Minireview *Highlight article*

Connective tissue growth factor: Role in trabecular meshwork remodeling and intraocular pressure lowering

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Impact statement

Connective tissue growth factor (CTGF) overexpression is involved in the pathophysiology of fibrotic diseases involving extracellular matrix (ECM) remodeling leading to excessive ECM deposition. Increased intraocular pressure (IOP) in glaucomatous optic neuropathy has been associated with excessive expression of CTGF and its upstream signaling molecule transforming growth factor beta (TGF-β). Imbalance in ECM regulation increases resistance at the aqueous humor outflow pathway leading to elevation of IOP, which subsequently damages the optic nerve. Therefore, CTGF could potentially be a target for antiglaucoma agents. This article describes the biology of CTGF, its regulation, signaling, and association with the pathophysiology of glaucoma. The impact of this review article lies in the possibility of utilizing CTGF as a potential therapeutic target for glaucoma and other fibrotic diseases.

Abstract

Connective tissue growth factor (CTGF) is a distinct signaling molecule modulating many physiological and pathophysiological processes. This protein is upregulated in numerous fibrotic diseases that involve extracellular matrix (ECM) remodeling. It mediates the downstream effects of transforming growth factor beta (TGF-β) and is regulated via TGF-β SMAD-dependent and SMAD-independent signaling routes. Targeting CTGF instead of its upstream regulator TGF-β avoids the consequences of interfering with the pleotropic effects of TGF-β. Both CTGF and its upstream mediator, TGF-β, have been linked with the pathophysiology of glaucomatous optic neuropathy due to their involvement in the regulation of ECM homeostasis. The excessive expression of these growth factors is associated with glaucoma pathogenesis via elevation of the intraocular pressure (IOP), the most important risk factor for glaucoma. The raised in the IOP is due to dysregulation of ECM turnover resulting in excessive ECM deposition at the site of aqueous humor outflow. It is therefore believed that CTGF could be a potential therapeutic target in glaucoma therapy. This review highlights the CTGF biology and structure, its regulation and signaling, its association with the pathophysiology of glaucoma, and its potential role as a therapeutic target in glaucoma management.

Keywords: Antiglaucoma, CTGF, extracellular matrix, fibrosis, remodeling, TGF-β

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Introduction

Connective tissue growth factor (CTGF) is a unique signaling modulator that is involved in multiple physiological processes such as cell proliferation, differentiation, adhesion and survival, angiogenesis, wound healing, and production of extracellular matrix (ECM). In addition, it plays a crucial part in pathophysiological processes, including tumorigenesis, fibrotic disorders, atherosclerosis, rheumatoid arthritis, and cardiac failure. The involvement of CTGF in pathophysiological processes leading to fibrotic disorders has been demonstrated in various studies, particularly those that utilized small-interfering RNA (siRNA) or antisense-mediated knockdown of CTGF.[1,2] Since CTGF is the downstream effector to transforming growth factor beta (TGF-β), a profibrotic cytokine, it may serve as a useful target for suppressing some of the TGF-β's profibrotic actions without interfering with TGF-β's other essential physiological functions.

From this standpoint, role of CTGF in glaucoma pathophysiology is important to investigate. Glaucoma causes irreversible blindness and is the worldwide second leading cause of visual loss and is characterized by optic nerve degeneration and visual field defects in affected individuals. Intraocular pressure (IOP) is regulated by maintaining the balance between aqueous humor (AH) formation and drainage. IOP is the most critical risk factor for the disease.

Ciliary body produces AH and this colorless fluid passes from the posterior chamber to the anterior chamber and exits the eye via the trabecular meshwork (TM) into Schlemm's canal. In primary open-angle glaucoma (POAG), there are no detectable structural abnormalities and IOP elevation is an outcome of the abnormally high resistance to AH outflow within TM. The IOP is also elevated in primary closed-angle glaucoma; however, this subtype of glaucoma often results from the structural abnormalities causing narrowing of iridocorneal angle and blocking the drainage of AH. POAG accounts for 74% of all cases of glaucoma.[3]

The increased TM resistance in POAG patients is linked to an increase in the deposition of extracellular fibrillar material consisting of elastic fibers and fibronectin, the major ECM constituent in the TM tissue. A large percentage of these patients have elevated AH TGF-β2 level, indicating that TGF-β2 is part of the pathogenesis of increased resistance in TM and elevated IOP in POAG eyes.[4–6] *In vitro* and *ex vivo* treatment using TGF-β2 increases the synthesis of ECM components and this effect has been shown to be largely mediated through CTGF.[7–10] Notably, CTGF is abundantly expressed in the TM of human eyes[11,12] and its expression is triggered by mechanical stimuli in a diverse range of cell types. Hence, increased IOP in glaucomatous eyes may serve as a mechanical stimulus inducing CTGF expression and the resultant pathological changes in TM. Therefore, CTGF could be a target for potential antiglaucoma drugs aiming for modifying the disease process in the TM of POAG eyes and other diseases associated with ECM remodeling.

In this brief review, we recapitulate recent knowledge relating to the regulation of CTGF expression in TM, its effects on the expression of ECM proteins, and its role in IOP elevation in glaucomatous eyes. We also highlight the possible role of CTGF as a therapeutic focus in the currently used antiglaucoma drugs and also for future drug development.

