Role of IGF-1 signaling in the pathology of diabetic retinopathy

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Diabetic retinopathy (DR) is a common eye disease caused by diabetic mellitus and can lead to blindness of eye. Currently, researches on the mechanisms and clinical interventions of DR are at an early stage. Under the condition of DR, insulin is usually used for the control of blood glucose. Similar to insulin, Insulin-like growth factor-1 (IGF-1), a protein which has been reported to lower the level of blood glucose, may also promote cell survival and proliferation in various tissues. However, effects of IGF-1 on DR are complex and controversial. The effect of IGF-1 on the normalization of blood glucose is beneficial for the patients, especially those with severe insulin resistance. However, IGF-1 mediates a strong mitogenic signaling and long term application of IGF-1 may aggravate retinal deterioration through promoting the proliferation of retinal endothelial cells. IGF-1 enhances the expression of vascular endothelial growth factor and erythropoietin. The binding of IGF-1 to IGF-1R also promotes the activation of multiple signaling pathways, including phosphatidylinositol 3-kinase/protein kinase B pathway, mitogen activated protein kinase pathway and nuclear factor-kB signaling pathway. All of these have been shown to be associated with the pathology of DR. In this review, we discuss the signaling pathway of IGF-1, evidence for a functional relationship between the IGF-1 and DR, as well as a possible role of IGF-1 signaling pathway in the process of DR.

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Introduction

Diabetes is a serious metabolic disease characterized and diagnosed by hyperglycemia. Hyperglycemia is the common property of the two major forms of diabetes – the immune-system-dependent Type 1 diabetes and insulin-resistance and beta cell dysfunction of Type 2 diabetes. Both forms of diabetes are associated with elevated risks of cardiovascular disease and this is due to hyperglycemia and interactions between hyperglycemia and other cardiovascular risk factors such as blood pressure and dyslipidemia and life style factors such as cigarette smoking\textsuperscript{[1]}. The vascular complications are usually described as microvascular and macrovascular disease with the former being expressed clinically as nephropathy, neuropathy and retinopathy and the latter as coronary artery disease and myocardial infarctions as well as strokes and amputations due to peripheral vascular disease\textsuperscript{[2]}. Hyperglycemia is very closely associated with microvascular disease and most likely a mediator of the pathology. The relationship between hyperglycemia and macrovascular is much less strong and the pathology most likely arises from interactions between hyperglycemia and other cardiovascular risk factors and other factors associated with the insulin-resistant state\textsuperscript{[3, 4]}. 

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Diabetic retinopathy (DR), a complication of diabetes that affects the eyes, results primarily from damage to the blood vessels caused by the hyperglycemia of diabetes. DR results in the gradual loss of sight leading potentially to blindness [5]. Pathologically, DR occurs as a result of leakage of blood from the blood vessels at early stage which is termed non-proliferative DR. Late stage DR is characterized by abnormal growth of vessels and termed proliferative DR [5]. Proliferative DR is the more severe type of DR and is one of the most common leading causes of blindness. Due to its serious impact on the function of eyesight, the mechanisms mediating the proliferation of endothelial and vascular smooth muscle cells and the resulting angiogenesis have been the subject of considerable interest and experimental investigations.

Insulin-like growth factor-1 (IGF-1) is a polypeptide with 70 amino acids and its chemical structure is similar to insulin. IGF-1 is associated with early development and it is anabolic in adults. IGF-1 is a critical regulator of many physiological functions, including glucose metabolism, cell survival and proliferation [6]. In vitro and in vivo investigations have shown that IGF-1 promotes the uptake of blood sugar and the synthesis of glycogen. On the other hand, IGF-1 inhibits protein catabolism [7, 8]. Most importantly, IGF-1 administration has actually normalized the insulin sensitivity and systemic administration of recombinant IGF-1 reduces the levels of insulin and glucose in the blood of diabetic patients with decreased response to insulin, which indicates that IGF-1 is capable of increasing insulin sensitively [9]. Thus, IGF-1 has the potential to have broad effects on insulin resistance and hyperglycemia as factors mediating the cardiovascular complications of diabetes including potentially an effect of the development and progression of DR.

