• Review •

Research progress on the role of connective tissue growth factor in fibrosis of diabetic retinopathy

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Abstract

• Diabetic retinopathy (DR) is one of the most important types of diabetic microangiopathy, which is a specific change of fundus lesions and is one of the most serious complications of diabetes. When DR develops to proliferative DR, the main factors of decreasing vision, and even blindness, include retinal detachment and vitreous hemorrhage caused by contraction of blood vessels by fiber membrane. Recent studies reported that the formation of fiber vascular membrane is closely related to retinal fibrosis. The connective tissue growth factor (CTGF) is a cytokine that is closely related to DR fibrosis. However, its mechanism is poorly understood. This paper summarizes the recent studies about CTGF on DR fibrosis for a comprehensive understanding of the role and mechanism of CTGF in PDR.

• KEYWORDS: proliferative diabetic retinopathy; fibrosis; connective tissue growth factor; vascular endothelial growth factor

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INTRODUCTION

With the improvement of living standards, the incidence of diabetes yearly increases and its accompanying microvascular complications, specifically diabetic retinopathy (DR), gradually become one of the four major blinding eye diseases[1-3]. When DR develops to the stage of proliferative diabetic retinopathy (PDR), clinical manifestations show the emergence of new blood vessels, which leads to bleeding, leakage, the release of various inflammatory and immune factors, and the hyperplasia of fibrous tissue around the new blood vessels. A fiber vascular membrane is eventually formed. Retinal detachment and vitreous hemorrhage caused by the contraction of fiber membrane are the main factors of PDR that cause decreased vision and even blindness[4]. Connective tissue growth factor (CTGF) is a secretory protein matrix that is rich in cysteine. CTGF plays an important role in trauma repair or as a key cell factor that promotes fibrosis. Its high expression levels are visible in lung, kidney, and heart fibrosis, atherosclerosis, and tumor lesions[5-8]. Content analysis showed that the expression of CTGF in retinal vascular fiber membrane and vitreous body of PDR patients is increased[9-10]. It also has been reported that the mRNA expression of CTGF in the fibrous membranes taken from the vitreous body of PDR patients is positive detected by *in situ* hybridization[11]; in addition, the degree of the positive correlation between CTGF concentration and vitreous retinal fibrosis confirms that CTGF is involved in and promotes the vascular membrane formation[12]. Thus, CTGF plays an important role in DR fibrosis.

STRUCTURE AND FUNCTION OF CONNECTIVE TISSUE GROWTH FACTOR

CTGF, also known as CCN2, is a secretory polypeptide that belongs to the immediate early gene CCN family (CTGF, Cyr61, and Nov are the first members of this group, now we still call these members the CCN family). The CNN family currently includes CCN1 (Cyr61), CCN2 (CTGF), CCN3 (Nov), CCN4, CCN5, and CCN6. CTGF is rich in cysteine, contains 349 amino acids, and has a molecular weight of 36-38 ku. Its gene is found on chromosome 6q23.1 and contains five exons and four introns. These exons code one signal peptide and four domains, which include insulin-like growth factor binding sites, Von Willebrand factor C repeat area, the thrombospondin type I repeat area, and cysteine rich C-terminal domain (CT). CTGF exert its biological function by combining these four modules with the extracellular matrix (ECM) components and receptors on the cell membrane surface[13]. Its multiple functions include participation in the regulation of many growth factors, formation and reshaping of ECM trauma repair, and fibrosis, as well as association with many physiological or pathological fibrosis of tissue cells[14]. In normal adult tissue or cells, it exists at a low level which may be undetectable. CTGF can regulate many growth factors and ECM proteins, leading to tissue reorganization

such as ECM formation, thickening of the basal layer, pericyte apoptosis, angiogenesis, trauma repair and fibrosis[15]. In PDR, CTGF promotes thickening of the retinal capillary basal layer and participates in pericytes loss, its expression is induced by advanced glycation end products and some growth factors such as vascular endothelial growth factor (VEGF)[15]. Other studies suggested that the mechanism of CTGF in PDR fibrosis includes the stimulation of the growth of fibroblasts and endothelial cells, adhesion, and promotion of the deposition of ECM[16-17].

BIOLOGICAL FUNCTIONS OF CONNECTIVE TISSUE GROWTH FACTOR

Zavadil *et al*[18] found that transforming growth factor-beta 1 (TGF-β1) is the key factor in epithelial mesenchymal transition (EMT); TGF-β1 induces the EMT and ECM synthesis through the phosphorylation of the Smad2/3 signaling pathways. The upstream cytokines of CTGF is TGF-β, which adjusts the fibrosis of signaling pathways and plays an important role in PDR[15].

