• Review Article •

# Molecules related to diabetic retinopathy in the vitreous and involved pathways

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## Abstract

● Diabetic retinopathy (DR) is one of the most common complications of diabetes and major cause of blindness among people over 50 years old. Current studies showed that the vascular endothelial growth factor (VEGF) played a central role in the pathogenesis of DR, and application of anti-VEGF has been widely acknowledged in treatment of DR targeting retinal neovascularization. However, anti-VEGF therapy has several limitations such as drug resistance. It is essential to develop new drugs for future clinical practice. The vitreous takes up 80% of the whole globe volume and is in direct contact with the retina, making it possible to explore the pathogenesis of DR by studying related factors in the vitreous. This article reviewed recent studies on DR-related factors in the vitreous, elaborating the VEGF upstream hypoxia-inducible factor (HIF) pathway and downstream pathways phosphatidylinositol diphosphate (PIP2), phosphoinositide-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways. Moreover, factors other than VEGF contributing to the pathogenesis of DR in the vitreous were also summarized, which included factors in four major systems, kallikrein-kinin system such as bradykinin, plasma kallikrein, and coagulation factor XII, oxidative stress system such as lipid peroxide, and superoxide dismutase, inflammation-related factors such as interleukin-1β/6/13/37, and interferon-γ, matrix metalloproteinase (MMP) system such as MMP-9/14. Additionally, we also introduced other DR-related factors such as adiponectin, certain specific amino acids, non-coding RNA and renin (pro) receptor in separate studies.

● **KEYWORDS:** diabetic retinopathy; vitreous; molecular pathway; vascular endothelial growth factor
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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia and the damage to vessels. Metabolic disorders in carbohydrates, fats and proteins could cause multi-organ damage, which led to organ dysfunctions, further resulting in diabetic complications. According to the 2019 International Diabetes Federation (IDF) Global Diabetes Map (Ninth Edition), there are currently approximately 463 million people with diabetes worldwide, and the prevalence among adults (aged 20-79y) is 9.3%. Among them, China has the largest population of patients with about 116.4 million, accounting for a quarter of the total number of patients in the world. Among them, 35.5 million people are over 65 years old.

Diabetic retinopathy (DR) is one of the most common complications of diabetes, as well as the most common retinal vascular disease. It was reported that DR was the main blindness-causing diseases in people over 50 years old[1]. The basic pathological changes of DR are retinal microangiopathy, including fundus neovascularization and fibrous proliferation. Current studies on DR have shown that the core molecule involved in the pathophysiology is vascular endothelial growth factor (VEGF), which can specifically stimulate the proliferation of vascular endothelial cells and promote neovascularization[2]. A great number of studies have confirmed that concentrations of VEGF in the vitreous of DR patients is significantly higher than that of ordinary people[3], and drugs targeting VEGF have already been developed, one of which were anti-VEGF drugs and it has been widely accepted as a mainstream treatment method yet. However, recent studies

discovered that there are many deficiencies in the application of anti-VEGF drugs, such as long-term drug resistance, high cost of treatment, and potential retinal detachment risk caused by intraocular injection[4]. In recent years, molecular research on the pathogenesis of DR has become a hot spot. Existing studies have found that although VEGF is the key molecule in the development of DR, there are hundreds of other molecules in the vitreous that participate in the process. Some of them are VEGF-dependent and others work independently[5]. Here, we present an overview focusing on the pathways and molecules discovered in the vitreous which are found to involve in the development of DR. We hope this review could reveal the intrinsic relationships between these molecules and provide inspirations for research interests and new therapeutic targets.

## VITREOUS AND ITS ROLE IN DIABETIC RETINOPATHY

### Vitreous and Its Relationship with Diabetic Retinopathy

Vitreous is the major content of the eye accounting for 80% of the inner volume of the whole globe which could transmit light to the retina and maintain the shape of the ocular tissue. Nowadays, lots of studies find that vitreous contains a variety of molecules that is associated with the function of other parts of the eye. These molecules can provide nutrition, perform anti-oxidant effect and may play a role in the development of some eye diseases[6]. For the vitreous body is in direct contact with the retina, the factors involved in the development of DR can diffuse into the vitreous, making it possible to study DR by exploring the relevant factors in the vitreous body[1]. As is widely accepted that VEGF is the core molecule in the development of DR, knowing how VEGF in the vitreous performs its biological function can greatly elaborate the mechanism of DR progression.

**Role of Vascular Endothelial Growth Factor in Diabetic Retinopathy** DR is clinically divided into non-proliferative DR (NPDR) and proliferative DR (PDR) according to the emergence of neovascularization, in which VEGF is highly involved. In NPDR stages, hyperglycemia causes damage to small blood vessels in the retina, thus leading to the secretion of VEGF, which acts as a protective role on rescuing the retinal neurons, but it also affects blood vessels negatively[7]. If the damage factors persist for a longer time, high-level of VEGF breaks the balance between pro-angiogenic and anti-angiogenic factors, after which VEGF acts destructively on vascular endothelial cells and stimulates the formation of new blood vessels, which is the symbol of PDR stage[7]. Meanwhile, VEGF in the vitreous can induce new blood vessels to grow into the vitreous cavity, leading to serious complications such as vitreous hemorrhage and tractional retinal detachment.

