Connective Tissue Growth Factor in Idiopathic Pulmonary Fibrosis

Subjects: Respiratory System

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Connective Tissue Growth Factor, also known as the cellular communication network 2 (CCN2), is a TGF-β-target gene and a member of the CCN family of secreted proteins that regulate matricellular protein. Matricellular proteins are expressed at higher levels during physiological and pathological processes, with distinct functions that bind to multiple receptors, other growth factors, and proteases, modulating their activity and mediating cross-talk between the ECM and cells. Idiopathic Pulmonary Fibrosis is a chronic, devastating, irreversible lung disease, characterized by injury-induced alveolar epithelial cell stress, progressive pathogenic myofibroblast differentiation, and imbalanced macrophage polarization, resulting in ECM deposition.

CTGF IPF fibrosis

1. Structure, Regulation, and Function of Connective Tissue Growth Factor

Connective Tissue Growth Factor (CTGF or CCN2) is a cysteine-rich, heparin-binding protein containing 349 amino acids, with an apparent molecular weight of 36–38 kDa. CCN family members have six members of multifunctional proteins, labeled CCN1 to CCN6. The CCN acronym is composed of the first three proteins members of the family: Cyr61 (cysteine-rich protein 61), CTGF, and NOV (nephroblastoma overexpressed gene) [1]. CCN proteins have a typical modular structure with four conserved domains, i.e., insulin-like growth factor (IGF)-binding proteins (IGFBPs) next to a von Willebrand factor type C repeat (VWC) (both are N-terminal fragments) and thrombospondin type I repeat (TSR) next to a C-terminal cystine-knot (CT) (forming a C-terminal fragment together) [2]. These domains each have specific binding partners, including an IGF protein for IGFBP, the TGF-β family for VWC, specific integrins (α4β1, α5β1, α6β1, and αvβ3) and sulfated glycoconjugates for TSP, and heparin-sulfate-containing proteoglycans (HSPGs), such as syndecan 4 and perlecan for CT [3][4].

CTGF expression is regulated at the transcriptional, post-transcriptional, and translational levels by various physiological and pathological factors [5]. Directly or through cross-talk with cell surface receptors, such as TGF-β and angiotensin, external stimuli initiate signaling pathways that recruit transcription factors to the nucleus, inhibiting or stimulating the expression of CTGF [6]. The critical transcription factors for the regulation of CTGF expression were found to be SMAD2, Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ)/transcriptional enhancer factor TEF-1 (TEAD), ETS proto-oncogene 1 (ETS-1), PI3K-AKT, Fox0, and mitogen-activated protein kinase (MAPK)/Id-1 [7][8][9][10].

The biological function of CTGF is that it binds to specific receptors to initiate signal transduction, directly binding cytokines, regulating their availability and activity, mediating the matrix turnover by binding to ECM proteins, and regulating the activity of cytokines and growth factors through modulation cross-talk between signaling pathways [6]. CTGF is expressed in mesenchymal cell lineage and mediates physiological tissue regeneration and pathological fibrosis via ECM deposition, fibroblast proliferation, matrix production, angiogenesis, and granulation tissue formation [5][11]. Depending upon the microenvironment condition and cell type, CTGF is involved in several pathologic processes such as carcinogenesis and tumor development [12], diabetes [13], neuromuscular disorders [14][15], systemic sclerosis [16], ocular diseases [17], cardiac fibrosis [18][19], renal fibrosis [20], liver fibrosis [21], and lung fibrosis [22]. The regulation of CTGF is described in **Figure 1**.

