



Connective tissue growth factor (CTGF) from basics to clinics

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Abstract

Connective tissue growth factor, also known as CCN2, is a cysteine-rich matricellular protein involved in the control of biological processes, such as cell proliferation, differentiation, adhesion and angiogenesis, as well as multiple pathologies, such as tumor development and tissue fibrosis. Here, we describe the molecular and biological characteristics of CTGF, its regulation and various functions in the spectrum of development and regeneration to fibrosis. We further outline the preclinical and clinical studies concerning compounds targeting CTGF in various pathologies with the focus on heart, lung, liver, kidney and solid organ transplantation. Finally, we address the advances and pitfalls of translational fibrosis research and provide suggestions to move towards a better management of fibrosis.

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CTGF structure and regulation

Structure of the CTGF gene and protein

CTGF (connective tissue growth factor), also known as CCN2, is one of the best-studied members of the CCN family [1], a family of regulatory proteins of the extracellular matrix (ECM) sharing a structurally related composition of functional domains. The acronym **CCN** was conceptualized after the initials of the first three proteins discovered in this family, namely: **C**ysteine rich angiogenic inducer 61 (CYR61), **C**TGF and **N**ephroblastoma overexpressed protein (NOV) [2]. Other members of the family include the WNT1 inducible signaling pathway proteins (WISP1, WISP2 and WISP3) also known as CCN4, CCN5 and CCN6, respectively [1]. The CCNs are involved in the control of a variety of important biological functions, including

cell proliferation, differentiation, adhesion and angiogenesis, as well as multiple pathological processes, such as tumor development and tissue fibrosis [3]. Apart from CCN5, all CCN family members are composed of four functionally distinct conserved regions: an insulin-like growth factor (IGF)-binding protein domain next to a von Willebrand factor type C repeat (together forming the N-terminal fragment), and a thrombospondin type 1 repeat next to a cysteine knot (together forming the C-terminal fragment). The non-conserved hinge between domains 2 and 3 is subject to proteolytic cleavage [4] (Fig. 1).

The *CTGF* gene (6q23.2) is a relatively short gene composed of 5 exons that code for a 349-amino acid protein. A novel splice variant of *CTGF* encoding the C-terminal domain has been identified in the pediatric precursor B cell acute lymphoblastic leukemia which may be involved with tumorigenesis [5].

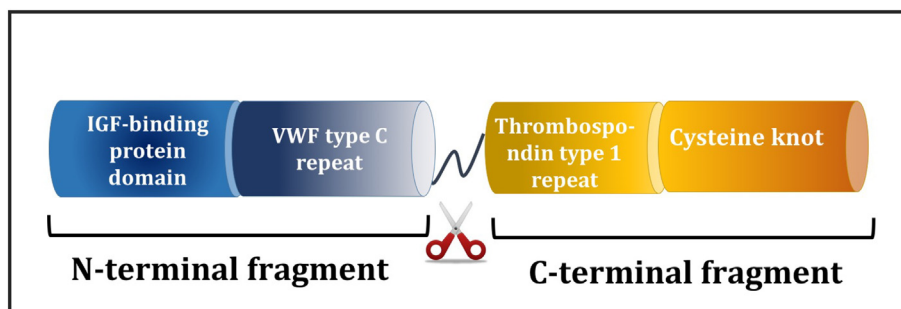


Fig. 1. With the exception of CCN5, all CCN family members are composed of four conserved regions: an insulin-like growth factor (IGF)-binding protein domain, a von Willebrand factor type C repeat, a thrombospondin type 1 repeat, and a cysteine knot. While each domain can have its own biological function(s), the different domains together may provide scaffolds for specific orchestration of crosstalk between multiple signaling pathways. The hinge between domains 2 and 3 can be cleaved by the activity of number of different proteases.

The CTGF gene and protein are highly conserved among vertebrate species. When comparing to human CTGF, the mouse ortholog exhibits 91% base pair identity and 95% amino acid similarity, while the zebrafish ortholog shows 78% identity and 87% similarity compared to humans. CTGF expression was initially discovered in endothelial cells and fibroblasts associated with connective tissue regeneration and wound healing [6,7]. *CTGF RNA* has been detected in many tissues, including smooth muscles, thyroid, spleen, kidney, prostate and endometrium, while high levels of CTGF protein have been reported in the cerebral cortex, smooth muscles, lymph nodes, lung, liver, gastrointestinal tract, endometrium and skin [8].

Regulation of CTGF

Contrary to the embryonic stage, where CTGF is highly expressed mediating renal, cardiovascular and skeletal development, the expression of *CTGF* is rather limited during adulthood. It is regulated at the transcriptional, post-transcriptional and (post-)translational levels by a variety of factors. At the transcriptional level, *CTGF* expression is induced by stimuli such as growth factors, cytokines, hormones, mitogens, ultraviolet light, oxygen deprivation and mechanical stress [9]. These external stimuli induce directly - or through crosstalks with cell surface receptors - the recruitment of transcription factors that drive the expression of *CTGF*. Most often, *de novo* synthesis of transcription factors is not necessary [4]. Transforming growth factor β (TGF β) is a master regulator of tissue growth, regeneration, remodeling and fibrosis and most TGF β responses involve CTGF stimulation at some level, such as the stimulation of ECM components, the proliferation of fibroblasts, osteoblasts and astrocytes, wound healing, and fibrosis [10–12]. The signaling molecules involved in crosstalk and integration of TGF β and CTGF effects are numerous and variable, depending on the cell type and the physiological or pathological process involved. For instance, while

TGF β stimulated Smads are necessary for induction of CTGF in normal fibroblasts and basal CTGF induction in scleroderma fibroblasts, maintenance of CTGF expression is independent of Smads [13].

Key transcription coactivators and transcriptional factors inducing CTGF expression are YAP (yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif)/TEAD (transcriptional enhancer factor TEF-1) and Ets1 (ETS proto-oncogene 1). YAP/TAZ induce the expression of *CTGF* in corneal epithelial, kidney epithelial and endothelial cells among others [14–16]. Very recently, it was demonstrated that YAP causes activation of hepatic stellate cells in mice and that TAZ, in a TGF β -dependent mechanism, upregulates *CTGF* in kidney epithelial cells [17,18]. Ets1 is another potent stimulator of *CTGF* in modulating ECM remodeling in fibroblasts, endothelial cells and cancer cells [19–22] (Fig. 2).

Table 1 provides the overview of the chemical compounds with therapeutic potential that are experimentally capable of reducing CTGF expression as well as different members of the micro RNA (*mi-RNA*) family that are discovered to be important in regulation of *CTGF*.