CTGF: biology and structure

CTGF was found by Bradham *et al.*[13] in 1991 as an ECM belonging to the CCN family, a simple acronym based on its three classical members "*C*onnective tissue growth factor (CTGF), *C*ysteine-rich protein (Cyr61), and *N*ephroblastoma-overexpressed gene (NOV)."[14] These proteins were later renamed as cellular communication network (CCN).[15] There are a total of six CCN family members, including CCN2 (CTGF), Cyr61 (CCN1), NOV (CCN3), WISP1 (CCN4), WISP2 (CCN5), and WISP3 (CCN6).[16] The CCN family of proteins have a multimodular structure that enables them to communicate with other proteins in order to execute their functions.[17] These interactions include those with cytokines, growth factors, cell surface integrins, matrix metalloproteinases (MMPs), and ECM proteins such as fibronectin.[18–20] The CCN family is involved in regulating cellular processes, such as cell proliferation, differentiation, death, survival, migration, and adhesion.[17,21–23]

CTGF is widely expressed and is pivotal in the embryonic development and wound healing process following injury.[24] The absence or lack CTGF expression has been implicated in neurodevelopmental disorders, including tuberous sclerosis, skeletal dysmorphism, and pulmonary hypoplasia,[25–27] whereas over-expression or excessive amount of this protein is associated with malignancies, such as osteosarcoma, breast cancer, and hepatocellular carcinoma,[28–31] and fibrotic disorders, such as glaucoma, cardiac and pulmonary fibrosis, as well as renal fibrosis.[32–35]

CTGF, like other members in the CCN group, consists of four cysteine-rich domains and is present in the cellular stroma.[22,36] CTGF is a mosaic protein due to its modular structure consisting of five exons that code for 394 amino acid residues. Both of its N- and C-terminals are made up of two domains known as module. The N-terminal comprises domain 1, the insulin-like growth factor–binding protein (IGFBP), and domain 2, the von Willebrand factor type C (vWC). The C-terminal comprises domain 3, the thrombospondin-1 (TSP-1), and domain 4, the C-terminal cystine knot (CT).[37,38] In between N- and C-terminals, there is a hinge, the site of proteolytic cleavage (Figure 1(B)). Proteases, such as elastase and plasmin, and MMP group of enzymes, such as MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-13, cause cleavage at the hinge-breaking CTGF between N- and C-terminals.[39]

The regulators of CTGF expression

Diverse variables modulate CTGF expression at the transcriptional, post-transcriptional, and post-translational stages. The extracellular stimuli including growth factors; cytokines, for example, TGF-β and bone morphogenetic protein (BMP)-2; hormones including angiotensin II and glucocorticoids, monocyte chemotactic protein (MCP)-1, and interferon (IFN)-γ promote CTGF expression.[40–42]

TGF-β

TGF-β is considered a master regulator of tissue growth, regeneration, remodeling, and fibrosis. Most TGF-β responses, including fibrosis, wound healing, ECM remodeling, fibroblasts proliferation, involve CTGF activity.[43–45] The intracellular signaling involving TGF-β and CTGF varies according to different kind of cell and the physiological or pathological outcomes. The TGF-β comprises isoforms, including TGF-β1, TGF-β2, and TGF-β3, and exerts its effects via intracellular signaling that is largely mediated via the SMAD-dependent (canonical) and the non-SMAD-dependent (non-canonical) pathways. TGF-β ligands exert their action via binding to TGF-β receptor type II (TGF-βRII), a transmembrane protein with protein kinase domain. This binding promotes TGF-β type I receptor (TGF-βRI) and phosphorylates it to form a ligand receptor complex.[46] Thereafter, downstream signaling involves either SMAD proteins of the canonical pathway or the mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/AKT, and Rho-like GTPase of the non-canonical pathway (Figure 1(A)).

TGF-β interacts with CTGF by binding at the vWC domain (Figure 1(B)). CTGF serves as a chaperone to transport TGF-β to its receptors by binding TGF-β with a low affinity and thus creating a positive feedback loop, which magnifies the TGF-β effects inducing higher CTGF expression.[47,48] TGF-β signaling via canonical or non-canonical pathways may lead to CTGF expression depending on the