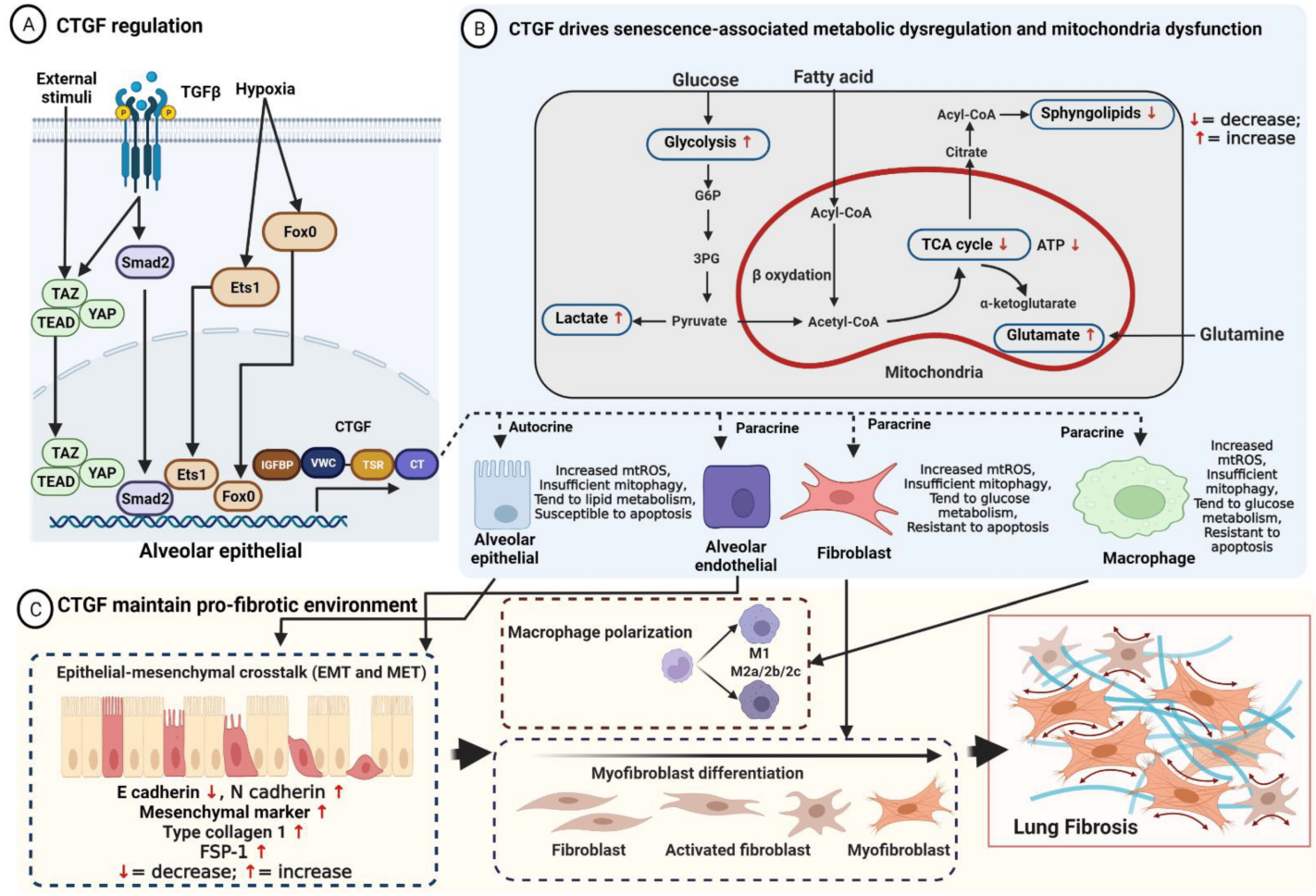

Figure 1. (**A**) Regulation of CTGF. CTGF expression is mainly regulated at the transcriptional level by various stimuli factors either directly or through cross-talk with cell surface receptors (TGF-β) that induce signaling pathways that recruit transcription factors (YAP/TAZ/TEAD, SMAD2, Ets-1, PI3K-AKT, and Fox0) to the nucleus, inhibiting or stimulating the expression of CTGF; (**B**) CTGF regulates aberrant metabolic responses associated with senescence of alveolar epithelial cells, endothelial cells, fibroblasts, and alveolar macrophages. As an essential downstream mediator of TGF-β1-induced mitophagy, CTGF induces mtROS and increases glycolysis, lactate, and glutaminolysis, leading to apoptosis resistance in macrophages and fibroblasts. Conversely, accumulation of mtROS inhibits mitophagy to promote alveolar epithelial apoptosis; (**C**) CTGF maintains pro-

fibrotic environment. Injured AECII secretes CTGF via autocrine and paracrine, inducing alveolar epithelial cells undergoing EMT to promote fibroblasts' migration and proliferation, regulating myofibroblast differentiation, and driving macrophage polarization, resulting in ECM deposition and lung fibrosis.