When hyperglycemia damages the microvascular in the eye tissue, hypoxia occurs. Meanwhile, the level of intracellular hypoxia-inducible factor 1 (HIF-1), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and oxygen regulated protein 150 (ORP150) increases[8-9]. HIF-1 is a DNA-binding protein that binds to the VEGF gene region and initiates transcription of the VEGF gene, thereby causing a large amount of VEGF to be produced. After that, ORP150 begins to play a role as molecular chaperone which binds to VEGF in the endoplasmic reticulum and transports it to the Golgi apparatus, promoting the release of VEGF to the extracellular matrix.

After that extracellular VEGF binds to VEGFR and activates the receptors through transphosphorylation, thereby activating downstream pathways. In this section, we mainly discussed three downstream pathways: phosphatidylinositol diphosphate (PIP2), phosphoinositide-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathway. The detailed pathway and key molecules are presented in Figure 1.

## OTHER DIABETIC RETINOPATHY-RELATED MOLECULES IN THE VITREOUS BODY

In this section, we will introduce newly explored molecules in the vitreous which may have relationship with the development of DR, as well as pathways they involved: Kallikrein-Kinin System (KKS), oxidative stress, inflammation and matrix metalloproteinase (MMP). Also some other molecules apart from these four pathways will be briefly introduced in this section (Figure 2).

**Molecules From Kallikrein-Kinin System** KKS is a set of regulatory systems including kallikrein and kinin in the human body. Kallikrein is a collective name for polypeptides including a variety of bradykinins (BK). KKS is composed of two independently regulated proteolytic pathways mediated by tissue kallikrein (TK) and plasma kallikrein (PK) in human, both of which are expressed in eye and found to be related to the progression of DR *via* the production of bradykinin and spontaneous stimulation of bradykinin receptors[10]. After the activation of BK receptors, KKS performs a variety of physiological effects such as coagulation, fibrinolysis, angiogenesis, *etc*[10].

Up to now, there have been a few studies on the role of KKS in the vitreous of DR patients. In normal eyes, with the function of blood-retinal barrier, some circulating substances can't diffuse into vitreous. However, under DR conditions, substances diffuse becomes easier since the breakdown of the barrier. A group of molecules in plasma KKS family, including high molecular weight kininogen, prekallikrein (PK), coagulation factor XII (FXII) and complement 1 esterase inhibitor (C1-INH) have been identified to accumulate in the vitreous of DR patients by proteomic analysis[11]. First three molecules participate in the activation of KKS, while C1-INH can inhibit KKS by preventing PK from activation. In retinal tissues, the activation of KKS promotes the aggregation