1) Shi *et al*[19] studied alveolar epithelial cells, and the results showed that TGF-β1/PI3K/CTGF signaling pathway is also important in pulmonary fibrosis: CTGF promotes the accumulation of overexpressed ECM as a downstream influencing factor of TGF-β1/PI3K pathways, and activate fibroblasts and further accelerate fibrosis. Pulmonary fibrosis is prevented by the PI3K inhibitors' functions of the inhibition of aberrant proliferation and migration of epithelial or fibrogenic cells through reversing EMT and reducing the expressions of CTGF and collagen fibers.

2) Jun and Lau[14] found that the high expression of CCN2 only exists in the initial inflammatory period of wound healing, but declines in the proliferation and maturation period of myofibroblasts. However, it leads to cell senescence and senescence-associated secretory phenotype though the direct application of CCN2 protein on skin wounds, such as the up-regulation of matrix metalloproteinases (MMPs), the down-regulation of collagen fibers and TGF-β signaling pathway, and eventually the reduction of collagen fibers.

3) The study of Chatzifrangkeskou *et al*[17] about heart diseases showed that the activation of extracellular regulated protein kinases (ERK1/2) may upregulate the expression of CTGF leading to promote TGF-β/Smad signaling pathways in a positive feedback loop which regulates cardiac fibroblasts fibrosis. Research showed that CTGF is the downstream regulatory factor of TGF-β pathways and stimulates the synthesis of ECM components[20].

4) Li *et al*'s[4] study reported that TGF-β-RhoA-MMP3-CTGF-VEGF shaft is the mesenchymal stem cells (MSC)-ECM molecular basis in artery remodeling. The RhoA/ROCK signaling pathway is activated by TGF-β and has an effect on the molecular switch on MSCs during the repair of blood vessel damage. The MSCs are transformed into fibroblasts when the RhoA/ROCK signaling pathway opens and into endothelial cells when the pathway closes, respectively. The former is a process of pathological intimal hyperplasia, whereas the latter is a physiologic endothelial repair.

The activation of MSCs RhoA protein promotes the formation of CTGF-VEGF complex in the ECM. The inactivated RhoA/ROCK signaling pathway increases the levels of the MMP3, splits the CTGF-VEGF complex, and down-regulates the level of CTFG, which leads to the release of VEGF and the endothelial differentiation of MSCs.

In conclusion, CTGF promotes cell proliferation, collagen synthesis, ECM deposition, mediation of cell adhesion and migration, and angiogenesis.

CONNECTIVE TISSUE GROWTH FACTOR INVOLVED IN THE REGULATION OF RETINAL MEMBRANE FIBROSIS

Connective Tissue Growth Factor and MMPs, Müller Cell Müller cell is one of the cells present before the formation of diabetic retinal membrane, and Bringmann *et al*[21] suggested that the membrane formation is related to reactive glial cell proliferation, fibrosis, and cell migration. Barcelona *et al*[22] found that the expression of MMPs, including MMP2 and MMP9, promotes the transfer of Müller cells. Erythropoietin (Epo) slows down the kidney fibrosis by inhibiting the transformation from renal tubular epithelial cells to mesenchymal cells in the kidney. Hu *et al*'s[23] research reported that Epo slows down the reactive hyperplasia of the Müller cells. Luo *et al*[24] suggested that Epo prevents the synthesis of CTGF in Müller cells and slows the severity of fibrosis.

Connective Tissue Growth Factor and the Thickening of the Basal Lamina The retinal vascular changes during early DR include capillary base layer thickening, perithelial cell loss, and acellular capillaries. The retinal capillary base layer is mainly composed of collagen type IV, layer adhesion proteins, and fibronectin. The expression levels of CCN family, especially CTGF, are up-regulated in the early stage of DR because these proteins potentially induce the expression of base layer compositions[15]. Kuiper *et al*[25] found that the loss of CTGF allele prevents the thickening of the retinal capillary basement membrane by reducing the protein expression of CTGF in the diabetic mice model induced by streptomycin. Van Geest *et al*[26] found that the lack of CTGF allele in mice with long term diabetes (6-8mo) reduces the perithelial cell and acellular capillary generation and controls the thickening of the retinal capillary basement membrane. CTGF plays a significant role in retinal vascular structural changes. This protein slows down the structural changes in DR by treating CTGF as a target.