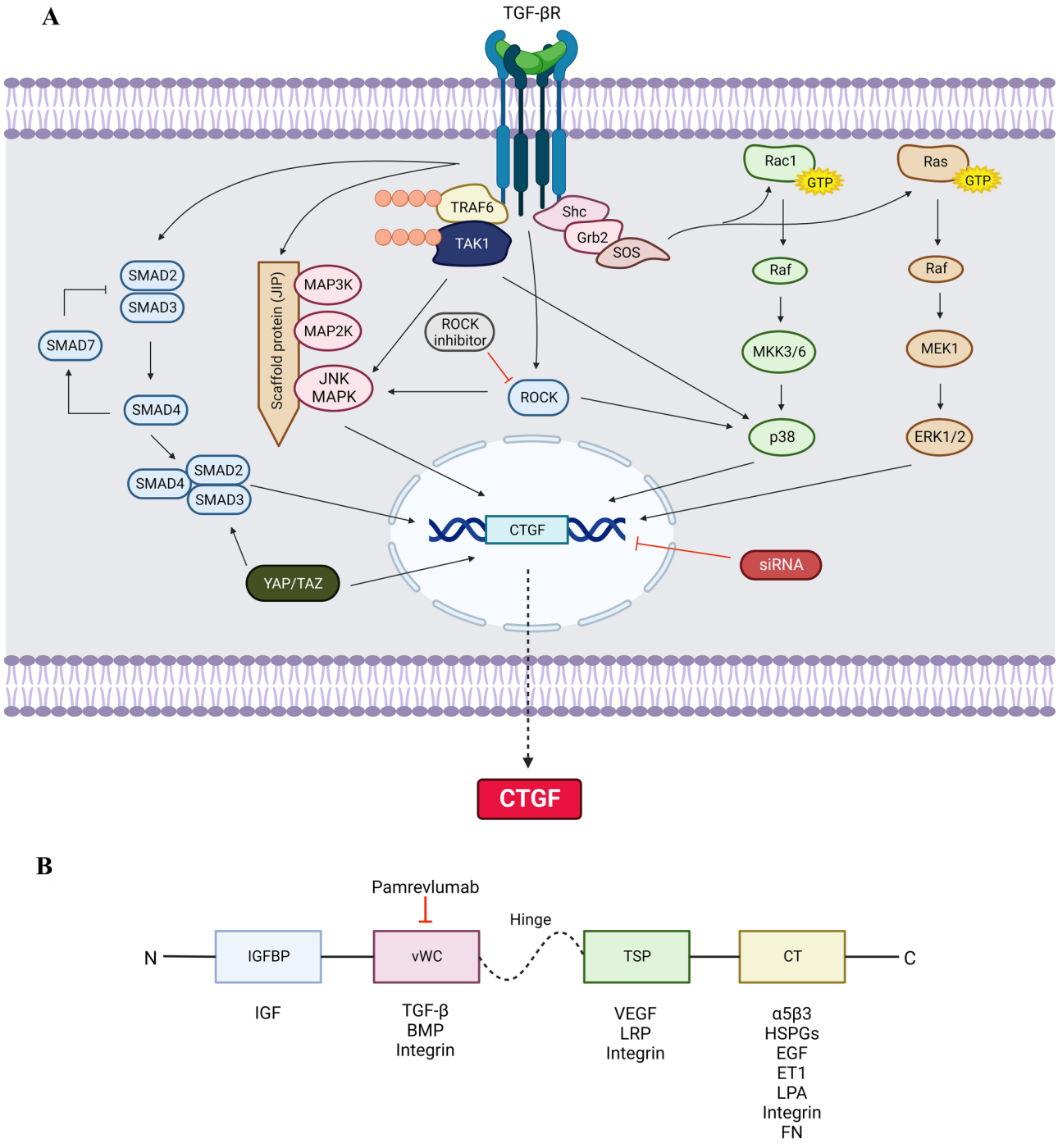

Figure 1. (A) TGF-β signaling via SMAD-dependent and SMAD-independent pathways to regulate CTGF-mediated ECM production. TGF-β signaling is initiated by binding of the ligand with the receptors. In SMAD pathway, the activated receptors phosphorylate SMAD 2 and SMAD 3 together with SMAD 4 to promote CTGF expression. SMAD 7 acts as inhibitory mediator for further stimulation of SMAD signaling. In SMAD-independent pathway, TGF-β activates MAP3K at scaffold protein, JNK-interacting protein-1, which facilitates the activation of MAP2K and JNK MAPK. JNK can also be activated by TAK1 upon polyubiquitylation with TRAF6 at the ligand-bound TGF-βR. Binding of the TGF-βR causing autophosphorylation of tyrosine residues recruits Shc and Grb2. The Shc-Grb2 complex binds to SOS that activates Rac1 and Ras GTPases. Rac1 activates Raf to phosphorylate MKK3/6 and stimulate p38. Ras activates Raf to phosphorylate MEK1 which stimulates ERK1/2. TGF-βR also activates ROCK signaling leading to phosphorylation of JNK and p38. The activation of these MAPK signaling molecules JNK, p38, and ERK1/2 induces the expression of CTGF for ECM synthesis. YAP/TAZ involves binding with the SMAD 2/3/4 complex and directly translocates into the nucleus. The siRNA inhibits the transcription of CTGF gene. The transcription of CTGF gene promotes the CTGF protein synthesis and secretes extracellularly. TGF-β: transforming growth factor β; TGF-βR: TGF-c receptor; SMAD: suppressor of mothers against decapentaplegic; MAP: mitogen-activated protein; MAPK: mitogen-activated protein kinase; JNK: c-Jun amino terminal kinase; TRAF6: tumor necrosis factor: TNF receptor–associated factor 6; TAK1: TGF-β-associated kinase 1; Shc: Src homology domain 2–containing protein; Grb2: growth factor receptor–binding protein 2; SOS: son of sevenless; GTP: guanosine triphosphate; MKK3/6: MAP kinase kinase 3/6; ERK: extracellular signal–regulated kinases; MEK1: MAP/ERK kinase 1; ROCK: RhoA/Rho-associated protein kinase; YAP/TAZ: yes-associated protein 1/transcriptional co-activator with PDZ-binding motif; siRNA: short-interfering RNA; CTGF: connective tissue growth factor. (B) Schematic representation of CTGF protein structure showing four structurally distinct domains and the proteins or growth factors that interact with specific domain. IGFBP: insulin-like growth factor–binding protein; vWC: von Willebrand factor type C; TSP: thrombospondin-1; CT: C-terminal cystine knot; IGF: insulin-like growth factor; TGF-β: transforming growth factor-β; BMP: bone morphogenetic protein; VEGF: vascular endothelial growth factor; LRP: lipoprotein receptor–related protein; HSPGs: heparan sulfate proteoglycans; EGF: epidermal growth factor; ET1: endothelin 1; LPA: lysophosphatidic acid; FN: fibronectin. Created with BioRender.com.
Pointed black arrows refer to stimulatory and red blunt arrows refer to inhibitory.

different cell types, tissues, or intended functional outcome. For example, the CTGF expression in normal fibroblasts is induced via the SMAD-dependent signaling, whereas in scleroderma fibroblasts, the induction of CTGF expression is SMAD-independent.[49]