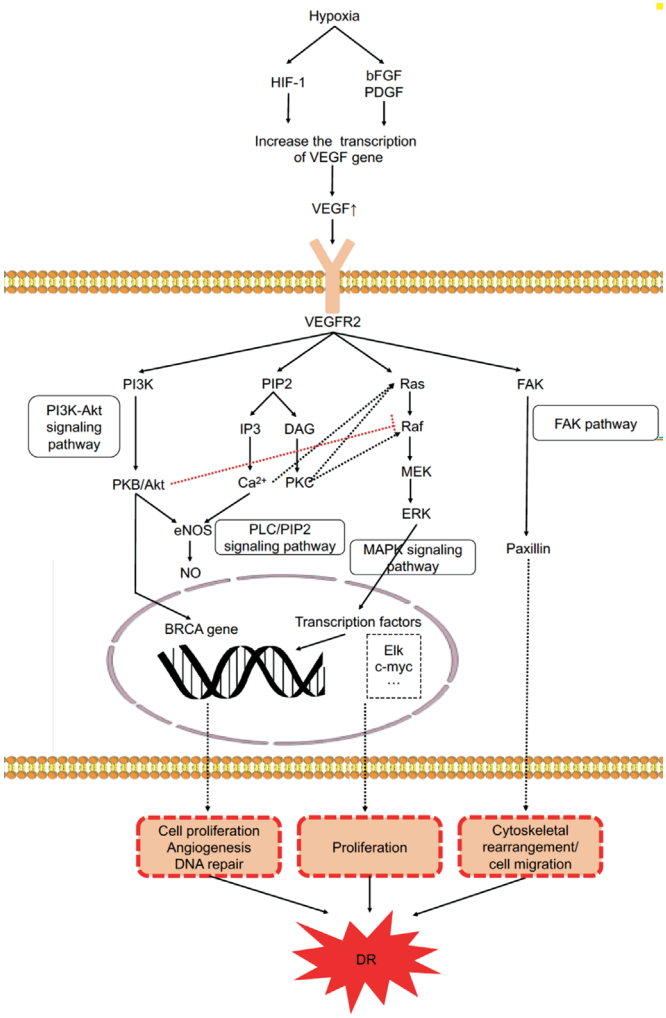


**Figure 1 Scheme of VEGF pathway** In the process of DR, VEGF acts as a core molecule, and is mainly induced by hypoxia. Hypoxia increases the expression of HIF and other growth factors (such as bFGF and PDGF), thereby increasing the transcription of the VEGF gene, translating more VEGF and secreting it out of the cell. Subsequently, VEGF is combined with VEGFR of the target cell (mainly *via* VEGFR2 in DR), and it performs physiological effect mainly through three ways: PI3K-Akt signaling pathway, PLC/PIP2 signaling pathway and MAPK signaling pathway. These pathways subsequently activate nuclear transcription factors and thereby changing gene expression, which ultimately leads to cell proliferation and vascular proliferation. In addition, VEGFR can also activate Paxillin through the FAK pathway, eventually leading to rearrangement of the cytoskeleton and cell migration. VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; DR: Diabetic retinopathy; HIF: Hypoxia-inducible factor; bFGF: Basic fibroblast growth factor; PDGF: Platelet derived growth factor; MAPK: Mitogen-activated protein kinase.

of neutrophils and microglia[12], and at the same time may increase vascular permeability and vascular edema, change the diameter of the vascular and the hemodynamics, and affect inflammation, angiogenesis, and neuronal functions[13]. The detailed mechanism of KKS system was shown in Figure 3.

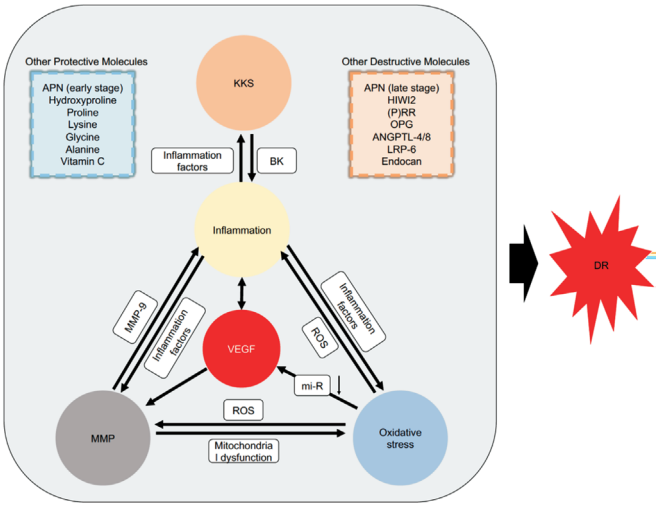


**Figure 2 Scheme of molecules and pathways involved in DR and their relationship** KKS pathway, inflammation, MMP, and oxidative stress are all involved in the process of DR, and they are closely related to each other. The inflammation response can activate the KKS pathway, MMP and oxidative stress, and bradykinin produced by KKS, MMP and ROS induced by oxidative stress in turn promote the inflammatory response. Besides, oxidative stress can activate MMP *via* ROS, and MMP can promote oxidative stress by damaging mitochondria. As for association with VEGF, KKS function independently in the vitreous, while inflammation elevate the expression of VEGF and in turn VEGF intensify the inflammation response. Oxidative stress downregulates certain mi-Rs, which ultimately increase the expression of VEGF, and VEGF can increase the expression of MMP-9. Apart from these four pathways, there are some other molecules are involved, some of them are protective molecules and others are destructive. DR: Diabetic retinopathy; KKS: Kallikrein-Kinin System; MMP: Matrix metalloproteinase; VEGF: Vascular endothelial growth factor; ROS: Reactive oxygen species.

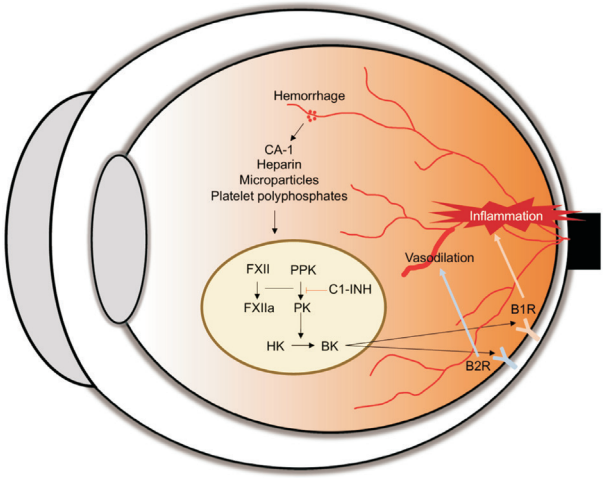


**Figure 3 Scheme of KKS pathway and functions** Retinal vascular hemorrhage activates a series of molecules such as CA-1, heparin, *etc*. These molecules then activate the KKS pathway. The KKS pathway then produces a large amount of bradykinin, which combines with bradykinin receptor 1 and bradykinin receptor 2 on the retina to play a pathogenic role. Among them, B1R mainly induces inflammatory response, while B2R mainly causes dilation of retinal blood vessels.