As a secreted soluble protein of 36–38 kD, CTGF can also be found in the extracellular body fluids including blood plasma, from where it is eliminated by glomerular filtration in the kidney, and in the liver by uptake in hepatocytes. Full length CTGF is eliminated through a rapid route, which is mediated by low-density lipoprotein receptor-related protein 1 (LRP1) binding on the hepatocytes and, to a lesser extent, (12% of total CTGF) by the kidney [53]. The C-terminal fragments of CTGF, similar to the full length CTGF, is cleared LRP1 binding on hepatocytes, which is a faster route than glomerular filtration [53]. Full length CTGF and its N-terminal and C-terminal fragments are filtered by the glomeruli and then reabsorbed completely from primary urine by the megalin-cubulin complex on proximal tubular epithelial cells. If reabsorption fails due to tubular dysfunction, especially the N-terminal CTGF fragment tends to accumulate in the voided

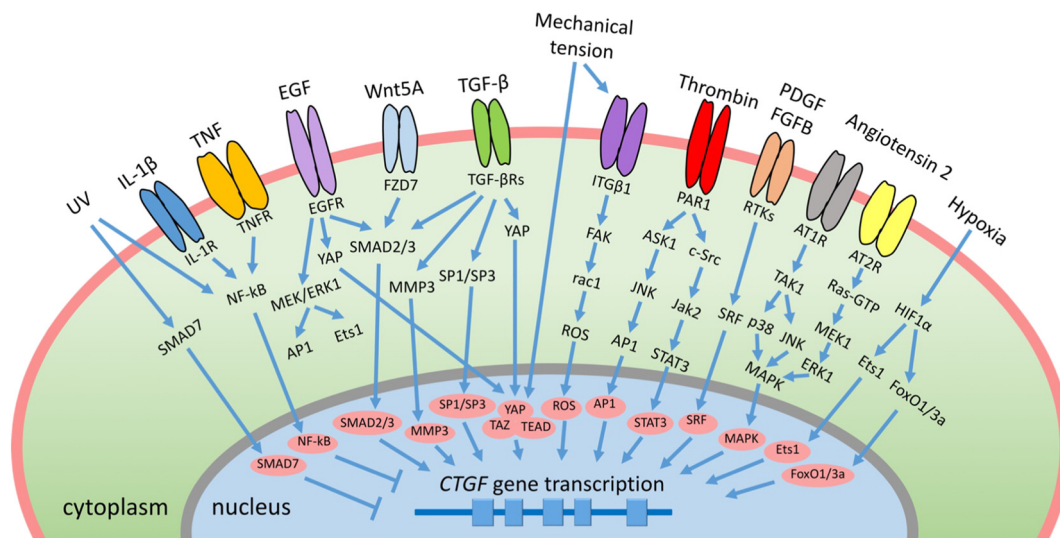


Fig. 2. External stimuli directly or through crosstalks, initiate signaling pathways that recruit transcription factors to the nucleus. These factors, based on the context in different cell types, inhibit or stimulate the expression of CTGF. CTGF expression is mainly regulated at the transcription level by the key transcription factors YAP/TAZ and Ets1 among others [9,23–35]. AP1: activator protein 1; ASK1: apoptosis signal-regulating kinase 1; AT1R: angiotensin 2 type 1 receptor, AT2R: angiotensin 2 type 2 receptor; c-Src: proto-oncogene c; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; Ets1: ETS proto-oncogene 1; FAK: focal adhesion kinase; FGFB: fibroblast growth factor 2; FOXO1/3a: forkhead box protein O1/forkhead box protein O3a; FZD7: frizzled 7; HIF1 α : hypoxia-inducible factor 1 α ; IL-1R: interleukin 1 receptor; IL1 β : interleukin 1 β ; ITG β 1: integrin α 3 β 1; JAK2: Janus kinase 2; JNK: c-Jun N-terminal kinase; MAPK: mitogen-activated protein kinase; MEK/ERK: mitogen-activated protein kinase/extracellular signal-regulated kinase; MMP3: matrix metalloproteinase 3; NF κ -B: nuclear factor kappa-light-chain-enhancer of activated B cell; PAR1: protease-activated receptor 1; PDGF: platelet-derived growth factor; Rac1: Ras-related C3 botulinum toxin substrate; RAS-GTP: RAS-guanosine triphosphate; ROS: reactive oxygen species; RTK: receptor tyrosine kinase; SP1/SP3: specificity protein 1/specificity protein 3; SRF: serum response factor; STAT3: signal transducer and activator of transcription 3; TAK1: transforming growth factor beta-activated kinase 1; TAZ: transcriptional coactivator with PDZ-binding motif; TEAD: transcriptional enhancer factor TEF-1; TGF β : transforming growth factor β ; TGF β R: transforming growth factor β receptor; TNF: tumor necrosis factor; TNFR: tumor necrosis factor receptor; UV: ultra violet; YAP: yes-associated protein.

urine [54,55]. The N-CTGF fragments do not bind to LRP1, thereby escaping hepatic elimination, and are less rapidly cleared than the full length and C-terminal

fragment. As a consequence, N-CTGF is more easily detected in blood plasma, and tends to accumulate when renal function is compromised.

Table 1. Overview of chemical compounds and mi-RNAs reducing CTGF expression.

Regulatory factor	Reference
Sinomenine	[36]
Curcumin	[37]
Caffeine	[38]
Statins	[39]
DN-9693 (a cAMP: phosphodiesterase inhibitor)	[40]
mi-RNA-15a/b	[41]
mi-RNA-18a	[42]
mi-RNA-18b	[43]
mi-RNA-19b	[44]
mi-RNA-26a	[44,45]
mi-RNA-26b	[44]
mi-RNA-29	[43]
mi-RNA-30	[46]
mi-RNA-133	[46]
mi-RNA-145	[47,48]
mi-RNA-205	[49]
mi-RNA-214	[50,51]
mi-RNA-375	[52]

Biological functions and mechanisms of action

The biological roles of CTGF are considered to be regulatory: I) It is capable to bind to specific receptors to initiate signal transduction, II) It can bind cytokines, mediating their binding to the cell-surface receptors and initiation of downstream signaling pathways, III) CTGF mediates the matrix turnover by binding to ECM proteins and IV) It is involved in the regulation of the activity of cytokines and growth factors through modulation of crosstalk between signaling pathways.

CTGF binding to cell surface receptors

CTGF binds several receptors, such as integrins (ITGs), heparan sulfate proteoglycans (HSPGs), lipoprotein receptor related proteins (LRPs) and tyrosine kinase (TK) receptors [4]. CTGF exerts many of its functions through binding to integrin receptors. In

some cases, LRP6 and HSPGs act as co-receptors [56]. For instance, in liver fibrosis, in activated hepatic stellate cells, CTGF binds to integrin $\alpha_5\beta_3$ with HSPGs acting as co-receptors [57]. Moreover, CTGF binds to $\alpha_5\beta_1$ and HSPG in pancreatic stellate cells to mediate their adhesion, proliferation, migration and collagen synthesis in pancreatic fibrosis [58].

CTGF, through direct binding to $\alpha_1\beta_3$ integrin, strengthens the adhesion of chondrosarcoma-derived chondrocytes to fibronectin [59].

CTGF binds to LRP1 on hepatocytes and LRP6 on kidney cells, which is discussed in depth later in this review. CTGF mediates fibrosis in cardiomyocytes partly through binding to TK receptor A [60]. Interaction of CTGF with syndecan 4, another receptor in mouse embryonic fibroblasts, is necessary for the motility of these cells [61]. CTGF binds to fibroblast growth factor receptor 2 and, in collaboration with fibroblast growth factor 2, regulates bone metabolism in osteoblasts [62]. Receptor activator of nuclear factor κ B (RANK), osteoprotegerin and dendritic transmembrane protein are other receptors to which CTGF binds, and through which it enhances osteoclast differentiation [63,64]. In human corneal fibroblasts, binding of CTGF to yet another receptor, mannose 6 phosphate/insulin like growth factor 2 receptor is necessary for fibroblast proliferation [65].