As well as potentially systemic effects, IGF-1 also has direct actions on the cells associated with DR. IGF-1 may also act on and enhance glucose transport in retinal endothelial cells and this process is associated with the major signaling proteins, protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) [10]. A pro-survival cellular effect is another recognized role for IGF-1. IGF-1 induces the activation/phosphorylation of protein kinaseB/Akt in PC12 cells and mediates anti-apoptotic functions [11]. In our recent research, we found that IGF-1 confers a neuroprotective effect in human retinal pigment epithelial cells exposed to sodium nitroprusside [12]. As toxic free radicals destroy the vascular endothelial cells in the early stage of DR, the protective effect of IGF-1 or its signaling agonists against cell death may be beneficial for the prevention or treatment of DR. As well as these metabolic and pro-survival effects, in the context of its role in DR, the effects of IGF-1 on cell division and growth are important to consider [13, 14]. In the retina, IGF-1 is linked to the proliferation and growth of retinal endothelial cells and this action is considered to be a strong contributor to the development of DR, especially in the later stages of DR [15].

As discussed above, IGF-1 may lower blood glucose and thus have a favorable systemic effect on the development and progression of DR, however the direct pro-survival and proliferation-prompting effects of IGF-1 may be undesirable responses in the context of DR. Thus, on the background that the pathogenesis of DR is complex and the role of IGF-1 in DR is complicated, the balance between the actions to control blood glucose and the direct actions on the endothelial cells involved in retinal neovascularization need to be explored and fully understood before consideration can be given to the clinical use of IGF-1 in patients with DR. An enhanced understanding of the molecular pathways and mechanisms of IGF-1R signaling and its relation with DR is required to identify and potentially exploit a therapeutic target for the treatment of DR. In this review, we will examine the current understanding of the IGF-1 signaling pathway, the effect on the pathology of DR and the potential role of IGF-1 signaling in the pathogenesis of DR with a view to identifying a potential therapeutic modality or target.

**IGF-1 and IGF-1 receptor signaling**

IGF-1 plays important roles in cellular proliferation and survival [16]. In the clinic, IGF-1 deficiency in children is viewed as a pivotal mediator of dwarfism [17], and IGF-1 is viewed as a pivotal factor promoting the effect of growth hormone, as it is responsible for the synthesis of various proteins [18]. The drug, mescaline, which is a peptide analog of IGF-1, is approved for increasing final height in children with short stature caused by severe deficiency of endogenous IGF-1 in the body [19]. In addition to growth stimulation, IGF-1 has many important metabolic actions, including actions of glucose metabolism and glycaemia. Systemic IGF-1 decreases blood glucose levels by direct inhibition of renal gluconeogenesis and by binding to IGF-1 receptors distributed in skeletal muscle, and thus, the glucose transport activity is enhanced [20]. This effect is viewed as an important mechanism to maintain glucose homeostasis in the body [20]. IGF-1 is mainly produced and secreted by the liver and fibroblasts and most of IGF-1 is bound to IGF-1 binding proteins (IGFBP) in the blood, which serve as the carrier protein for IGF-1. There are at least 6 kinds of IGFBP (IGFBP1-IGFBP6) and IGFBP3 is the major IGFBP in the serum [21]. Most of IGF-1 binds to its receptor in the serum. Binding of IGF-1 to IGFBP hinders the contact with IGFR1 in the cell surface and extends the lifespan of IGF-1 [22].
Therapeutic Targets for Neurological Diseases

About 2% IGF-1 is free and the unbound IGF-1 is unstable in plasma where the N-terminal can be cleaved and metabolized to des-(1-3) IGF-1 and the tripeptide glycine-proline-glutamate [23, 24]. The concentration of IGFBP in the serum is also highly correlated with the level of blood glucose and it may be used for monitoring insulin sensitivity [25].