In addition, some proteomics studies have shown that about 30 kinds of protein related to KKS increased and had significant correlation with diabetic macular edema (DME), and the correlation coefficient was greater than that between VEGF and DME. It is suggested that KKS may be more related to the occurrence of DME than DR[14]. Besides, KKS and VEGF are thought to act independently in the development of DME, for the levels of PK and VEGF in the vitreous samples of DME patients were not correlated[14].

Currently, no human experiments confirmed that KKS was related to retinal neovascularization, but KKS has been proven to promote angiogenesis in other tissues[15]. Therefore, the role of KKS in retinal neovascularization needs to be further explored.

**Molecules Related to Oxidative Stress** Diabetes induced oxidative stress in retinal and capillary cells plays a significant role in the development of DR[16]. Oxidative stress represents the imbalance between oxidants and antioxidants, resulting in generating a huge amount of reactive oxygen species (ROS)[17]. Excessive ROS can inhibit mitochondrial biogenesis and mitochondrial DNA (mtDNA) repair, lower the levels of antioxidants such as Mn-superoxide dismutase (Mn-SOD) and glutathione (GSH), methylate mtDNA to alter the protein expression, which results in mitochondrial dysfunction and changes the permeability of the outer membrane, thus increasing the release of cytochrome C and further inducing cell apoptosis[18]. Besides, ROS can promote the production of inflammatory factors such as nuclear factor kappa-B (NF-κB), protein kinase C (PKC), MAPK *etc.*, intensifying the inflammatory response[19] (Figure 4). Recently, oxidative stress related molecules have been focused by many researchers.

Some oxidative stress-related biomarkers have been found to have altered concentrations in DR patients, which may help patients get earlier and more precise diagnosis. In plasma and vitreous of patients with PDR, the content of superoxide dismutase (SOD) and lipid hydroperoxidation (LPO) were significantly higher than those in the control group, and there was a positive correlation between the two molecules, while other oxidative stress-related biomarkers such as advanced oxidized protein product (AOPP) and malondialdehyde (MDA) showed no differences between groups. Researchers suggested that the combined increase of SOD and LPO can be a biomarker of the progression of DR[20]. Animal studies and human studies of DR asymptomatic patients are needed to further confirm the availability.

Besides, a newly discovered protein, oxidative stress-responsive apoptosis-inducing protein (ORAIP), was found to play a role in DR progression[21]. Under the oxidative stress, ORAIP is rapidly secreted from cells and acts as a pro-apoptotic ligand to induce apoptosis. Some studies have found that the ORAIP

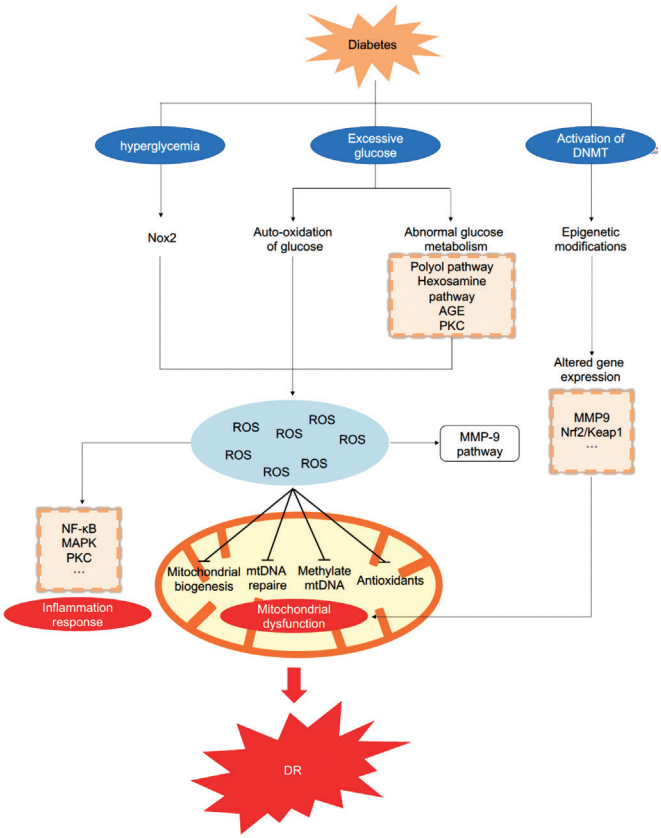


**Figure 4 Scheme of oxidative stress pathway** Under the condition of diabetes, hyperglycemia induce the activation of Nox2 in the cell, and at the same time, the excessive glucose will be metabolized by auto-oxidation and four other glucose metabolism pathways, thereby generating a large amount of ROS. These ROS subsequently disturb mitochondrial biogenesis and mitochondrial DNA repair, abnormally methylate mitochondrial DNA, and reduce the expression of antioxidants. The series of reactions ultimately lead to mitochondrial dysfunction and induce cell apoptosis. In addition, ROS can promote the expression of NF-κB, MAPK and other molecules, thereby intensifying the inflammatory response. ROS can also activate the MMP-9 pathway, which also contributes to DR. NF-κB: Nuclear factor kappa-B; MMP: Matrix metalloproteinase; ROS: Reactive oxygen species; DR: Diabetic retinopathy.

in the vitreous of patients with PDR is significantly higher than that in the control group. It was speculated that ORAIP may cause retinal damage through oxidative stress, but the specific mechanism needs to be further explored[22].