SMAD-dependent signaling in TGF-β-mediated expression of CTGF

The SMAD-dependent downstream signaling is activated after the TGF-β ligands stimulate the phosphorylation of TGF-βRI. The SMAD pathway comprises series of phosphorylation of several SMAD proteins belonging to three functional classes: these include the receptor-regulated SMAD (R-SMAD), the co-mediator SMAD (Co-SMAD), and the inhibitory SMAD (I-SMAD). The ligand–receptor complex phosphorylates and then actuates R-SMADs (SMAD 2 and 3), which heterodimerize and form a complex with Co-SMAD (SMAD 4). The activated complexes then translocate into the nucleus, where transcription of target genes will be regulated by binding of the complex directly to DNA, interacting with diverse DNA-binding proteins, and employing either transcriptional co-activators or co-repressors. The I-SMADs block phosphorylation of R-SMADs[50,51] (Figure 1(A)).

There are a total of nine SMAD proteins including SMAD 1 to SMAD 9. The R-SMADs include SMAD 1, 2, 3, 5, 8, 9; the SMAD 4 is Co-SMAD, and I-SMADs include SMAD 6 and 7. SMAD 2 and 3 specifically mediate the TGF-β signaling pathway that phosphorylates after receptor activation at the cell membrane. SMAD 4 functions as a central sensor, forming heteromeric multimeric complexes with activated R-SMADs, and regulates transcription of target genes. SMAD 7, originally localized in the nucleus, inhibits TGF-β signaling as it blocks the formation and activation of R-SMAD and Co-SMAD complexes by adhering to the TGF-βRI at the cell membrane.

SMAD proteins conduct key functions in signal conduction and regulation of downstream target genes in the canonical TGF-β pathway.[52] CTGF has been recognized as one of these target genes that mediates the development of fibrosis, including myofibroblast activation, excessive ECM synthesis, and ECM degradation inhibition. CTGF reinforces TGF-β receptor by binding directly to the TGF-β receptor type III (TGF-βRIII). Thus, blockade of TGF-β/SMAD-dependent expression of CTGF is likely to be a more reasonable therapeutic target in preventing fibrogenesis rather than the upstream blockade of TGF-β/SMAD that is susceptible to interfere with a wide range of physiological functions.[53]

SMAD 2/3 phosphorylation has been reported to be stimulated by TGF-β in human lung fibroblasts[54] and orbital fibroblasts.[55] Aberrations of TGF-β1 have been shown to upregulate the gene expression of ECM proteins, α-smooth muscle actin (α-SMA),[56] as well as the downstream CTGF proteins[57] via SMAD 2/3 phosphorylation. Moreover, inhibition of TGF-β/SMAD signaling was shown to decrease CTGF expression, leading to reduction of the fibrotic response in endometrial fibrosis.[53] Similarly, it was observed that inhibition of SMAD 2/3 led to the reduction in TGF-β1-induced increase in CTGF expression in human granulosa cells.[58] Interestingly, the rise of CTGF expression in rat progenitor cells activated by TGF-β1 was not mediated by the SMAD signaling pathway.[59] This finding was also supported by O'Donovan *et al.*,[60] who have observed elevated CTGF level and reduced SMAD 2/3 phosphorylation in renal mesangial cells in streptozotocin (STZ)-induced diabetic mice. These findings insinuate that TGF-β/SMAD signaling regulates CTGF expression differently depending on the cell types, the species, and the experimental conditions.

SMAD-independent signaling in TGF-β-mediated expression of CTGF

The stimulation of SMAD-independent or the non-canonical pathway following the activation of TGF-β receptors occurs via sequential phosphorylation or direct interaction of signaling proteins. These non-canonical signaling largely includes MAPK pathways leading to the activation of extracellular signal–regulated kinase (ERK)1/2, p38 MAPK, and c-jun N-terminal kinase (JNK) via Ras superfamily of small GTPase. In addition, TGF-β signaling may also activate Rho subfamily of small GTPase which has been shown to crosstalk with some constituents of MAPK[61] (Figure 1(A)).

ERK1/2

The MAPK pathway is activated when TGF-β ligand binds to TGF-βRII, causing phosphorylation of tyrosine residues. Following this, Src homology domain 2–containing protein (Shc) and growth factor receptor–binding protein 2 (Grb2) are recruited by TGF-βRII, bringing forth the stimulation of ERK1/2 through sequential activation of Ras, Raf, and MEK1/2.[62,63] ERK1/2 activation leads to phosphorylation of transcription factors that modulate gene expression. Interestingly, ERK1/2 signaling may also interact with the TGF-β/SMAD pathway.[64] In TM cells, TGF-β-activated ERK signaling increases expression of plasminogen activator inhibitor-1 (PAI-1), a tissue plasminogen activator (tPA) primary inhibitor.[65] Inhibition of tPA prevents the activation of pro-MMP and that inhibits ECM degradation causing accumulation of ECM within the TM tissue. In human lung epithelial cells, TGF-β has been proven to induce CTGF expression via the ERK signaling pathway.[66] Inhibiting CTGF in dextran sulfate sodium-induced ulcerative colitis could improve inflammatory response via blocking ERK signaling pathway.[67]

p38 MAPK

The GTPase Rac1 activates p38 MAPK pathway, thus stimulating the expression of Secreted Protein Acidic and Rich in Cysteine (SPARC) in TM cells.[68] The binding of SPARC to ECM proteins regulates the growth factor efficacy and MMP expression.[69] Upon activation of TGF-β receptors, tumor necrosis factor receptor–associated factor 6 (TRAF6), an E3 ubiquitin ligase, binds with TGF-βRI.[70,71] This subsequently recruits TGF-β-activated kinase 1 (TAK1) through association and induced activation allowing the phosphorylation of MAP kinase kinase (MAP2K) MKK3/6. This resulted in the activation of the p38 MAPK pathway.[72,73] p38 MAPK mediates expression of CTGF, ECM proteins such as fibronectin, as well as MMP-2.[74–76] SB203580, a p38 MAPK inhibitor, has

been demonstrated to attenuate type I collagen production induced by TGF-β2 in human TM cells.[77]