The action of IGF-1 is primarily mediated through activating and binding to its specific cell surface receptors [26]. The IGF-1R is a transmembrane protein which is comprised of two α and two β sub-units. Binding of IGF-1 to the IGF-1R leads to the activation of multiple intracellular signaling pathways which play roles in apoptosis, cell proliferation, cellular differentiation and migration during development and in adults [27]. Phosphorylation of the IGF-1R is crucial for the activation of survival-promoting signaling [28]. IGF-1 is able to bind to both the IGF-1R and the insulin receptor, both of which are composed of two α subunits and two β subunits, α subunits are exposed out of the membrane and β subunits are transmembrane proteins, which possess the tyrosine kinase functional signaling domains [29]. The affinity of IGF-1 is much higher for IGF-1R than for the insulin receptor. IGF-1R is a receptor tyrosine kinase, thus, IGF-1 initiates the activation of IGF-1R leading to the auto trans phosphorylation of the IGF-1R and the initiation of multiple signaling pathways. The PI3K/protein kinase B (PI3K/Akt) pathway is one of the key signaling pathways downstream of the IGF-1R [30].

The binding of IGF-1 to its receptor triggers the activation of PI3K, which subsequently phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) located on the cellular membrane and converts PIP2 to phosphatidylinositol 3,4,5 trisphosphate (PIP3). PIP3 recruits the kinase Akt to the membrane, so that phosphoinositide-dependent kinase-1 (PDK1) could contact Akt directly and phosphorylate it [31]. Mammalian target of rapamycin complex 2 (mTORC2) is the other major upstream kinase for the activation of Akt [32]. PDK1 phosphorylates Akt at Thr308 [33], while mTORC2 phosphorylates Akt at Ser473 [34]. Akt is fully activated (as a kinase) when both of these sites are phosphorylated. Activated Akt recognizes Ser/Thr motifs within the consensus sequence, RXRXXS/T, and as this sequence is quite common, Akt promotes the phosphorylation of many downstream substrates containing this motif. One of the main functions of PI3K/Akt signaling is to promote cell proliferation and protein synthesis, in most cases, these effects were mediated through regulation of the mTOR and ribosomal S6 kinase [32]. Akt also protects against cell apoptosis directly by inactivating pro-apoptotic molecules, such as Bad, Bim and the forkhead (FoxO1/3a) transcription factors as well [35].

IGF-1 plays an important role in mutagenesis and cell proliferation through governing PI3K/Akt signaling. Some of these effects may be mediated through the Mitogen-activated protein kinase kinase/extracellular regulated protein kinases (MEK/ERK) signaling pathway. IGF-1 triggers ERK pathway activation through the Ras/Raf/MEK cascade [36]. Activated ERK (phosphoERK) directly activates various downstream kinases and transcription factors, including ribosomal S6 kinase, cyclin-dependent kinase and myc. Both of these signaling pathways have been implicated in the stimulation of cell proliferation [37-39]. The IGF-1R signaling is illustrated in Figure 1.

Pathology of Diabetic Retinopathy

DR is a damage to the retina caused by diabetes and prolonged hyperglycemia may result in blindness in adults. The retina functions to receive various images and send these signals to a specific region of brain. External environmental factors (such as sunlight exposure and hypoxia) and internal