2. Connective Tissue Growth Factor Maintains the Pro-Fibrotic Environment in Idiopathic Pulmonary Fibrosis

A recent hypothesis in the understanding of the pathogenesis of IPF stated that aberrant epithelial and epithelial–mesenchymal cross-talk responses to chronic alveolar epithelial injury might induce fibrosis independently of inflammatory events [23][24]. Alveolar epithelial injury provides an epithelium-associated pro-fibrotic environment. Recurrent injuries lead to epithelial apoptosis and drive the aberrant activation of epithelial cells to transdifferentiate into fibroblast epithelial–mesenchymal transition (EMT) [25][26][27]. There are phenotype changes characterized by downregulated epithelial markers, such as E-cadherin, whereas fibroblast-specific genes, such as *α*-smooth muscle actin (*α*-SMA), N-cadherin, fibroblast-specific protein 1 (FSP-1), and type I collagen, are upregulated [28]. Myofibroblasts can also modulate epithelial apoptosis, preserving a pro-fibrotic environment [29]. As a result, bidirectional EMT cross-talk assists the pro-fibrogenic positive feedback loop, resulting in fibrosis progression rather than wound resolution [30]. AECs become "vulnerable and sensitive to apoptosis," but myofibroblasts become "apoptosis-resistant and immortal" [31].

Usual interstitial pneumonia (UIP) is a histopathologic and radiologic hallmark pattern for IPF. It is characterized by variations in temporospatial heterogeneity fibrosis, the accumulation of fibroblasts (fibroblast foci), and subpleural and paraseptal honeycombing [32]. Vanstapel et al. showed high expression of CTGF in fibrotic regions of restrictive allograft syndrome (RAS) lungs [33]. Furthermore, CTGF was found to be upregulated in cultured fibroblasts [34], injured epithelial cells [35], bronchoalveolar lavage and lung tissue [33], and plasma [36]. CTGF is upregulated in patients with IPF as well as in pro-fibrotic mediators and pro-fibrotic environments that contribute to fibrogenesis [37]. CTGF likely maintains aberrant responses of alveolar epithelial cells, fibroblasts, and alveolar macrophages in the development and progression of IPF (**Figure 1**). Many studies have reported that CTGF plays direct and indirect roles in accelerated aging, mitochondria dysfunction, and metabolic reprogramming.

2.1. Activated Alveolar Epithelial Cells Initiate a Cycle of Fibrosis through Connective Tissue Growth Factor

The precise mechanism of how CTGF-related activated epithelial cells induce fibrogenesis remains poorly defined. Following environmental injury, alveolar epithelial cells trigger their apoptosis and become active by secreting pro-fibrotic factors TGF-β to attract fibroblasts [38]. Type II alveolar epithelial cells (AECII) undergo EMT induced by EGFR–RAS–ERK signaling via zinc finger E-box-binding homeobox 1 (ZEB1)–tissue plasminogen activator (tPA), which augments fibroblast recruitment and activation [39]. AECII and activated fibroblasts secrete CTGF via autocrine and paracrine secretion, which contributes to the capacity of injured alveolar epithelial cells undergoing EMT to promote fibroblasts' migration and proliferation [40][41]. The knockdown of the CTGF gene was shown to attenuate inflammatory responses induced by silica in bronchial epithelial cells [42].

Kasai et al. showed that CTGF might play a role in mediating the EMT process initiated by TGF-β1 [43]. Conversely, Shi et al. did not find evidence of the involvement of CTGF in the process of EMT induction via TGF-β1 [44]. However, a recent study proved that the effects of paracrine in secreted CTGF play an essential role in the EMT-like transition of epithelial cells into mesenchymal cells [45]. Therefore, the deletion of CTGF in mice lung epithelial cells attenuated the fibrotic response to bleomycin [41].

CTGF-induced EMT requires complex multiple signaling pathways to augment fibroblast migration and activation. Xu et al. demonstrated that CTGF contributes to fibroblast activation and matrix protein accumulation via phosphoinositide 3-kinase (PI3K) [35]. Integrin-linked kinase (ILK)-mediated CTGF was shown to induce EMT in AECII cells [46]. Cheng et al. reported that hypoxia-induced CTGF generated α-SMA and collagen expression via the MAPK–MAPK kinase (MEK)–extracellular-signal-regulated kinase (ERK) pathway [47]. Even though the role of ERK is unclear, the activation of the ERK signaling pathway in TGF-β1-induced EMT is crucial [48]. In addition, TGF-β-induced CTGF induces EMT-like changes in the adjacent epithelial cells through ERK, ADAM17, RSK1, and C–EBPβ pathways [49]. Therefore, the inhibition of the MAPK–MEK–ERK pathway might prevent the progression of pulmonary fibrosis [50]. The administration of CTGF was also followed by upregulated tenascin C, an element involved in modulating ECM integrity and cell physiology [51][52].

