cytokines, chemokines, and adhesion molecules [69]. Although there is no pathogen involved in the pathogenesis of DR, there is a low-grade inflammation, hence suggesting a role of hyperglycemia in induction of inflammation. Increased generation of superoxide is considered as a strong activator for release of inflammatory mediators [71, 72], and it can also stimulate various pathways leading to facilitate inflammation, such as an increase in vascular permeability and release of cytokines by feedback loop mechanism [73]. High glucose-treated retinal endothelial cells showed an increased level of IL-1 β [74], and incubation of these cells with recombinant active IL-1 β also leads to increased production of ROS and NF κ B activation, therefore suggesting a continuous feedback loop [75, 76]. Furthermore, in experimental diabetic models, the early signs of diabetic retinopathy include vascular leakage, the mechanism by which hyperglycemia-induced vascular leakage is believed to be an inflammatory reaction between ROS and cell adhesion molecules such as CD18 and ICAM-1. This reaction induces breakdown of blood-retinal barrier and causes tissue ischemia, resulting in neuronal or retinal cell death [77]. Result of a recent research documented an important proinflammatory role of MMPs including MMP-2 and -9 in retina of diabetic animal [69, 78]. Although the precise mechanisms have yet to be elucidated, data from numerous studies suggest that MMPs support penetration of inflammatory cells by dissolving the blood-retinal barrier, a hallmark of early changes in DR [41, 69]. As leukocytes transmigrate across the vascular wall, they release MMPs, which in turn degrades tight junction-

related proteins and the surrounding basal lamina, inducing vascular leakage, similar features seen in diabetic retina. Zonulae occludens-1, VE-cadherin, and occludin are substrates for MMP-2, MMP-3, MMP-7, and MMP-9 [79–81]. Basal lamina proteins, such as fibronectin, laminin, and heparin sulfate, are also degraded by MMPs [82]. Involvement of MMPs in barrier disruption is supported by the finding that hyperglycemia disrupts the cell to cell contact. It has been shown that in rodents, broad-spectrum MMP inhibitor BB-3103 decreases endothelial gap formation and occludin loss [83]. The proinflammatory role of MMP-2 and -9 is further supported by that high glucose-activated NF κ B, inducing MMP-9 induction and inhibition of MMP-9 by specific chemical inhibitors and inhibiting production of inflammatory mediators [56, 84, 85]. Knockout mice showed significant reductions in the expression of macrophage inflammatory protein-1 alpha (MIP-1 α), MIP-1 β , and MCP [86]. Additionally, neutrophil and macrophages are known to activate the MMP-2 and -9 secretion [87, 88]. However, addition of inflammatory cytokines to cells upregulates the expression of MMP-2 and -9 along with other various cytokines and chemokines, and administration of IL-1 β to diabetic animal upregulates the expression of MMP-2 and -9.

MMPs and angiogenesis

Diabetic retinopathy is characterized by abnormal angiogenesis which results in immature new vessel leading to vascular leakage, neovascularization, and vitreous hemorrhage [89, 90]. Angiogenesis is a complex multistep process, and it requires proper co-ordination of several growth factors and chemokines. Hyperglycemia induces hypoxia in retinal tissues; hypoxia is a key regulator of angiogenesis by inducing the disruption of balance between proangiogenic and antiangiogenic content and leading ocular neovascularization [91]. Vascular endothelial growth factor (VEGF) is a major mediator of neovascularization in physiological and pathophysiological conditions; it plays a crucial role in the formation of new blood vessels, and it also regulates the hypoxia-induced tissue angiogenesis [92–94]. VEGF, a key angiogenic inducer, may cause pathological neovascularization in retinal microvascular tissue under hyperglycemic condition: in the vitreous of human or retina in rodents, the level of VEGF and its receptor is elevated under hyperglycemia [95, 96]. To initiate retinal angiogenesis, the delicate balance between proangiogenic and antiangiogenic factors is likely to be shifted, such that mitogenic factors are enhanced and/or inhibitory factors are decreased. In the retina of a diabetic rodent, the antiangiogenic factor, pigment epithelium-derived factor mRNA, and protein expression are reduced and upregulate the angiogenic factor

(VEGF) [97–99]. The increased levels of MMP-2 and -9 are often found in angiogenesis [100, 101]. Association of MMP-9 to the cell membrane is considered important in tumor growth and angiogenesis. During angiogenesis, activated endothelial cell secretes proteases, chemokines, cytokines and MMPs. These proteins dissolve the vascular basement membrane, allowing capillary endothelial cell to migrate through basement membrane into the extravascular organization. This helps in providing space for the formation of new vessel [101, 102]. Diabetes-increased VEGF in the retina or vitreous is positively correlated with MMP-2 and -9 upregulation [103–105]. Activated endothelial cells secrete MMP-2 and -9 during angiogenesis, and this might be via the PI3K/Akt and ERK1/2 pathways because it was shown that activation of these signaling molecules stimulate MMP-2 and -9 productions, which have been shown to regulate angiogenesis. Interestingly, the regulation of VEGF is also mediated by PI3K/Akt and ERK1/2 signaling pathway in the retina of diabetic animals [106, 107]. Additionally, a study suggests that interaction of Akt with RhoB may subserve endothelial cell survival during vascular development and maybe pathological angiogenesis, leading to the microangiopathies characteristic of diabetic microvascular disease [58]. Thus, imbalanced MMP-2 and -9 activities have been implicated in the pathogenesis of diabetic retinopathy, and the use of MMPs inhibitors has been suggested as a relevant pharmacological target, especially to prevent the vascular alterations associated with DR. Endogenous inhibitor of MMP-2 and -9 including TIMP1, TIMP2, and specific chemical inhibitors against MMP-2 and -9 could be promising to treat diabetic retinopathy. Overall, it suggests that during pathogenesis of diabetic retinopathy, enhanced MMP-2 and -9 activation is caused by increase in production of ROS/MAPK/angiogenic factors and decrease in production of antioxidant and antiangiogenic molecules. This switches on the button of the progression of DR, and regulation of MMP-2 and -9 by genetic manipulation or chemical inhibition could have potential therapeutic value in preventing the progression of diabetic retinopathy.

Conclusion

Collectively, these studies showed that in diabetes, MMP-2 and -9 appear to play a multiple role as a proapoptotic, proinflammatory, and proangiogenic in retinal microvasculature. Because early blockade of MMPs stabilizes the oxidative stress, apoptosis, inflammation, and angiogenesis to confer both early and long-term protection of retina from hyperglycemia, it suggests that early inhibition of MMPs may be an effective strategy to prevent the progression of DR.

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Conflict of interest None.

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