CTGF binding to cytokines

CTGF can directly bind to cytokines regulating their availability and activity. For instance, CTGF binds vascular endothelial growth factors (VEGFs), fibroblast growth factor 2 (FGF-2), bone morphogenic protein 4 (BMP4), TGF β and platelet-derived growth factor B (PDGFB) [66–69]. The downstream effects of binding of CTGF to the cytokines and growth factors are context-dependent. For instance, while CTGF inhibits the VEGF-A signaling pathway in angiogenesis (induced by hypoxia or ischemia), in kidney fibrosis, it induces the VEGF-C induced lymphangiogenesis [66,69].

CTGF binding to ECM proteins

CTGF binds to components of the ECM such as fibronectin, aggrecan and HSPGs [70]. The binding of CTGF to ECM components is central to the functions of CTGF in cell adhesion and motility (discussed further in detail) as well as its role as the mediator of ECM turnover during tissue remodeling in (patho) physiological conditions [71].

CTGF modulating crosstalk between signaling pathways

There is not much known about the role of CTGF as the mediator in linking signaling pathways. However, based on multiple evidence on the binding

of CTGF to soluble growth factors on the one hand and its binding to multi-ligand receptors on the other hand, CTGF fulfills the common features of a matricellular protein very well. CTGF can facilitate specific downstream effects *via* binding to context-dependent receptors such as ITGs, HSPGs and LRP6 (see above) [56].

CTGF functions in development

CTGF appears to play an important role in a myriad of biological processes active in different stages during development. *CTGF* knockout mice demonstrate developmental skeletal malformations, including impaired palatogenesis and rib-cage deformity. These mice die shortly after birth due to respiratory failure [72]. Although the responsible mechanisms are not well elucidated, CTGF seems to be important for cellular proliferation, adhesion and cell spreading during of palatogenesis [73]. During development, CTGF is highly expressed in vasculature, heart, lung and bone tissue [74]. Interestingly, CTGF was found to be involved in regulation of energy metabolism in bone tissue, and CTGF deficiency in murine chondrocytes caused reduced expression of several glycolytic enzymes. CTGF deficiency also imbalanced protein synthesis, with excessive consumption of essential amino acids and thereby critically reducing the availability of essential amino acids [75]. CTGF also induces differentiation of murine neural progenitors to astrocytes through p44/42 mitogen MAPK signaling and fibronectin deposition [10]. In addition, CTGF drives the early differentiation of myoblasts in mice, while it inhibits the terminal differentiation of myoblasts [76].

CTGF as mediator of cellular events

Cell adhesion and motility

Inhibiting CTGF *in vitro* using anti-CTGF neutralizing antibody inhibits the attachment of cells on the surface, indicating the necessity of CTGF for adhesion of the cells to the ECM; however, as previously stated, CTGF is neither a cell-membrane molecule nor a structural component of the ECM, but rather bridges the latter (including fibronectin, perlecan, vitronectin, and decorin) to integral cell surface molecules like ITGs and connexin 43 [4,77]. In fibroblasts, CTGF binds to ITGs and HSPGs and initiates phosphorylation of focal adhesion kinase (FAK) and extracellular signal regulated kinase (ERK), activates F-actin, paxillin and RhoA and enhances the formation of focal adhesions [77].

CTGF overexpression in some cancers enhances the motility of cancer cells and metastasis. For instance, CTGF induces the motility of breast cancer cells through integrin $\alpha_5\beta_3$ and phosphorylation of ERK 1/2 and through P38 phosphorylation in non-small cell

lung cancer [77,78]. Although CTGF has been associated with metastasis of several cancers, it actually enhances anoikis and reduces metastasis in lung cancer. This implies the context-dependent role of CTGF in regulating cell motility [79].

Cell survival, cell cycle arrest and senescence

It has been demonstrated that CTGF, by activating mitogen activated protein kinase (MAPK) phosphatase 1 (MPK1, or Dual Specificity Phosphatase 1) and stabilization of anti-apoptotic protein B-cell lymphoma 2 (BCL-2), enhances cell survival in kidney mesangial cells [80]. This is contrary to the prior observation that CTGF causes cell cycle arrests in kidney mesangial cells [81]. In cellular senescence, typically identified as prolonged cell cycle arrest, CTGF is produced in large amounts, and might stimulate senescence-associated inflammation and fibrosis [82].

CTGF in connective tissue remodeling

The process of wound healing occurs through the recruitment of immune cells to the site of injury and activation of injured epithelial/endothelial cells. These cells produce profibrotic and pro-inflammatory cytokines, which induce trans-differentiation of epithelial cells, local or recruited mesenchymal cells (fibroblasts and pericytes) and endothelial cells, to become myofibroblasts, and increase the production of ECM. This process will cease by elimination of the initial insults by the immune system. However, if the injury persists, repeats, or gets too intense, or if the process

of repair becomes dysregulated, the normal tissue repair will proceed to excessive, and at some point, irreversible fibrosis [83,84].

Wound healing and tumor-induced connective tissue responses are indistinguishably similar comprising inflammation, cell proliferation and tissue remodeling. Cells involved in wound healing and tumor stroma induction produce several cytokines and chemokines to enhance cell proliferation, ECM production, and formation of new blood vessels [85]. CTGF is one of the main regulators of the molecular events that occur in the spectrum of wound healing to fibrosis through its interactions with several receptors, growth factors and signaling molecules (Fig. 3).

Upon injury in the kidney, damaged tubular epithelial cells get activated and produce proinflammatory and profibrotic factors such as CTGF and TGF β . These cytokines further induce the dedifferentiation of proximal tubule epithelial cells to acquire a phenotype resembling contractile myofibroblasts which are the main mediators of matrix turnover [84]. In several *in vitro* and *in vivo* kidney fibrosis models during the maladaptive response of the proximal tubule cells to injury, these cells get arrested in the G2/M phase of the cell cycle with activation of the DNA damage repair (DDR) mechanisms that result in the production of profibrotic factors [86,87]. This pathway has now been demonstrated to essentially involve several components of the DDR [33,88,89]. It will be interesting to further elucidate how production of CTGF by cell cycle-arrested tubular epithelial cells may relate to their trans-differentiation to myofibroblasts and other aspects of kidney fibrosis. For this,

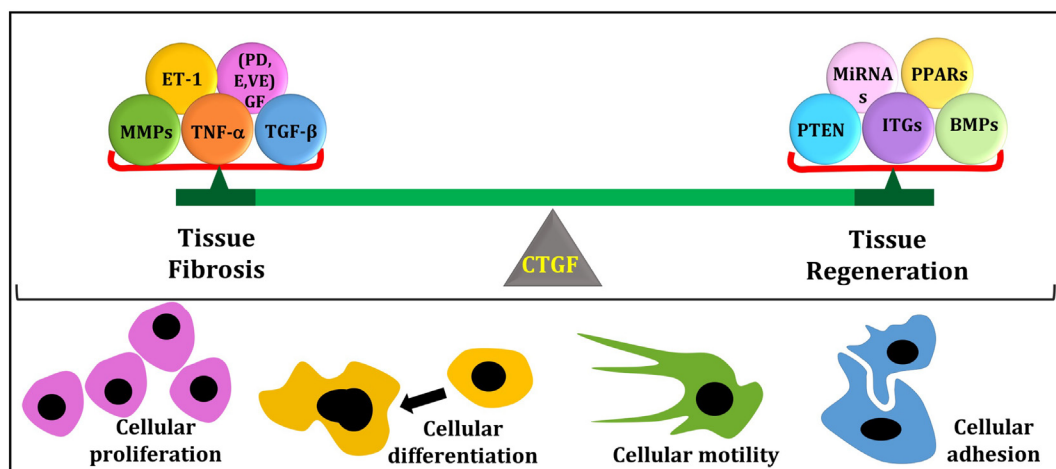


Fig. 3. CTGF mediates both tissue regeneration and fibrosis through its interaction with several factors which results in cell proliferation, differentiation, motility, adhesion and matrix turnover. The pathways involved in fibrosis are complex and tissue-specific. However, the following factors are commonly involved in manifestation of fibrosis in different tissues: ET-1: endothelin-1; (PD, E, VE) GF: platelet-derived, epidermal and vascular endothelial growth factor; MMPs: matrix metalloproteinases; TNF- α : tumor necrosis factor α ; *mi-RNA*: micro RNA, PPAR: peroxisome proliferator-activated receptor; PTEN: phosphatase and tension homolog; ITGs: integrins; BMPs: bone morphogenic proteins.