2.2. Connective Tissue Growth Factor Stimulates the Differentiation of Lung Fibroblasts

Fibroblasts are tissue mesenchymal cells that are fundamental in establishing and maintaining an ECM. Fibroblast migration and activation, followed by myofibroblast differentiation, is the central pathogenesis of pulmonary fibrosis [53][54]. TGF-β regulates the mechanism of myofibroblast differentiation and connective tissue formation during physiological repairment and fibrotic processes. CTGF acts as a downstream mediator of TGF-β action, but CTGF does not act as a direct mediator to induce myofibroblast differentiation and collagen matrix contraction [55][56]. Several studies reported that CTGF triggered fibroblast proliferation and migration and myofibroblast differentiation [57][58][59]. The deletion of CTGF reduced ECM production, characterized by the low expression of COL1α2, COL3, and EDA-fibronectin mRNA [60].

As described previously, myofibroblasts may enhance the apoptosis of AECII. Although the primary source of oxidative stress is inflammatory cells, myofibroblasts generate reactive oxygen species (ROS) [61]. Shibata et al. demonstrated that secreted protein acidic and rich in cysteine (SPARC) promotes hydrogen peroxide (H_2O_2) secretion by TGF-β, leading to epithelial apoptosis [62]. Previously, Wang et al. demonstrated that the expression of CTGF and SPARC were increased in fibroblasts; therefore, SPARC might regulate the collagen expression by affecting the expression of CTGF [63]. Next, SPARC and CTGF seemed to be involved in the same biological pathway that upregulated collagen expression in mice fibroblasts [64].

2.3. Connective Tissue Growth Factor Modulates Dysfunction of Macrophage Polarization

Macrophage homeostasis is needed in the early phases of injury and the resolving phase. In IPF, there is an aberrant wound-healing process following an alveolar epithelial injury that involves the alteration of the polarization of M1 macrophages (pro-inflammatory) and M2 macrophages (anti-inflammatory) [65]. The continuous release of various pro-inflammatory cytokines and chemokines (M1 phenotypes) will preserve the fibrotic environment and induce the secretion of anti-inflammatory/pro-fibrotic cytokines (M2 phenotypes), leading to aberrant wound healing and tissue repair [66].

CTGF-associated macrophages drive polarization. CTGF was shown to be involved in the mechanism of an increase in M1 and a decrease in M2 macrophage markers in the pancreas [67]. Wang et al. also proved that CTGF regulates the polarization of macrophages in hepatocellular cells [68]. Furthermore, Zhang et al. revealed that the secretion of CTGF by M2 macrophages promotes fibroblast proliferation, migration, adhesion, and ECM production via activating the AKT–ERK1/2–STAT3 pathway in lung fibrosis [69]. Therefore, a CTGF blockade abolished M2-polarized macrophage influx [70].

2.4. Connective Tissue Growth Factor Increases Endothelial Growth

Although the mechanisms are not entirely clear, a study reported the possibility of endothelial cells being a source of myofibroblasts and undergoing endothelial–mesenchymal transition (EndoMT) [71]. The increased proliferation of endothelial cells was followed by fiber formation and ECM deposition via sterol regulatory element-binding protein 2 (SREBP2) [72]. Moreover, protein C3ar1 and galectin-3 induced EndoMT in vivo and in vitro [73].

CTGF regulated endothelial cell function and angiogenesis under certain pathological conditions [74][75]. CTGF interacted directly with vascular endothelial growth factor (VEGF) in driving the development of fibrosis and associated lymphangiogenesis/angiogenesis [76][77]. Kato et al. found that the level of CTGF protein was higher in bleomycin-treated mouse lungs than those in saline-treated lungs [78]. It was revealed that CTGF helps the transition of endothelial cells in EndoMT through direct and direct interaction with other pro-fibrotic proteins via hypoxia or inflammatory factors.

2.5. Fibrocyte Differentiation Involved in Connective Tissue Growth Factor

Fibrocytes are the precursors of fibroblasts. The expression of fibrocytes in patients with IPF was high, but the expression of lung fibrocytes was significantly higher compared with circulating fibrocytes [79]. The association between the increased number of circulating fibrocytes and the mechanism of fibrocyte differentiation remains unclear.

However, several studies support the involvement of CTGF in fibrocyte differentiation. CTGF contributes to fibrocyte proliferation and enhances fibrocyte differentiation into a myofibroblast phenotype through SMAD2 and ET_A receptor (ET_AR) [80][81]. Under hypoxia conditions, CTGF was shown to induce the expression of circulating fibrocytes through hypoxia-inducible factor-1α (HIF-1α) and histone deacetylase 7 (HDAC7) [82].

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