As for the relationship between oxidative stress and VEGF, oxidative stress can change the expression of VEGF *via* alter the levels of microRNAs (miR) such as miR-126, miR-146a and miR-200b. Oxidative stress downregulate all these three miRs, which promotes the expression of VEGF[23-24]. Thus oxidative stress along with VEGF contribute to the progression of DR.

**Molecules Related to Inflammation** During the development of PDR, infiltration and adhesion of inflammatory cells are common pathological manifestations. Inflammation is

the central role in DR, and lots of immune cells, molecular mediators are also involved. In early stages of DR, inflammation plays a protective role against the apoptosis caused by hypoxia, which presents as a defensive reaction of the tissue, but in later stages, uncontrolled inflammation interplays with angiogenesis and coagulation, contributing to the development of DR[25]. Many inflammatory molecules have been detected in the vitreous of DR patients, and most of them are proteins regulated by nuclear transcription factors such as NF-κB. Here we summarize some inflammatory molecules in the vitreous which are involved in DR.

Previous studies have measured various inflammatory molecules in the vitreous specimens and evaluated their role in the development of DR. We divided these inflammatory molecules into three groups: pro-inflammatory molecules, anti-inflammatory molecules and pleiotropic cells factors. Pro-inflammatory molecules referred to factors which can promote inflammation, including interleukin (IL)-1β, IL-6, IL-8, Interferon-γ (IFN-γ), CCL-2/MCP-1, tumor necrosis factor α (TNF-α), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), chemokines CCL2, CCL5, CXCL8, CXCL10, CXCL12[26], CXCL16, ADAM10 and ADAM17[27]. Among them, studies have found that the levels of all above factors except IL-6 in the vitreous of patients with DR are higher than those in the control group, but there is no significant difference between IL-6[26,28]. However, other studies draw different conclusions. One study has shown that in patients with PDR after partial vitrectomy, the levels of MCP-1/CCL-2 and IL-6 in the vitreous increase. It is speculated that the increase of these two substances in the postoperative period is indicative of long-term inflammation, thus having a certain predictive value on postoperative DME[29]. Anti-inflammatory molecules referred to factors which can inhibit inflammation, including IL-37. A study pointed out that IL-37 is also elevated in the vitreous of PDR patients, and its content is related to VEGF-A and Ang-2, suggesting that it plays a role in PDR[30]. Although anti-inflammatory immune regulators like IL-37 are found to be up-regulated, they are more of a passive compensation and are not strong enough to reverse the inflammatory process[28]. Pleiotropic cells factors referred to factors which can both promote and inhibit inflammation under different conditions, include IL-2, IL-4, IL-13 and NO. Among them, studies have found that IL-13 is down-regulated, NO is up-regulated in the vitreous of PDR patients, and there is no significant difference as for IL-2 and IL-4[26,28].

In addition, neutrophil extracellular traps (NETs), another inflammation related products, has been found in the vitreous of DR patients. NETs is the product of neutrophil NETosis, a novel process of neutrophil death differing from necrosis and apoptosis. Studies have shown that under the induction of high glucose, NADPH oxidase participates in the formation of NETs in eye tissues, it is found that NETs in the vitreous of PDR patients is higher than those in normal people. Further experiments found that the content of NETs in the vitreous decreased after anti-VEGF treatment, suggesting that NETs may play a certain role in the occurrence and development of PDR and have a certain relationship with VEGF[31].

Another inflammatory mediator leukotriene (LT) also plays a critical role in DR. LTs in retina contribute to inflammatory response, oxidative stress and the expression of pro-angiogenic agents. As for relationship with inflammatory response, hyperglycemia induces inflammation, increases retinal leukostasis, and recruits LTs-producing leukocytes, which further aggravates the inflammatory response and forms a vicious circle. For oxidative stress, after ischemia reperfusion injury of retina, leukocytes produce more LTs which bind to receptors and reduce the scavenging of free radicals and activate NADPH oxidase, thus inducing oxidative stress[32]. Besides, LTs can activate MAPK pathway and induce the production of TNF-α, which increases the production of VEGF[33] and MMP-9[34], together with Nox-1 after the activation of NF-κB in LTs[35], these mechanisms contribute to retinal neovascularization.