Table 2. List of molecular interventions targeting CTGF in different pathological contexts.

Disease setting	Source/level of intervention	Intervention	Results	Reference
<i>Carcinogenesis and tumor development</i>				
Acute lymphoblastic leukemia	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab; together with vincristine, L-asparaginase and dexamethasone)	Prolongation of survival	[218]
Metastatic melanoma	Human/melanoma cell lines	Anti-CTGF antibody (FG-3019/pamrevlumab)	Inhibition of progression	[99]
Pancreatic tumor	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab)	Reduction of tumor size	[219]
High grade serous ovarian cancer	Human/HGSOC tumor tissue	Anti-CTGF antibody (FG-3019/pamrevlumab)	Inhibition of migration and peritoneal cell adhesion	[220]
Ductal pancreas cancer	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab; together with gemcitabine)	Reduction of tumor size	[221]
<i>Ocular and neural diseases</i>				
Glaucoma	Human/cribrosa cells	Anti-CTGF antibody (FG-3019/pamrevlumab)	Reduction of (fibronectin, fibrillin-1, collagen 1, and α -smooth muscle actin)	[102]
<i>Muscular diseases</i>				
Duchenne muscular dystrophy	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab)	Amelioration of impaired muscle function and fibrosis	[111]
<i>Skin fibrosis (Scleroderma)</i>				
Skin fibrosis	Mouse/skin fibroblasts	Anti-CTGF antibody (FG-3019/pamrevlumab)	Attenuation of inflammation, fibrosis and vascular damage	[117]
<i>Lung fibrosis</i>				
Lung fibrosis	Mouse/systemic	Anti-CTGF antibody	Reduction of collagen type I and α 2 promoter activity	[125]
Lung fibrosis	Rat/pulmonary microvascular endothelial cells	Anti-CTGF antibody	Inhibition of fibroblast transformation and decrease in collagen	[222]
Lung fibrosis	Human/embryonic lung fibroblast and mouse/systemic	Anti-CTGF antibody	Reduction of inflammatory leukocytes and hydroxyproline content of lung tissue, attenuation of fibrosis	[223]
Lung fibrosis	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab)	Attenuation of excessive collagen disposition	[224]
Lung fibrosis	Human/embryonic lung fibroblasts	Anti-CTGF antibody	Decrease in endothelin- induced α -smooth muscle actin expression	[225]
<i>Cardiac fibrosis</i>				
Myocardial fibrosis in cardiomyopathy	Mouse (systemic)	Anti-CTGF antibody (FG-3019/pamrevlumab)	Improvement of left ventricular function	[146]
<i>Liver fibrosis</i>				
Liver fibrosis	Mouse/systemic	Antisense CTGF oligonucleotide	Reduction of collagen type I induced by carbon tetrachloride	[226]
Liver fibrosis	Rat/stellate cells	Antisense CTGF oligonucleotide	Attenuation of stellate cells' activity, reduction of TGF β	[174]
Liver fibrosis	Rat/primary stellate cells	Antisense CTGF oligonucleotide	Inhibition of stellate cells' activation and reduction of ECM genes	[175]
Hepatitis C-induced liver fibrosis	Human/hepatoma cell lines	Short hairpin-RNA mediated knockdown of CTGF	Reduction of α -smooth muscle actin and MMP2 expression	[227]
Liver fibrosis	Rat/immortalized stellate cells	Short hairpin-RNA mediated knockdown of CTGF and antisense CTGF oligonucleotide	Reduction of proliferation and activity of stellate cells and reduction of collagen expression and liver fibrosis	[176]
<i>Kidney fibrosis: tubulointerstitial diseases</i>				
Interstitial fibrosis in diabetic nephropathy	Human/kidney fibroblasts	Anti-CTGF antibody	Partial inhibition of glucose-induced matrix formation	[228]
Renal interstitial fibrosis	Rat/interstitium	Antisense CTGF oligonucleotide	Decrease of ECM proteins and myofibroblast proliferation	[229,230]

Table 2 (continued)

Disease setting	Source/level of intervention	Intervention	Results	Reference
<i>Kidney fibrosis: tubulointerstitial diseases</i>				
Renal interstitial fibrosis	Human/tubule epithelial cells	Antisense CTGF oligonucleotide	Inhibition of TGF β -induced and direct trans differentiation of epithelial cells to myofibroblasts	[231]
Renal interstitial fibrosis	Mouse/systemic	Antisense CTGF oligonucleotide	Reduction of plasminogen activator inhibitor 1 (PA-1) and tissue inhibitor of metalloprotease 1 (TIMP1)	[214]
Renal interstitial fibrosis	Human/tubule epithelial cells	Antisense CTGF oligonucleotide	Inhibition of TGF β induced increase of plasminogen activator inhibitor 1	[232]
Renal interstitial fibrosis	Human/tubule epithelial cells	Antisense CTGF oligonucleotide	Reduction of lymphangiogenesis through decrease of VEGF-C	[188]
<i>Kidney fibrosis: glomerulonephropathies</i>				
Fibrosis	Rat/mesangial cells	Antisense CTGF oligonucleotide	Inhibition of cell proliferation and reduction of fibronectin	[233]
Crescentic glomerulonephritis	Rat/glomerular parietal epithelial cells	Antisense CTGF oligonucleotide	Inhibition of TGF β and PDGF-induced ECM production	[234]
Diabetic Nephropathy	Human/mesangial cells	Antisense CTGF oligonucleotide and anti-CTGF antibody	Reduction of TGF β induced increase of fibronectin (stimulated by high glucose)	[235]
Diabetic nephropathy	Rat/mesangial cells	Anti-CTGF antibody	Inhibition of Fibronectin and collagen IV production	[236]
Diabetic nephropathy	Rat/interstitial fibroblasts	Antisense CTGF oligonucleotide	Inhibition of advanced glycation end products (AGE)-induced collagen 1 production and leptin-induced mitogenesis	[237,238]
Diabetic nephropathy	Mouse/renal cortex	Antisense CTGF oligonucleotide	Reduction of fibronectin, collagen I and V and PAI-1	[205]
Diabetic nephropathy	Human/mesangial cells	Antisense CTGF adenoviral transduction	Reduction of tissue inhibitor of matrix metalloprotease and fibronectin induced by advanced glycosylated end-products	[239]
Diabetic nephropathy	Mouse/podocytes	Anti-CTGF antibody	Restoration of EMT induced by high glucose	[240]
Diabetic nephropathy	Mouse/glomeruli	Anti-CTGF antibody (FG-3019/pamrevlumab)	Amelioration of β -catenin overexpression, reduction of podocytes' EMT	[207]
<i>Transplantation</i>				
Heart transplantation	Mouse/systemic	Anti-CTGF antibody (FG-3019/pamrevlumab)	Reduction of fibrotic lesions in allografts and cardiomyocyte hypertrophy	[241]

staining kidney biopsies for CTGF and other markers may yield important insights.