JNK

TGF-β is capable in inducing the activation of the MAP kinase kinase kinase (MAP3K) family. Components of the MAP3K, the MAP2K family members, and JNK are retained closely by the scaffold protein, JNK-interacting protein-1. This enables a quick series of phosphorylation that leads to the JNK active site phosphorylation.[78] JNK can also be activated via TAK1 through phosphorylation.[63] Stimulation of the JNK signaling pathway can initiate the SMAD 3 phosphorylation, translocation to nuclei, which finally lead to recruitment of SMAD 3 to the CTGF promoter, inducing CTGF expression in both human and orbital fibroblasts.[79–81]

Rho GTPase/Rho kinase signaling

TGF-β can stimulate Rho-like GTPases pending on different cell types. Studies have shown that in epithelial cells and primary keratinocytes, TGF-β actuates Rho A in its GTP-bound state, an activation that is likely to be independent of SMAD 2 or SMAD 3.[63,82,83] JNK and p38 MAPK can directly be activated by Rho GTPases. Rho kinase may also mediate TGF-β-induced CTGF expression by phosphorylating ERK1/2 and JNK.[84] Hence, TGF-β and CTGF may also increase the ECM component expressions such as fibronectin, type I collagen, and MMP-2 by stimulating the RhoA/Rho-associated protein (ROCK) signaling pathway.[85]

Other regulators of CTGF

CTGF expression may also be regulated by multiple ligands and extracellular signaling molecules since specific receptors of CTGF have not been identified. These ligands include BMP, α5β3 integrin, insulin-like growth factor (IGF) 1, vascular endothelial growth factor (VEGF), low-density lipoprotein receptor–related protein 1 (LRP-1), heparan sulfate proteoglycans (HSPGs), decorin, epidermal growth factor (EGF), hemodynamic endothelin (ET) 1, secreted frizzled-like protein-2 (sFRP-2), antiproliferative factor, thrombin, histamine, serotonin, and prostaglandins.[41,86–89]

Hypoxia induces CTGF expression via p38 signaling.[90,91] In fact, TGF-β was shown to synergize with hypoxia for induction of CTGF expression and muscle fibrosis.[92] Mechanical stresses, such as mechanical stretch and fluid flow, can cause CTGF elevation in different types of the cells.[93–95] CTGF is elevated in mesangial cells and myofibroblasts cultivated under high-glucose conditions, and this is regulated by TGF-β.[96–98] In addition, CTGF expression was found to be increased by metabolic factors, such as advanced glycation end-products (AGE) and free fatty acids.[99,100] Another inducer of CTGF is lysophosphatidic acid (LPA). LPA is a potent bioactive phospholipid that plays a significant role in development of fibrosis including that in skeletal muscle by inducing CTGF expression and fibroblasts activation and proliferation.[101–103]

Tumor necrosis factor α (TNF-α) is a known powerful inhibitor of CTGF in cells of mesenchymal origin.[104] In a recent study, level of CTGF in TNF-α-exposed osteoblasts was reduced in a concentration- and time-dependent manner.[105] CTGF expression in non-involved fibroblasts in chronic wounds of human tissue was also reduced by TNF-α.[106] TNF-α was reported to inhibit CTGF-promoter activity in endothelial cells of human lung in a dose-dependent manner.[107] Nitric oxide was also shown to downregulate CTGF in rat mesangial cells.[108,109]

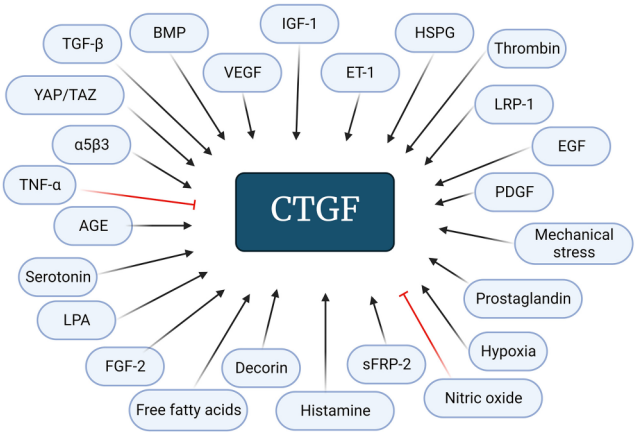

Figure 2. Factors involved in the regulation of CTGF. TGF-β: transforming growth factor-β; BMP: bone morphogenetic protein; VEGF: vascular endothelial growth factor; IGF-1: insulin-like growth factor 1; ET1: endothelin 1; HSPGs: heparan sulfate proteoglycans; LRP: lipoprotein receptor–related protein; EGF: epidermal growth factor; PDGF: platelet-derived growth factor; sFRP-2: secreted frizzled-like protein-2; FGF-2: fibroblast growth factor 2; LPA: lysophosphatidic acid; AGE: advanced glycation end-products; TNF-α: tumor necrosis factor α; YAP/TAZ: yes-associated protein 1/transcriptional co-activator with PDZ-binding motif. Pointed black arrows refer to stimulatory and red blunt arrows refer to inhibitory. Created with BioRender.com.