Apart from NETs and LT, other inflammation-related molecules such as sCD200, nucleotide binding domain containing Pyrin domain 3, and leucine-rich repeat receptor (NLRP3) inflammasome have been found to have a higher level in the vitreous of PDR patients than that in control group[36-37], and NLRP3 inflammasome pathway was associated with decreased vitamin D concentrations in the vitreous of PDR patients[38]. Besides, recent study has shown that content of 7 kinds of oxylipins including lipoxygenase (LOX)- and cytochrome P450 (CYP)-derived oxylipins in PDR patients discriminated from non-diabetic control, indicating an underlying imbalanced inflammation-resolution homeostasis in PDR[39].

In conclusion, inflammatory molecules play an important role in DR, and lots of factors have been identified (Figure 5). For VEGF takes part in the inflammatory response of the tissue, levels of most of these factors have close relationship with VEGF. It's promising that these inflammatory molecules being the fundament to develop new treatment. But current studies concerning these factors haven't figured out the exact mechanisms, so further researches are needed.

**Molecules Related to Matrix Metalloproteinases** MMPs are a large family of proteases that require the involvement of metal ions such as $Ca^{2+}$, $Zn^{2+}$ to perform their functions. The concentration of MMPs in tissue is very low in normal adults, but under the condition of inflammation, high glucose or oxidative stress, the concentration of MMPs will be up-

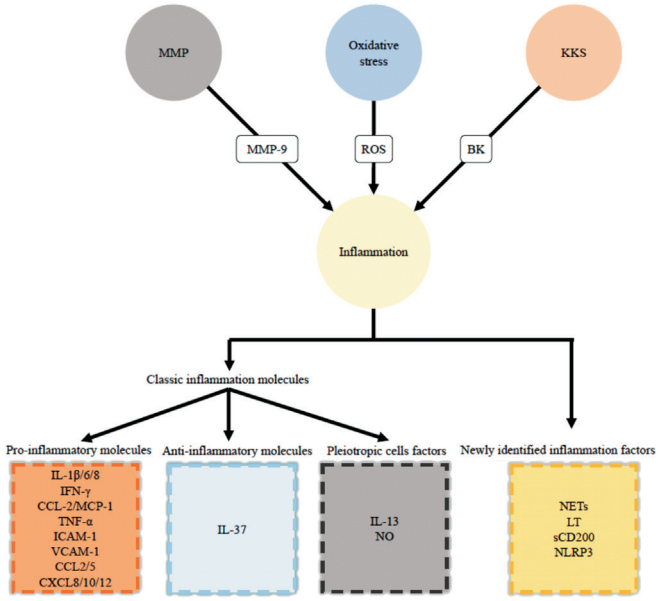


**Figure 5 Inflammation factors involved in DR** Inflammation is closely related to the other three systems in this article. MMP-9, oxidative stress products ROS and KKS system products BK can all induce the production of inflammatory factors. We divide the classic inflammatory factors into three categories: pro-inflammatory molecules, anti-inflammatory molecular and pleiotropic cytokines, at the same time we introduce some newly discovered inflammatory factors related to DR that exist in the vitreous. DR: Diabetic retinopathy; KKS: Kallikrein-Kinin system; BK: Bradykinins; MMP: Matrix metalloproteinase; ROS: Reactive oxygen species.