In the following section, we will elaborate on the roles of CTGF in multiple pathologies. Studies of the impact of molecular interventions to increase or impair CTGF availability and activity are listed in Table 2.

Roles in specific pathological conditions

Carcinogenesis and tumor development

The role of CTGF in carcinogenesis has been extensively studied with a focus on its interaction with TGF β , but it is becoming clear that the role of CTGF in

cancer development is also determined by the synergistic effect of the network of CTGF with other CCN proteins and other molecules in tumor environment [79]. CTGF has been both positively and negatively correlated with evolution of tumors and metastases. For instance, while CTGF expression enhances the growth of hepatocellular carcinoma and early stages of colon carcinoma, it seems to have a protective effect against metastasis in later stages of colon cancer [90,91]. Of note, high CTGF expression is also associated with the aggressive inflammatory and stroma-rich phenotype of colorectal cancer [92]. CTGF expression is also increased in breast cancer (where it might regulate chemo-resistance) [93,94], chondrosarcomas, enchondroma, glioma, pancreatic cancer, thyroid carcinoma, chondrosarcoma, esophageal cancer, intrahepatic cholangiocarcinoma, neuroendocrine

tumors, and squamous cell carcinoma of the tongue [95–97]. In a model of murine melanoma, the absence of CTGF impaired the ability of the malignant cells to dynamically remodel the ECM and metastasize. Moreover, in wild type mice injected with melanoma cell lines lacking CTGF, no metastasis was observed in the lungs [98]. CTGF is known to be vital for the progression of melanoma through the hypoxic induction of hypoxia-inducible factor 1 and 2 α (*HIF1* and *HIF2 α*). In accordance, blocking CTGF with the anti-CTGF monoclonal antibody, FG-3019/pamrevlumab, could inhibit the migration of human melanoma cell lines emphasizing the potential of CTGF as a therapeutic target in metastatic melanoma [99].

There are several mechanisms proposed for involvement of CTGF expression in different aspects of tumor biology, involving inflammation, cell proliferation, survival and motility but further discussion is beyond the scope of this review and is elaborated elsewhere [79]. To maintain the focus of this article on fibrosis, we have only mentioned few examples of such studies in Table 2. For a broader overview of drugs that modulate the tumor microenvironment, interested readers are encouraged to read a recent review in this field [85].

Ocular and neural disorders

CTGF is shown to be involved in the pathology of several ocular diseases where it has been implicated primarily in modulation of fibrosis and neovascularization through matrix metalloproteinase 2 (MMP2) and VEGF. In proliferative diabetic retinopathy, an increasing amount of CTGF in disequilibrium with VEGF, causes a switch from neovascularization to fibrosis, an event known as angiofibrotic switch, which promotes scarring of the retina [100]. Synergistically with TGF β , CTGF causes cellular changes (myofibroblastic phenotype) and changes in extra-cellular matrix in myopia and glaucoma [101]. In glaucoma, pre-treatment of human cribriform cells with anti-CTGF antibody FG-3019/pamrevlumab, reduced CTGF-induced ECM-associated gene expression (fibronectin, fibrillin-1, CTGF, collagen 1, and α -smooth muscle actin) [102]. In a mouse model of diet-induced insulin-resistant diabetes, elevated levels of CTGF in the brain were detected and correlated with the clinical progression of Alzheimer's disease [103]. In Duchenne muscular dystrophy (DMD), elevated levels of CTGF in animal models and biopsies of patients correlate with inflammation and fibrosis and with clinical severity of this disease [104–107]. In addition to a role for TGF β , [108], YAP/TAZ/TEAD and, subsequently, CTGF are elevated in human and mouse failing hearts [109]. In these pathologies, transcription factors Smad3 and SP1/SP3 are important in regulation of CTGF in response to TGF β stimulation in myoblasts [26]. In skeletal muscle cells, CTGF causes increased accumulation of ECM through ERK phosphorylation possibly *via* the interaction with HSPGs since the addition of heparan could

reverse CTGF effects [110]. Finally, treating dystrophic mice with the aforementioned FG-3019/pamrevlumab could ameliorate impaired muscle function and fibrosis, and a clinical trial for this treatment in DMD patients has been started (clinical trial: NCT02606136) [111].

Systemic sclerosis

Systemic sclerosis (SSc) is an autoimmune disease characterized by progressive fibrosis of skin and internal organs. Although the underlying mechanisms of the disease remain largely unknown, CTGF, may play an important role, since a polymorphism in the *CTGF* promoter region (rs6918698) that enhances CTGF and subsequent collagen I production was associated with systemic manifestation of the disease, and increased levels of CTGF in serum of patients with SSc correlate with the progression of skin and pulmonary fibrosis [112,113]. A later study in an animal model demonstrated that the synergic effect of CTGF and basic fibroblast growth factor contributes to skin fibrosis by increasing the levels of monocyte chemoattractant protein, which is important for infiltration of macrophages [114]. The role of CTGF in SSc fibroblasts is reported to be dependent on TGF β and together, TGF β and CTGF (induced by TGF β) cause persistent fibrosis in animal models of SSc [115]. Moreover, other evidence suggests that CTGF, TGF β and endothelin-1 (ET-1) together induce myofibroblast differentiation [116]. It has recently been demonstrated that blocking CTGF by FG-3019/pamrevlumab antibody in mouse skin fibroblasts attenuates inflammation, fibrosis and vascular damage induced by angiotensin II [117]. Although direct blockade of CTGF seems to be a promising treatment option of SSc, additional studies have suggested that indirect modification of CTGF expression with the help of other potential therapeutic targets such as phosphatase and tension homolog (PTEN) and P2X purinoceptor 7 is a valid alternative approach [118,119]. In SSc skin fibroblasts, PTEN, a protein and lipid phosphatase and the inhibitor of PI3-Akt signaling, is downregulated and CTGF is important in the fibrosis induced by loss of PTEN. PTEN is also reduced in the fibroblasts of patients with idiopathic pulmonary fibrosis, leading to the hypothesis that it is also involved in lung fibrosis. This hypothesis was further tested by the genetic deletion of this gene together with CTGF in collagen expressing murine fibroblasts. These cells did not develop significant collagen deposition, highlighting the regulatory effect of CTGF downstream of PTEN [118].

Lung fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic condition of the lung with yet unknown etiology. The gradual loss of lung function can only be treated

with lung transplantation, highlighting the necessity of finding other treatment options for this disease.

Although the etiology of the disease is not known, injury to the lung alveolar epithelial cells seems to be the primary event which results in excessive ECM disposition and fibrosis due to the imbalance between the profibrotic and antifibrotic events [120].