Another signal transduction pathway, the yes-associated protein 1/transcriptional co-activator with PDZ-binding motif (YAP/TAZ) signaling pathway, regulates cell proliferation, differentiation, and organ size.[110–112] These two are closely related transcriptional co-activators and are activated by various extracellular signals, including TGF-β, LPA, integrins, mechanical cues, and many others. Activation of YAP/TAZ pathway by TGF-β is mediated through activation of Rho GTPase signaling and SMAD complex, which associate with YAP/TAZ and translocate into the nucleus.[113] Importantly, one of the key target genes of YAP/TAZ pathway is CTGF as YAP/TAZ binds directly to the promoter region of CTGF and enhances its downstream signaling including ECM production and cell proliferation.[114] (Regulators of CTGF expression are summarized in Figure 2.)

CTGF mediates tissue remodeling and fibrosis

The regulatory actions of CTGF result from its binding with several receptors, including integrins, HSPGs, lipoprotein receptor–related proteins (LRPs), and tyrosine kinase (TK) receptors, to initiate signal transduction.[115] In liver fibrosis, CTGF binds to integrin $\alpha_5\beta_3$ in hepatic stellate cells and with HSPGs acting as co-receptors.[116] In pancreatic fibrosis, CTGF joined to $\alpha_5\beta_1$ and HSPG regulates pancreatic stellate cells' adhesion, propagation, migration, and collagen synthesis.[117] CTGF also binds to a receptor known as IGF-2 receptor leading to proliferation of human corneal fibroblasts.[118]

CTGF may also directly bind to cytokines such as VEGFs, fibroblast growth factor 2 (FGF-2), platelet-derived growth factor B (PDGFB), TGF-β, and BMP for regulating their activity.[47,119,120] In addition, it adheres to ECM elements such as fibronectin. The binding of CTGF to fibronectin boosts the fibronectin affinity to fibrin for ECM accumulation in wound healing and tissue fibrosis.[121] Over-expression of CTGF in skeletal muscle is associated with skeletal muscle dystrophy, such as Duchenne muscular dystrophy (DMD), leading to increased collagen deposition and muscle fibrosis, which eventually impairs muscle function and reduces myocyte survival.[122–124]

CTGF in the eye

CTGF is normally expressed in the ocular tissue.[123] Studies have elucidated the presence of CTGF in healthy ocular tissues such as the corneal endothelium, lens subcapsular epithelium, vasculature of iris and retina of mice and rabbits,[124] tear fluid of horses,[125] and the rat retinas.[126] Presence of CTGF-immunoreactivity (CTGF-IR) was shown by van Setten *et al*.[127] confirming the presence of CTGF in various structures of the human eye. This study detected CTGF in the cornea (majority in the basal layers), stromal keratinocytes, and endothelial cells. The sphincter and dilator muscle of the iris and conjunctiva epithelium also displayed a positive CTGF-IR.[124] These findings supported the presence of CTGF in human tear fluid.[128] A weak CTGF-IR was observed in the ciliary body but it was prominent in the vascular endothelium which may point to the origin of CTGF in the AH.[123] Further studies detected the presence of CTGF in the choroid mainly at the choriocapillaris, retinal nerve fiber layer, optic nerve head, lamina cribrosa, and endothelium of the central retinal artery in healthy human eye.[127]

The presence of CTGF in many parts of the normal eye is well known; its increased expression, however, is frequently linked to pathological conditions involving the anterior and/or posterior eye segments. These include pseudoexfoliation glaucoma, corneal and conjunctival scarring, Graves' ophthalmology, age-related macular degeneration, diabetic retinopathy, and proliferative vitreoretinopathy.[129–134] In patients with age-related macular degeneration, CTGF has been expressed in endothelial cells and retinal pigment epithelial cells of choroidal neovascular membranes.[135] CTGF expression in retinal microglia and pericytes has also been shown in patients with diabetic retinopathy.[136] In addition, CTGF gene and protein expressions were found to be significantly upregulated in Graves' ophthalmology orbital fibroblasts when compared to normal fibroblasts.[134]

Glaucoma

Glaucoma is an optic neuropathy and the major risk factor associated with this condition is the elevated IOP.[137] IOP fluctuations lead to mechanical stress on the posterior ocular structures, and this in turn triggers activation of apoptotic pathways leading to degeneration of the retinal ganglion cells.[138] A critical balance between AH production and drainage maintains the IOP in its normal range (10–21 mmHg). The drainage of AH from the anterior segment at the iridocorneal angle occurs largely through TM and to a little extent via alternative uveoscleral pathway.[139]

In the TM of POAG patients, excessive deposition of extracellular fibrillar material also known as "sheath-derived plaque material" has been detected. The plaque material consists of ECM elements such as elastic fibers, fibronectin, and collagen type IV.[140–142] The TM cells also have a distinct actin-myosin skeleton with contractile ability. A dysfunction of TM cell skeleton leads to their relaxation, which results in increased AH outflow resistance.[143]