Interaction of Connective Tissue Growth Factor and Vascular Endothelial Growth Factor With the development of PDR, a variety of growth factors is associated with the newly formed blood vessels and the proliferation of fibrosis. VEGF is one of the most important growth factors that promote angiogenesis. Retinal neovascularization is reduced after inhibiting VEGF, and this method is clinically confirmed and widely used[27-29]. Anti-angiogenesis therapy is applied in the clinical work of PDR to reduce the intraoperative and postoperative bleeding. However, we observed in the clinical work that there is an increasing tendency of fibrous membranes after the anti-VEGF treatment, with the neovascularization subsided. This phenomenon increases the probability of iatrogenic hole and indirectly leads to the inefficiency of the operation. Previous studies[30-31] confirmed the acceleration of fibrosis with the increasing compositions of fibrosis after the injection of anti-angiogenic drugs in the vitreous body. The progression of fibrosis also occurred in therapy patients with age-related macular degeneration[32]. Hence, some scholars suggested that a transformation mechanism exists between the angiogenic proliferation and fibrosis in the development of PDR, and this mechanism is called the "blood vessels-fibrosis switch"[33].

CTGF and VEGF are two key factors that promote angiogenesis and fibrosis. Studies showed that in the interaction between VEGF and CTGF, VEGF induces the expression of CTGF[34-35]. CTGF adjusts the VEGF-CTGF protein to inhibit the angiogenesis induced by VEGF[36]. Our previous work revealed that in the group with injection of anti-VEGF 10d before the operation, the level of VEGF in proliferative membrane was significantly reduced and the levels of CTGF were increased (compared with the control group). This finding is consistent with the "blood vessels-fibrosis switch"[37].

Kuiper *et al*[33] suggested a dynamic balance between CTGF and VEGF in patients with PDR. When the threshold of CTGF is obtained, the activities of CTGF increase and those of VEGF decrease. The CTGF/VEGF ratio breaks through the threshold, and the manifestation of PDR develops in the direction of fibrosis proliferation. CTGF/VEGF ratio is a strong predictor of vascular fibrosis transformation.

Van Geest *et al*[38] confirmed the results of Kuiper *et al*[33] research and reported that CTGF/VEGF ratio is also a predictor of fibrosis in patients with the anti-VEGF treatment into the vitreous body. For these patients, even in a short period of time, the ratio is also a risk factor for postoperative fibrosis complications of high CTGF and low VEGF expressions.

Zhang *et al*[39] found that the stain on the retinal membrane of PDR patients with treatment of anti-VEGF is more intense than that of the control group after three weeks. The increased CTGF was detected in the human adult retinal pigment epithelial (ARPE-19) and BV2 microglial cells and lysate supernatant. Hence, they thought that the treatment of intravitreal bevacizumab up-regulates the expressions of TGF-β2, CTGF and consequently accelerates fibrosis. Zhang *et al*'s[40] study found that the expression of CTGF increased in human umbilical vein endothelial cell after the anti-VEGF treatment. Their finding suggests that bevacizumab potentially induces CTGF expression.

Our research showed that the expression level of VEGF protein in fiber vascular membrane significantly decreased in PDR patients with intraocular anti-VEGF treatment; however, the CTGF level significantly increased, and the fiber vascular membrane grew larger[37]. Hence, we simultaneously introduce VEGF and CTGF to observe their effects and influences on fibrosis. Our recent research showed that the synthesis levels of CTGF and VEGF were greatly reduced in diabetic mice with the double-target treatment of intravitreal injection with VEGF inhibitors ranibizumab and anti-CTGF shRNA[41]. The conclusion of the previous study, which states that the anti-VEGF treatment reduces the level of VEGF expression and simultaneously increases the level of CTGF expression, was verified. In addition, the double-target therapy also plays a positive role in reducing the damage on the retinal microvascular structure[41].

CONCLUSION

CTGF is an important cytokine that is involved in DR fibrosis. In the PDR stage, the CTGF/VEGF ratio is a strong predictor of vascular fibrosis transformation. This indicates that the therapy of combing anti-VEGF injection and CTGF shRNA intervention is a possible effective method to prevent the pathological fibrosis in PDR. It provides new insights into the treatment of DR and introduces a clear definition of the molecular mechanisms of the involvement of CTGF in regulating PDR.

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