Increase of CTGF levels in fibroblasts of bleomycin-induced mouse model of fibrosis indicates a potential role of CTGF in pathology of IPF [121]. CTGF-deficient animals have less myofibroblasts and ECM disposition, indicating that CTGF is necessary for induction of fibrosis in these animals [122]. However, histological analyses of the skin lesions of bleomycin-induced pulmonary fibrosis animals demonstrate that CTGF induces the most severe fibrotic effects when administered with TGF β [123]. Moreover, in the lungs of fibrosis-resistant mice, stimulation of fibrosis with bleomycin and CTGF together, and not each individually, causes fibrotic responses [124]. Therefore, CTGF seems to stimulate lung fibrosis in function of a second trigger, such as bleomycin and thus could be targeted to ameliorate fibrosis. Indeed, administration of anti-CTGF antibodies in several animal models of fibrosis reduces the expression of ECM components, enhances the survival after inducing lung damage by radiation and preserves the morphology of alveolar epithelial cells [125,126].

In patients with IPF, CTGF is shown to be increased in fibroblasts [127], bronchoalveolar lavage cells [128], plasma [129] and lung tissue [130] which makes CTGF a valid therapeutic target and possible biomarker for companion diagnostics for treatment of this disease. There is encouraging evidence for the potential of anti-CTGF monoclonal antibody, FG-3109/pamrevlumab, in treatment of IPF. In an open-label phase 2 trial, FG-3109/pamrevlumab was proven to be safe and well-tolerated by patients [131]. A double-blind placebo-controlled trial demonstrated significantly reduced decline of the forced vital capacity in patients treated with FG-3109/pamrevlumab compared to the placebo arm. Moreover, combining FG-3109/pamrevlumab with stable doses of pirfenidone, a TGF β suppressor [132] and nintedanib, a TK inhibitor [133], was also well-tolerated by IPF patients [134].

Cardiac fibrosis

Cardiac fibrosis often occurs in the context of hypertension and diabetes mellitus. Cardiac fibrosis is a huge global burden with limited treatment options, highlighting the importance of discovering new treatment options [135].

CTGF is expressed in cardiac fibroblasts and cardiomyocytes, where its promoter activity and expression is enhanced by TGF β . Increased CTGF causes upregulation of ECM proteins including fibronectin and collagen I and III following myocardial infarction in animals [136] and both TGF β and CTGF

were found to be expressed in fibroblasts of the scar tissue in a mouse myocardial infarction model [137]. CTGF is also involved in diabetic heart disease; it exerts an endocrine regulatory role on rat cardiomyocytes treated with high glucose and fatty acids. CTGF in these cells induces apoptosis and hypertrophy by phosphorylating the TK A receptor [138].

Blocking CTGF by RNA interference in primary mouse cardiomyocytes causes attenuation of the expression of several genes such as stretch-induced chemokine, matrix metalloproteases, extracellular and cell-adhesion proteins, suggesting the involvement of CTGF in various pathways [139]. Ubiquitous or heart-specific CTGF knockdown or overexpression in mouse models of cardiac fibrosis did not significantly impact the progression of fibrosis, suggesting other possible drivers of cardiac fibrosis [140,141]. Conversely, CTGF overexpression in the myocardium of mice has also been observed to improve the left ventricular function after myocardial infarction through inhibition of inflammatory responses and apoptosis. Consistently, improved heart function in patients with elevated serum CTGF levels suggested that CTGF might indeed have some protective role in left ventricular remodeling after myocardial infarction [142].

A number of different *in vivo* interventions have linked CTGF with important molecular pathways of cardiac fibrosis. Cardiac-specific overexpression of serum response factor (SRF), acts as the upstream inducer of CTGF by reducing *mi-RNA-133*, which in turn downregulates both SRF and CTGF [27]. Moreover, circular RNA 000203 increased CTGF expression in mouse diabetic cardiomyopathy by inhibiting *miR-26b-5p*, which attenuates fibrosis by downregulating CTGF [143]. *MiR-455* and *-132* are still other *mi-RNAs* recently reported to attenuate fibrosis by decreasing CTGF and offering new potential drug targets [144,145].

As for targeting CTGF for treatment of heart disease, administration of the human IgG CTGF-neutralizing antibody FG-3019/pamrevlumab for two weeks in the lamin A/C-deficiency mouse model of cardiomyopathy, significantly improved cardiac fibrosis and enhanced left ventricular function [146]. A similar protective effect was observed for the rodent equivalent FG-3149 in the PKC ϵ mouse model of dilated cardiomyopathy [147]. Thus far, CTGF inhibition for treatment of heart diseases has not been studied in clinical trials.

Liver fibrosis

Although the liver parenchyma has a large regenerative capacity, chronic liver disease can lead to the permanent loss of hepatocyte mass and replacement by fibrotic tissue. This can ultimately lead to severe architectural disturbance called cirrhosis, which is associated with complications such as malnutrition,

portal hypertension, gastrointestinal bleeding, ascites, hepatorenal syndrome and encephalopathy, eventually resulting in the need for a liver transplantation. The underlying causes of cirrhosis worldwide are diverse,

but viral hepatitis (around 60%) and chronic alcohol abuse (around 20% of the etiology) are important factors [148]. The histopathological features of cirrhosis include diffuse hepatic fibrosis with the formation of