CTGF in the pathogenesis of glaucoma

TGF-β2 is commonly associated in the pathogenesis of glaucoma. Studies have revealed that patients with POAG have higher TGF-β2 level in their AH in contrast to healthy subjects.[4,144] TGF-β2 treatment of TM cells heightens the ECM component synthesis, including fibronectin and collagen type IV.[11,145] TM contractility was also shown to be affected following TGF-β1 treatment due to the development of actin stress fibers and increased α-SMA expression.[146] TGF-β target gene, CTGF, is a majorly expressed genes in the human TM.[12] It has been described as a significant mediator of TGF-β2-induced ECM accumulation in the human TM. Synthesis of fibrillar ECM such as fibronectin, collagen types III, IV, and VI was increased following CTGF and TGF-β2 treatment of human TM cells.[11] However, the expression of collagen I was found to be induced by CTGF but not by TGF-β2.[11,147] The effects of TGF-β2 on ECM were shown to be mediated by CTGF as it interacts with human TM cells surface molecules to form cell–matrix adhesions.[11] CTGF is upregulated when TM cells are exposed to mechanical stretch or high-pressure perfusion.[93] Increase of CTGF by abnormally activated TGF-β2 can result in over-expression of ECM in the TM, which contributes to the elevated AH outflow resistance. As TGF-β2 downstream mediator of ECM remodeling, CTGF has no effect on the proteolytic system such as MMP2, MMP9, and PAI-1.[11]

CTGF as target for the treatment of glaucoma

Prostaglandin analogues and β-blockers. The current management of glaucoma is largely based on the reduction of IOP within the target range using IOP-lowering medications, including prostaglandin analogues and β-blockers. Prostaglandin F2α receptors are extensively scattered in the eye especially in the ciliary muscle bundle, the site of unconventional route of AH outflow. Prostaglandin analogues such as latanoprost reduce IOP by increasing AH drainage through uveoscleral pathway, a non-pressure-dependent pathway unlike the conventional TM pathway. These analogues stimulate prostaglandin F2α receptors in the ciliary muscles and increase the release of MMPs. MMPs are enzymes that increase the degradation of ECM deposited within the ciliary muscle and hence reduce the non-conventional AH outflow resistance.

Timolol maleate and levobunolol are examples of β-adrenoceptor antagonist (β-blockers) and are clinically used topically to lower IOP. It has been proposed that β-blockers have a dual mechanism of action in which they lower AH

production and augment the AH drainage. β-blockers block the β-adrenergic receptors which cause a reduction in the intracellular second messenger, cAMP. cAMP elevation in the ciliary epithelial cells increases chloride ion (Cl^-) efflux, attracting water and hence increases AH production. These effects are blocked by β-blockers. Studies have also shown that timolol enhances AH outflow; the precise mechanism, however, remains unclear.[148]

Based on a meta-analysis by Li *et al.*,[149] prostaglandin analogues such as bimatoprost and latanoprost have the highest efficacy to lower IOP followed by β-blockers. A fixed prostaglandin analogues and β-blockers combination is commonly used in treating glaucoma patients when more than one antiglaucoma agents are needed to achieve the target IOP.[150] Recent studies have demonstrated that treatment with combined prostaglandin analogues and β-blockers more effectively suppressed the profibrotic genes expression in TM of patients with POAG compared to treatment with prostaglandin analogues alone or β-blockers alone. The study by Tejwani *et al.*[151] observed that combination of bimatoprost and timolol lowered the *TGFβ1*, *TGFβ2*, *CTGF*, *FN* gene expression. It was also observed that CTGF expression was lower in β-blockers alone group compared to prostaglandin-alone group. Furthermore, the messenger RNA (mRNA) expression of these genes was validated by quantification of the respective proteins showing reduced levels of CTGF and fibronectin in TM treated with combination of prostaglandin and β-blockers compared to the individual drug. The study also observed that the effects on profibrotic markers were modulated by inhibiting the phosphorylation of SMAD 3.[151]

Rho Kinase (ROCK) inhibitor. Rho GTPase proteins are from the Ras superfamily and consist of monomeric small GTP-binding proteins.[152] Rho GTPases and its effector Rho kinase (Rho-associated coiled-coil-containing protein kinase [ROCK]) are known to be linked in the pathophysiology of glaucoma. ROCK has two isoforms, that is, ROCK1 (ROKb/P160) and ROCK2 (ROKa). Activation of Rho GTPase stimulates ROCK to phosphorylate numerous intracellular substrates for cellular responses. ROCK controls the actomyosin contraction, actin cytoskeletal dynamics, cell morphology, cell adhesion, cell stiffness, and cell and ECM reorganization.[152,153] Ripasudil and netarsudil are two ROCK inhibitors currently approved as glaucoma therapy. Ripasudil was approved in 2014, whereas netarsudil was approved in 2017.[154,155]

Several studies have investigated the effects of ROCK inhibitors on TM cells, AH outflow, and IOP. ROCK inhibitors enhance the conventional outflow of AH by relaxing the smooth muscle, reducing deposition of ECM in TM and therefore attenuating AH outflow resistance. Both TGF-β and CTGF are known to upregulate the expression of ECM elements via the ROCK signaling pathway (Figure 1(A)). Inhibition of ROCK signaling inhibited CTGF-induced expression of ECM molecules including fibronectin.[85] In TM cells, the inhibition of ROCK was found to be dose- and time-dependent. They induce changes in cell shape associated with decreased actin stress fibers reversibly, focal adhesions, and cell–cell interactions.[156–158] ROCK signaling also modulates the expression of ECM and fibrotic cytokines, including TGF-β and CTGF.[159,160]

SiRNA Short-interfering RNAs (siRNAs) are small molecules of double-stranded RNA requiring a minimum length between 21 and 23 nucleotides to affect the target gene expression.[161] The siRNAs in the cytoplasm of eukaryotic cells form RNA-induced silencing complex, a multimeric RNA–protein complex that identifies the complementary mRNA and thus blocks the production of specific proteins.[162] SiRNAs can be introduced exogenously to mimic the endogenous RNA interference (RNAi) and these could silence the targeted gene at any stage of cellular process in specific regions without affecting other non-targeted regions[163] (Figure 1(A)).