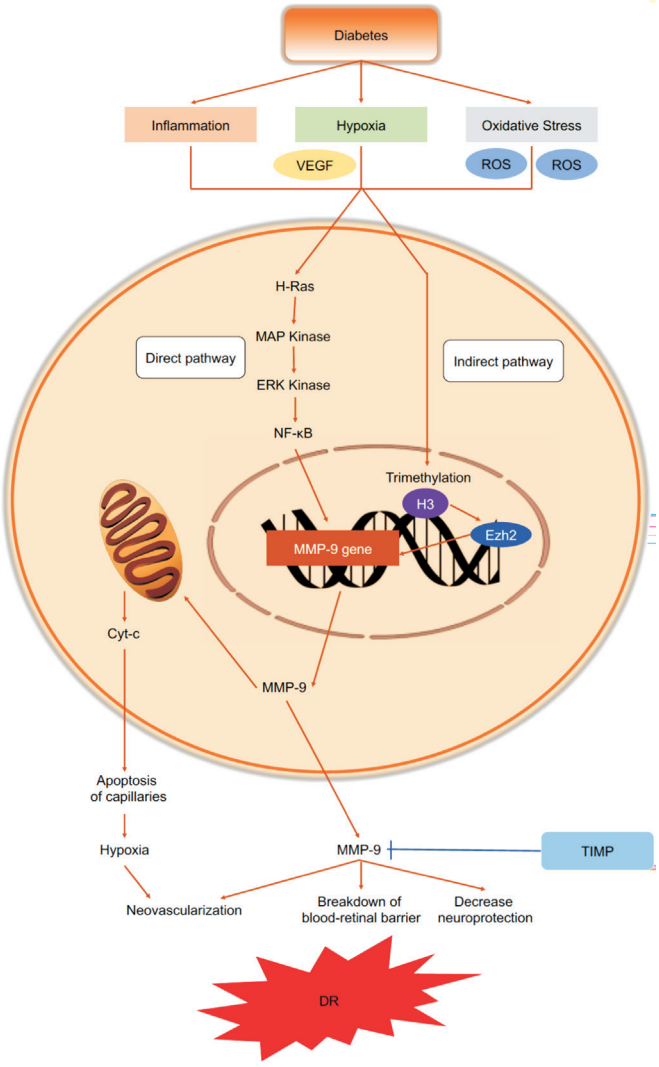


**Figure 6 Scheme of MMP-9 pathway** Inflammation, hypoxia and oxidative stress caused by diabetes can activate MMP-9 expression through direct and indirect pathways. Subsequently, MMP-9 caused mitochondrial dysfunction and the mitochondria released Cyt C to induce the apoptosis of vascular endothelial cells, further aggravating tissue hypoxia and inducing neovascularization. On the other hand, MMP-9 is secreted from cells and directly participates in angiogenesis, while degrading the blood-retinal barrier and reducing the ability of retinal neuroprotection, which ultimately leads to DR.

regulated. Generally, MMPs play a role in tissue remodeling, organ growth and development, angiogenesis, inflammation, and cell migration[40].

MMPs are also widely expressed in ocular tissue, and they are involved in the development of various ophthalmic diseases. Many studies have confirmed the vital role of MMPs in the process of DR. Although the specific mechanism is still unclear, it is widely accepted that in the presence of high glucose, hypoxia and inflammation, MMPs in the retina will be upregulated and degrade the extracellular matrix in the basement membrane of microvessels, thereby destroying blood-retinal barrier, leading to the changes in vascular permeability, which is the core pathological change of DR. At the same time, the degradation of the basement membrane will promote the migration of vascular endothelial cells, thereby forming new retinal vessels[41]. What's more, MMPs can reshape the extracellular matrix, leading to severe lesions such as retinal hemorrhage, edema, and vitreous hemorrhage during the PDR stage[40].

Current studies have found that levels of MMP-1, MMP-7, MMP-9 and MMP-14 are higher in the vitreous of PDR compared with control[42]. MMP-9, a gelatinase, is thought to be the most important MMPs in DR and has been greatly explored. High glucose induced inflammation, oxidative stress and hypoxia induced VEGF can stimulate MMP-9 through direct ways (induction of transcriptional factors to directly increase the expression of MMP-9) or indirect ways (epigenetic pathways). MMP-9 participates in the DR progression mainly through 3 ways (Figure 6). The first one is the angiogenesis. Interestingly, MMP-9 has dual role in angiogenesis. In early stages of DR, MMP-9 acts as an angiogenesis antagonist, for it can activate the angiostatin, which is an angiogenesis inhibitor[43]. Besides, MMP-9 contributes to the apoptosis of retinal capillary cells, thus inhibit the angiogenesis. The induction of apoptosis is mainly through the damage of mitochondria. Second, MMP-9 plays its role through the

destruction of prominin-1/CD133, which is an important molecule in development, protection and regeneration of retina. The third is the breakdown of blood-retinal barrier. MMP-9 can break junctional complex molecules such as occludins and claudins, as well as degrade components of basement membrane, causing the breakdown of blood-retinal barrier[42].

For the inhibition of MMPs, one research has analyzed the differences in the concentrations of four types of tissue inhibitor of matrix metalloprotease (TIMP), including TIMP-1, TIMP-2, TIMP-3, TIMP-4 in the vitreous of PDR patients. The results showed that compared with the control group, levels of TIMP-1 and TIMP-4 in the vitreous of PDR patients were significantly increased, while the TIMP-2 and TIMP-3 presented no significant difference, indicating that different types of TIMPs in the eye tissue expression differently and might play different role in DR[44].

In addition to MMPs and TIMPs, some studies have also investigated extracellular MMP inducers (EMMPRIN) in DR. One research found that in the vitreous of PDR patients, the level of EMMPRIN was significantly higher than that of the control group and was positively correlated with MMP-1, MMP-9 and VEGF levels. In human retinal microvascular endothelial cells cultured *in vitro*, EMMPRIN was found to induce the expression of MMP-1, HIF-1α and its downstream VEGF. It is speculated that EMMPRIN may have similar effect *in vivo*[45].

**Other Molecules that May be Related to Diabetic Retinopathy** Apart from these four groups of molecules introduced above, there are many studies focusing on other molecules recently, but they have not been carried out in depth, so we will briefly introduce these progressions in this section. These molecules include adiponectin, certain specific amino acids, PIWI-like protein, renin receptor, non-coding RNA and so on.

Some researchers have studied the role of adiponectin (APN) in the development of DR. In other tissues throughout the body, APN is closely related to tissue fibrosis, but its effect differs among different tissues. In patients with PDR, the APN concentration in the vitreous was significantly higher than that in the control group, while the concentration in serum was significantly lower than that in the control group. Meanwhile, it was found that intraocular APN has the function of inhibiting new blood vessels, and making DR progress toward fibrosis. In addition, by observing the role of APN in PDR at different periods, the researchers found that APN may play a therapeutic role in the early stages of PDR, while in the later stages, it promotes fibrosis in the posterior segment of the eye, suggesting that APN functions differently in different stages of the disease[46].