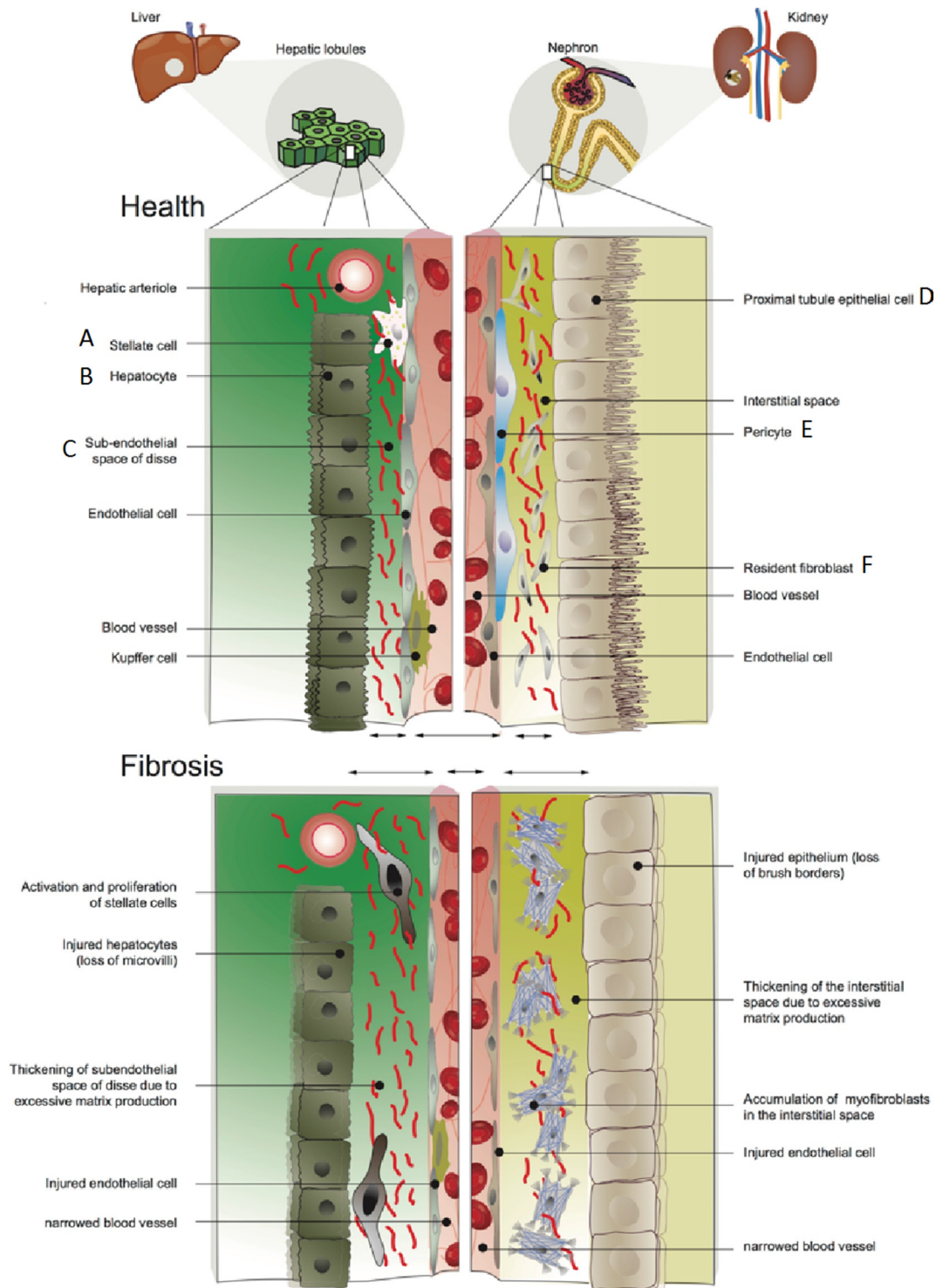


Fig. 4 (legend on next page)

nodules of regenerating hepatocytes. The increasing degree of connective tissue deposition results in destruction of the normal liver architecture, with an increased and altered composition of the cellular matrix of the subendothelial space of Disse, generating additional diffusion barriers between hepatocytes and liver sinusoids and an increased resistance for the portal blood flow.

Hepatic stellate cells (HSCs), which are pericytes situated in the perisinusoidal space in close contact with the endothelium and hepatocytes, are considered the primary source of ECM deposition. During normal physiology, HSCs are involved in vitamin A and fat storage and constitute *circa* 10–15% of cells in the liver [149]. In addition, it has been shown that stellate cells also play a central role in liver regeneration/repair, angiogenesis, vascular remodeling and the hepatic immune response through their ability to act as antigen presenting cells [150]. As a result of hepatocellular damage characterized by apoptosis, oxidative stress and the release of cytokines and growth factors by non-parenchymal liver cells (granulocytes and macrophages), HSCs are activated and start to proliferate, transform into myofibroblasts, migrate, contract and bring about fibrogenesis. However, stellate cells are not the only cell type involved in liver fibrosis. Depending on the underlying etiology also portal (myo)fibroblasts, circulating fibrocytes, hepatocyte progenitor cells, bone marrow derived cells, Kupffer cells and even endothelial cells (through epithelial to mesenchymal transition) are believed to function as fibrogenic cells (Fig. 4) [150,151].

HSC activation occurs in different stages of liver disease [149]. As already mentioned, the initiation is brought about by paracrine activation of stellate cells due to the release of cytokines by apoptotic hepatocytes. The stimulated HSCs are further activated by cytokines produced by invading leucocytes, thrombocytes, activated Kupffer cells and local hepatocytes. In addition, a progressive change in the constituents and density of the surrounding ECM occurs within the subendothelial space towards an environment con-

taining more fibril-forming collagens (particularly collagen type I and III) and fibronectin through the action of sinusoidal endothelial cells that are stimulated by TGF β [152,153] (Fig. 4). This altered ECM composition gives rise to several feedback mechanisms that further stimulate the fibrosis process.

Although a lot of attention has gone to TGF β , CTGF is another important and partially TGF β independent profibrotic stimulus in liver fibrosis. The range of actions of CTGF in liver fibrosis is broad and includes increased cell proliferation, differentiation, migration, adhesion, ECM synthesis, and direct interaction with matrix components [154]. Several studies have demonstrated that CTGF is overexpressed in fibrotic livers [155]. However, the cellular distribution of CTGF expression appears to be dependent on disease etiology and progression [151]. Again HSCs (together with fibroblast) are considered the most important source of CTGF in liver fibrosis, while also hepatocytes, portal fibroblasts and cholangiocytes can contribute to CTGF production [156,157]. Although TGF β signaling results in increased CTGF expression through Smad signaling pathways in hepatocytes, CTGF expression in HSCs occurs through both TGF β -dependent (*via* Smad3 and Stat3 signaling) and TGF β -independent pathways [158,159]. Like hepatocytes, hepatocyte progenitor cells have also been demonstrated to contribute to CTGF production in response to TGF β through Smad signaling pathways [160]. Next to TGF β , hepatic expression levels of CTGF can be upregulated by ET-1, TNF α , glucocorticoids, VEGF, acetaldehyde, nitrogen oxide, thrombin, lipid peroxidation products, PDGF, p53, hypoxia and mechanical factors such as shear and cell stretch [151,161].

While CTGF antagonizes BMP, it stimulates TGF β signaling [68] and promotes the phenotypic changes of HSC into myofibroblast and in addition, regulates collagen I and integrin expression in HSC and supports adhesion *via* interaction of a CTGF surface receptor with fibronectin and HSPGs [167–169]. On the other side, PPAR γ , curcumin and *microRNA-214* have been shown to downregulate CTGF expression in HSCs and

Fig. 4. Liver and kidney fibrosis as two examples of CTGF-related adverse tissue remodeling. Liver: cytokines and growth factors including CTGF are secreted upon injury from hepatocytes and inflammatory cells. CTGF is involved in the following phenomena: A) CTGF produced continuously by the HSCs, in cooperation with TGF β and fibronectin, mediate the proliferation, survival, migration and adhesion of the HSCs. B) Hepatocytes go through epithelial to mesenchymal transition (EMT) which contributes to the pool of myofibroblasts that produce ECM and bring about fibrogenesis. EMT is induced by TGF β and inhibited by BMP7. CTGF as the downstream inducer of TGF β , shifts the ratio of TGF β /BMP7 towards more EMT. C) Sustained production of CTGF in HSCs is responsible for fibrillary collagen production and thickening of the subendothelial space of Disse due to excessive matrix production [149,162]. Kidney: in response to injury, recruited inflammatory cells and the injured epithelial cells, produce CTGF and other profibrotic cytokines. CTGF is involved in the following phenomenon: D) Proximal tubule epithelial cells as a result of production of CTGF and through EMT, dedifferentiate to myofibroblasts. Although there is a controversy as to the contribution of EMT to ECM-producing mesenchymal cells, using BMP7 agonists, TGF β and CTGF antibodies, inhibits EMT and points to involvement of these pathways in the latter. E) Pericytes, in response to CTGF (among others including TGF β and PDGF), are stimulated to detach from the tubular basement membrane, migrate into the interstitial space and differentiate to myofibroblasts. F) Resident fibroblasts in response to increasing levels of CTGF, turn into pathological fibroblasts that contribute significantly to the production of ECM [163–166].

nearby hepatocytes [50,170–172]. As mentioned before, the liver also plays a role in the clearance of circulating CTGF through LRP1 receptor-mediated endocytosis by hepatocytes [173].