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Figure 1.IGF-1 receptor signaling pathway. Most of IGF-1 in the body is bound to IGF-1 binding protein (IGFBP), which makes it unable to contact cell surface receptor. Free IGF-1 binds to IGF-1 receptor (IGF-1R) with high affinity. While at high concentration, IGF-1 may also bind to insulin receptor (IR). Binding of IGF-1 to its receptor phosphorylates IGF-1R and activates phosphatidylinositol 3-kinase (PI3K), activated PI3K catalyzes the conversion of Phosphatidylinositol 4,5-Bisphosphate (PIP2) to Phosphatidylinositol 3,4,5-Trisphosphate (PIP3), elevated PIP3 activates phosphoinositide dependent kinase-1 (PDK1) and protein kinase B (Akt), phosphatase and tensin homolog (PTEN) is responsible for the dephosphorylation/innactivation of Akt, activated Akt acts on various downstream targets, such as Foxhead protein O, GSK-3, CREB and p90RSK, and this signaling is a particularly important survival-promoting signal. IGF-1R may also activate Ras/Raf/MAPK signal pathway and play a role in cell survival and proliferation.
pathological conditions (such as hyperglycemia) may cause damage to the retina [40]. In the condition of diabetes, the sustained hyperglycemia of uncontrolled diabetes may produce damage to the retina [41]. In most cases, DR results from alterations in the blood vessels of the retina, or DR is viewed as the result of microvascular retinal changes [42]. In the early stage of DR, many factors are involved in the process of eye complications in individuals with diabetes [43]. Production of advanced glycation end-products during the process of diabetes mellitus results in overload of oxidative stress and activates pro-inflammatory signaling [44]. Overproduction of oxygen free radicals decreases the thickness of vessel wall and impairs vascular functions [45]. Inflammation is usually accompanied by an increased production of cytokines and it was initiated as a secondary event by hyperglycemia [46]. Chronic inflammation leads to injury to the arterial wall in both the peripheral or ocular vascular system [46]. PKC also has been associated to vascular alterations, such as increase of permeability, leukocytes adhesion and synthesis of extracellular matrix, these disturbances are linked to the development of retinopathy [47]. High blood glucose also leads to apoptosis or death of pericytes and incompetence of the vascular walls, and damaged blood vessels in the retina may swell and leak fluid or blood into the eye, which is usually accompanied by macular edema or retinal hemorrhages, and leads to vision loss. In the late stage of DR, the lack of oxygen in the retina stimulates the expression of vascular endothelial growth factor (VEGF) and erythropoietin (EPO), both of which promote the proliferation of blood vessels, inducing growth of new blood vessels on the surface of the retina [48, 49]. However, these new blood vessels or capillaries are abnormal and can’t supply the retina with proper blood flow. The newly formed blood vessels are fragile with high risk of rupture, and blood may easily leak from the vessels causing the scarring which can impair eye sight, finally causing blindness [50].

Role of IGF-1 signaling in the pathology of Diabetic Retinopathy

Insulin is the most efficacious hormone for the treatment of hyperglycemia. It mediates a range of direct and indirect actions which lower hyperglycemia. IGF-1 is a polypeptide hormone about the same size as insulin and it has a similar structure and function to insulin. In contrast to insulin which is made and released from the beta cells of islets in the pancreas, IGF-1 is mostly produced in the liver and fibroblasts where insulin has a stimulatory effect on the production of IGF-1 [51]. In normal conditions, insulin is the major hormone regulating the level of glucose in the body, and IGF-1 plays a supplementary role. Under the conditions of DR, insulin or oral hypoglycemic drugs should be firstly considered to be used to control the level of blood glucose, however, the application of IGF-1 could be considered when there is an extreme insulin resistance or insensitivity whereby recombinant IGF-1 might be an alternative strategy to normalize the blood glucose level and prevent the acute complications of diabetes [52]. However, the application of IGF-1 has obvious side effects, including swelling of the optic nerve behind the eye and headache, and these side effects hinder its clinical application [53]. Another major limitation to the use of IGF-1 is the mitogenic property of IGF-1. Studies have shown that there is an increase in the incidence of proliferative DR after application of IGF-1 [54, 55].

Recently, reports have described that IGF-1 plays a role in the process of DR. Exogenous administration of IGF-1 through intravitreal or intracorneal injections accelerate neovascular changes in retina and cornea in animals [56]. In patients with DR, a higher concentration of IGF-1 has also been observed in the vitreous fluid [57, 58]. These researches highlight the damaging role of IGF-1. On the other hand, in the eye, IGF-1 receptors are widely distributed in many tissues, and treatment with an IGF-1 receptor antagonist, which blocks the role of IGF-1, decreases the formation of microvascular in the retina [15]. Moreover, disruption of the IGF-1 receptors leads to reduced growth of endothelial cells and slows down the progress of neovascularization in mice [59]. Consistent with the above results, in humans, mutations of the DNA sequences of IGF-1 receptor decrease retinal vascular changes [60]. These results demonstrate that IGF-1 signaling may participate in the process of DR and antagonism of IGF-1 signaling is beneficial for the patients with DR.