As for the role of certain amino acids in the development of DR in the vitreous, some studies have tested the concentration of hydroxyproline, proline, lysine, glycine and alanine in the vitreous, and found that the concentration of certain amino acids was significantly higher than that in control group. The possible mechanism for this phenomenon is these amino acids might induce the accumulation of triglycerides and APN, further produce adipogenesis, and at the same time increase the antioxidant capacity and reduce the levels of pro-angiogenic markers that may intensify disease. Therefore, researchers speculated that the increased content of these amino acids in the vitreous may have a protective effect[47].

Researchers from India studied the P-element-introduced Wimpy Testis (PIWI) -like protein, Piwi like RNA-mediated gene silencing 2 (HIWI2). Western blot analyses found that the level of HIWI2 protein in the vitreous of PDR patients significantly increased compared to non-PDR patients. Further studies on human retinal pigment epithelial cells cultured *in vitro* showed that the expression of HIWI2 protein was significantly increased under oxidative stress and VEGF condition, and the expression level was dose-dependent. In addition, after the HIWI2 gene was knocked out, VEGF and growth factor decreased significantly under oxidative stress, which is accompanied with the decrease of epithelial mesenchymal transition (EMT) biomarker. Thus, the HIWI2 protein may play several roles in the development of DR, including the regulation of VEGF, growth factors, and EMT[48].

New progress has also been made in research on the (pro) renin receptor [(P)RR]. (P)RR is a common receptor for renin and its precursors, and it is also a component of the renin-angiotensin system (RAS). Studies have found that a RAS-related protein system exists in human eye tissues, and the protein system can stimulate the expression of VEGF-A. At the same time, the concentration of (P)RR in the vitreous of PDR patients was positively correlated with renin, and VEGF, and was significantly higher than that in control group, suggesting that (P)RR is also closely related to VEGF-A-driven angiogenesis. This group of researchers also designed an RNA that can inhibit (P)RR, which they hope could depress angiogenesis by inhibit the translation of (P)RR. And further research is needed to clarify its therapeutic ability and feasibility of clinical application[49].

Besides these proteins, non-coding RNA was also found to participate in the progression of DR in vitreous. It is reported that increased expression of HOX antisense intergenic RNA (HOTAIR) was observed in vitreous of PDR patients. HOTAIR usually exerted its capabilities by preventing oxidative stress and modulating epigenetic pathways involving histone methylation, histone acetylation, DNA methylation, and transcription factors, thus may function as a critical

epigenetic mediator of angiogenesis in DR[50]. Another research also found that expression of non-coding RNA ANRIL was positively correlated with Ang II, p65 and VEGF expression in the vitreous of PDR patients[51], and previous study has found that overexpression of ANRIL may be a result of the activation of the RAS and NF-κB pathway, thus ANRIL was confirmed to be associated with the onset of DR[52]. Expression of miR-409-5p was also found to increase in vitreous of PDR patients. As overexpression of miR-409-5p promotes the proliferation, migration, and tube formation, and increased VEGF expression and secretion, anti-miR-409-5p therapy may provide a novel strategy in treating DR[53].

Another study focused on levels of vitamin C in the vitreous. Vitamin C is an anti-oxidant and thought to be related to oxidative stress in the eye. Researchers found that the level of vitamin C in PDR patients' vitreous showed a tenfold decrease, and had correlation with the degree of macular ischemia. But whether these two phenomenon has causal relationship remains unknown[54].

Other factors include osteoprotegerin (OPG)[55], angiopoietin-like Protein 4 (ANGPTL-4)[56] and ANGPTL-8[57], low-density lipoprotein receptor-related Protein 6 (LRP6)[58], endocan[59], *etc*. have been found to be related to DR progression. These factors are higher in the vitreous of PDR patients than in the control group and are related to VEGF, but further research is needed to reveal their mechanism of action.

## CONCLUSION

In the last decade, VEGF related research has made major breakthroughs in the pathogenesis of DR, and thus developed a highly specific anti-VEGF monoclonal antibody therapy, which is a milestone in the history of DR treatment. However, anti-VEGF treatment can only delay the disease progression, and it has problems such as high cost, short effective time, frequent intraocular injection, and drug resistance. Therefore, further exploration of DR-related pathogenesis and targeted treatment options are needed. This article reviews the clinical and basic research on the changes of intravitreal molecules in the development of DR, and summarizes researches on KKS pathway, oxidative stress, inflammation and MMPs-related pathways. In addition, the results of many studies on a single isolated molecule such as APN are also listed. By reviewing the research of DR-related molecules in the vitreous body in recent years, the author aims to provide inspirations for the study of DR mechanism and lay the molecular foundation for new treatment methods. Hope that related research will be applied to the clinic and set the next milestone for DR treatment.

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