Despite the potential application of siRNA as pharmacotherapy, several challenges remain unresolved. These include reducing off-target effects, ensuring stability of siRNAs, and establishing their route of administration.[162] One of the most challenging off-target effects is to prevent activation of innate immunity. This can be achieved with siRNA chemical structure modification or using shorter siRNA duplexes.[164] Chemical modifications of siRNA also improve the stability of siRNA.[165] Improvement of the bioavailability of siRNA-based drugs can be achieved with the use of cell-penetrating peptides which has been shown to improve the ocular delivery of siRNA.[166] The last decade has seen the advancement of viral and non-viral carriers for siRNA delivery systems and their extensive evaluation.[167]

Junglas *et al.*[168] showed that RNAi used to deplete CTGF in cultured human TM cells attenuates actin cytoskeleton leading to reduction of outflow resistance. The study utilized pSiCTGF, a vector coding for a short-hairpin RNA against CTGF mRNA. The normal human TM cells transfected with pSiCTGF showed significantly reduced CTGF mRNA expression compared to non-transfected cells. They further observed that this reduction was associated with decreased CTGF level and its target gene, the fibronectin.

A recent study by Dillinger *et al.*[169] evaluated effects of siRNA administration targeting CTGF. They used different types of siRNA nanoparticles layer-by-layer coated with polyethylenimine and hyaluronic acid. They observed that human TM cells treated with hyaluronic acid (siRNA) nanoparticles led to reduction of CTGF expression by 50%. This study also attempted to examine the role of siRNA in reducing the expression of tight junction proteins in the Schlemm's canal. It was observed that the reduction of tight junction proteins using siRNA was associated with IOP reduction; however, this effect lasted only for a short duration.[8] siRNA targeting CTGF has high potential as antiglaucoma agents. Development of improved formulation can further enhance delivery of the therapeutic agent to tissues of the AH outflow pathway with high efficiency.

Pamrevlumab (FG-3019). FG-3019 is a full human recombinant DNA–derived CTGF-reactive monoclonal $IgG_{1/K}$ antibody, isolated and cloned from genetically engineered mice immunized with recombinant human CTGF and expressing human immunoglobulin transgenes.[170] This

agent recognizes and binds to CTGF at the vWC domain,[171] thus intercepting the cytokine interactions with CTGF and subsequently inhibiting the downstream signaling (Figure 1(B)). Multiple studies have demonstrated the ability of pamrevlumab to diminish fibrotic response in conditions such as idiopathic pulmonary fibrosis,[172] diabetic kidney disease,[173] and DMD.[174] Experiments using FG-3019 as potential treatment for these conditions have reached clinical trials, and for idiopathic pulmonary fibrosis and chronic DMD, its evaluation has progressed to phase III.[175]

This agent has also been on the radar of researchers for glaucoma. The glaucomatous TM cells showed reduction of profibrotic genes and protein expressions of α-SMA and collagen 1A1 after treatment with FG-3019.[9] Unfortunately, due to the limitation imposed by the suitability of the route of administration, the effects of FG-3019 on the IOP remain unknown. This monoclonal antibody is administered parenterally since oral administration is unsuitable due to its large molecular size and vulnerability to degradation in the gastrointestinal system.[176] Furthermore, drug administration to the eye has always been a major challenge for drug delivery scientists due to the unique ocular anatomy and physiology. Since the target site to reduce IOP is in the anterior chamber of the eye, it is unlikely that the parenterally administered agent can reach target region in sufficient concentration. The bioavailability of drugs reaching the ocular tissue via oral/systemic delivery is often less than 2%.[177]

Search for drugs targeting CTGF has come a long way for the treatment for fibrotic diseases. Despite favorable data from various preclinical and clinical studies, their progression to clinical application is still awaited. Anti-CTGF strategies that reduce CTGF including siRNA, such as RXI-109, OLX 101, and OLX 201, or monoclonal antibody, such as pamrevlumab (FG-3019),[48] although are known to target CTGF expression and reduce fibrosis; their effectiveness for the same in clinical setting remains unclear. Furthermore, development of dosage forms suitable for clinical use remains challenging.

Conclusions

CTGF is an attractive target for future antiglaucoma agents. Its expression is regulated via several upstream regulators, of which TGF-β remains the prominent one. It increases the expression of several ECM proteins in TM causing an increase in AH outflow resistance. Better understanding of the involvement of CTGF in the IOP elevation in glaucomatous disease has led to development of new agents targeting CTGF in improving the drainage of AH through the TM tissue. Targeting CTGF only can avoid inhibition of its upstream regulators such as TGF-β that have pleotropic effects. This is expected to reduce the potential adverse drug reactions. Currently available drugs such as prostaglandin analogues, β-blockers, and ROCK inhibitors have been shown affect CTGF expression. However, contribution of this effect to their IOP-lowering capacity remains unclear. CTGF as a direct target rather than its targeting via upstream regulators could yield clinically useful antiglaucoma agents.

AUTHORS' CONTRIBUTIONS

All authors participated in writing and review of the manuscript. The idea conceptualization of this review was made by MDSH and NR. MDSH took the lead in writing the manuscript and was supervised by NR. The preparation of the original draft was written by MDSH, NR, ASAB, and NFAH. The manuscript was compiled by MDSH in consultation with NR and RA for critical review, commentary, revision, and approval of the final manuscript.

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