IGF-1 imparts a positive effect on cell division, growth promotion and angiogenesis. In the retina, IGF-1 is synthesized by various cells, including vascular endothelial cells, pericytes, glia cells, retinal ganglion cells, and retinal pigment epithelium [61]. IGF-1R is expressed widely in the eye, including RPE cells and retinal endothelial cells. Activation of IGF-1R by IGF-1 causes a stimulation of hypoxia-inducible factor 1a protein synthesis and enhances the expression of the downstream target, VEGF [62]. In the retina, IGF-1 also mediates the induction of VEGF in human retinal pigment epithelial cells [63]. VEGF is an important protein that helps to create a novel blood vessel, it is also involved in the regulation of blood vessel growth, maturation and function [64, 65]. The induction of VEGF by IGF-1 plays an essential role during the development of proliferative DR [65]. As mentioned above, in the condition of diabetes, the expression of EPO is increased as well. Similar to the effect of IGF-1 on VEGF, IGF-1 could induce the activation of hypoxia-inducible factor-1, leading to increased mRNA levels and the production of EPO [66]. This effect may
contribute to the formation of new blood vessels induced by IGF-1.

The biological actions of IGF-I are mediated by binding to the IGF-IR and the activation of several intracellular kinases, including PI3K. PI3K play a pivotal role in stimulating the growth and proliferation of vascular smooth muscle cells, and inhibition of PI3K by wortmannin reduced early vascular smooth muscle cell (VSMC) replication in rats [67]. Evidence suggests that PI3K activation modulates vascular smooth muscle cell growth, proliferation and survival through a series of phosphorylation events [68]. Akt, which is a critical enzyme that exerts translational control of protein synthesis, has been identified as a downstream target of PI3K [69]. In VSMCs, inhibition of Akt activation/phosphorylation blocks the stimulatory effect of serum on DNA synthesis, cell-cycle progression, and cell proliferation [70]. mTOR is a substrate of Akt. Inhibition of PI3K by LY294002 or blockage of mTOR by rapamycin could attenuate the level of phosphorylated mTOR and thereby reverse the effect of PI3K/Akt on the proliferation of retinal pigment epithelial cells [71]. Other components such as nuclear factor kappa B and ERK may also activate a mitogenic signal-transduction cascade and are also associated with the regulation of proliferation of VSMCs and epithelial cells [72]. The role of IGF-1 signaling in the pathology of diabetic retinopathy is illustrated in Figure 2.

Conclusions

Prolonged and chronic hyperglycemia is one of the leading causes of vascular complications in diabetes. DR is a serious complication occurring in the retina. Maintaining blood glucose levels within the normal range is the best way to decrease the risk of complications, including DR. Systemic administration of IGF-1 may be considered for the treatment of hyperglycemia when cells or insulin receptors fail to respond to the normal stimulation of insulin. IGF-1 might also be effective to prevent the apoptosis of normal endothelial cells in the eye before the new and abnormal vessels are formed [12]. However, IGF-1 is known to be mitogenic and plays a role in the growth and proliferation of vascular endothelial cells. In this condition, the therapeutic use of IGF-1 has to be considered in the light of the potential complications with DR for patients receiving recombinant IGF-1. Furthermore, blockade of IGF-1 signaling pathway at the early stage should be promising to prevent the formation and growth of newborn blood vessels [73]. Currently, laser treatment is the major effective way to treat DR; however, targeting on IGF-1 signaling may serve as an alternative or adjunct treatment. The role of IGF-1 signaling in the development of DR is complicated, Whether or not IGF-1 signaling will become a therapeutic target for the protection of retina under the condition of hyperglycemia will depend upon further research. Further researches will include studies of its analogs and downstream targets.

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