Due to its downstream localization in different pathways leading to fibrosis, CTGF may be an attractive target for anti-fibrotic therapies in liver fibrosis. Indeed, downregulation of CTGF expression with the help of siRNA has been demonstrated to reduce liver fibrosis in rodent models [174–176]. Other drugs that inhibit CTGF expression through PPAR γ and protein kinase A activity such as prostaglandin E2, prostaglandin J2 and pentoxifylline have been shown to attenuate fibrosis in experimental and clinical settings [177–179]. In 2016, a phase 2 study with the humanized anti-CTGF antibody FG-3019/pamrevlumab in patients with liver fibrosis was completed whose results are yet awaited (NCT01217632).

The secretory character of CTGF and its production by various cell types in liver disease makes it a potential biomarker for the diagnosis of liver fibrosis and its progression. Several clinical studies have demonstrated significantly increased serum or plasma CTGF in patients with fibrotic/cirrhotic liver disease. However, CTGF levels did not correlate with the stage of fibrosis, and appeared to be higher in patients with progressive fibrosis than in those with end-stage cirrhotic liver disease [149,151,180].

Kidney fibrosis

Kidney fibrosis is a final common pathway of chronic kidney disease (CKD) irrespective of etiology [181]. CKD is an important problem for patients, doctors and health administrators. An estimated 10% of the global population suffers from CKD and over 2 million people with an advanced stage of CKD currently receive renal replacement therapy (RRT). However, RRT delivery is not widely available, *i.e.* predominantly in the more economically developed countries. Unfortunately, for many patients in the rest of the world, the proper care for severe renal failure is still lacking. In addition, the number of people with CKD worldwide is rapidly rising and this is considered a global health crisis. Therefore, it is necessary to counteract kidney fibrosis in progressive kidney diseases before the point of irreversibility.

In the kidneys, tubular epithelial cells are the most vulnerable cell type to injury, but still, the excessive deposition of ECM in the interstitial compartment, which leads to scar tissue formation, is produced by myofibroblasts (Fig. 4). Upon injury, tubule epithelial cells may undergo a partial epithelial-to-mesenchymal transition (EMT) which results in tubular function impairment, triggers cell cycle arrest and promotes the release of critical fibrogenic cytokines [182] and growth factors such as CTGF.

Besides its direct action and as a co-factor for TGF β , CTGF also has the potential to modulate VEGF and

BMPs, which are integral to development and repair processes in renal fibrogenesis [183]. The mechanism of action of CTGF in renal fibrosis has been widely reviewed by others [84,182,183]. In brief, CTGF is necessary for induction of TGF β and, apart from that, exerts profibrotic effects by interacting with low-density lipoprotein receptor-related protein 6 (LRP6), activating the Wnt pathway and enhancing insulin growth factor and collagen expression [184]. Recently, it has been suggested that overexpression of CTGF after ureteral obstruction in mouse kidney, increases inflammation and fibrosis but does not affect nephrogenesis or kidney homeostasis. Also, the apparent lack of any renal dysfunction in the CTGF conditional knockouts is consistent with an aspecific role of CTGF largely restricted to modulating a tissue injury response [185]. In the same study, authors showed that pericytes isolated from mice overexpressing CTGF became hyper-migratory and hyper-proliferative. Treatment with recombinant domain 1, 1 + 2 (-N terminus), or 4 (-C terminus) independently activated myofibroblast differentiation and wound-healing responses in these pericytes. CTGF domain 4 showed the broadest profibrotic activity. Administration of blocking antibodies specifically against CTGF domain 4 or recombinant Dickkopf-related protein-1, an endogenous inhibitor of LRP6, effectively inhibited inflammation and fibrosis associated with ureteral obstruction *in vivo*, pointing to CTGF domain 4 as a therapeutic target in renal fibrosis [185].

Another therapeutic strategy for renal fibrosis is to reduce fibroblast proliferation. Lysophosphatidic acid (LPA) signaling through its receptor LPA1 directly induced fibroblasts recruitment. In a LPA1-deficient (LPA1 $^{-/-}$) or -sufficient (LPA1 $^{+/+}$) mouse model, CTGF expression in tubule epithelial cells was suppressed [186]. CTGF derived from proximal tubule epithelial cells (PTC) mediated both renal fibroblast proliferation and myofibroblast differentiation. Moreover, it was shown that LPA-LPA1 signaling directly induced CTGF expression in primary PTC, through a myocardin-related transcription factor (MRTF)-serum response factor (SRF) pathway. Administration of an inhibitor of this factor suppressed renal fibrosis in these cells. The authors suggested that targeting LPA-LPA1 signaling and/or MRTF-SRF-induced transcription could be a promising therapeutic strategy for renal fibrosis [186].

Another group showed the role of the protein Sirtuin 1 (Sirt1) in the protection against renal disease by inhibiting fibrosis and oxidative metabolism. In this study, the role of a Sirt1 activator, SRT1720 was investigated in unilateral ureteral obstruction (UUO)-induced tubulointerstitial fibrosis in mice. The administration of SRT1720 increased the Sirt1 levels and partially attenuated both UUO-induced renal fibrosis and apoptosis. SRT1720 also attenuated the levels of oxidative stress suggesting its protective potential against reactive oxygen species-induced damage. In

this context, SRT1720 effectively inhibited the levels of TGF β /CTGF induced by UO. Authors suggested that the mechanisms of action for SRT1720 may include, at least in part, the suppression of renal oxidative stress and the TGF β /CTGF signaling pathway. Therefore, Sirt1 activator may be another candidate therapeutic agent for the treatment of fibrotic kidney disease *via* downregulation of CTGF [187].

Very recently, another role for CTGF has been suggested in fibrotic tissue remodeling. Prominent expression of CTGF and VEGF-C were associated to lymphangiogenesis during tubule-interstitial fibrosis in obstructed nephropathy [188]. Using a CTGF knockout mouse model, the authors observed significantly reduced number of lymphatic vessels and VEGF-C expression in comparison to wild-type mice. Also in an ischemia-reperfusion injury mouse model, CTGF knockdown was associated with reduced lymphangiogenesis.

In vitro, CTGF induced VEGF-C production in HK-2 cells, while CTGF siRNA suppressed TGF β -induced VEGF-C upregulation. The authors used surface plasmon resonance analysis, which show that CTGF and VEGF-C directly interact, and that CTGF is highly involved in fibrosis-associated renal lymphangiogenesis through regulation of this direct interaction.

In glomerulonephritis, inhibition of CTGF seems to be fundamental, since its elevated expression leads to signaling of other growth factors and promotes fibrosis [188]. The investigators used 3 models of CTGF knockout mice to investigate the role and source of CTGF in anti-glomerular basement membrane glomerulonephritis and showed that macrophage migration into glomeruli with nephritis is mediated by CTGF derived from mesangial cells, which enhanced macrophage chemotaxis and adhesion in a CTGF-dependent manner. Systemic reduction of CTGF expression ameliorated proteinuria and glomerular injury in the mouse model of anti-glomerular basement membrane glomerulonephritis through suppression of inflammation and ECM accumulation [189]. Altogether, these very recent evidences of CTGF's role in fibrosis regulation in kidney demonstrate the urgency to efficiently reduce this signaling pathway in order to block renal fibrosis and improve kidney function upon injury.

Diabetic nephropathy

Diabetic nephropathy (DN) is one of the major causes of end-stage kidney disease for which dialysis or transplantation are the only treatment options [190]. The incidence of diabetes and subsequently diabetic nephropathy is increasing worldwide imposing a large burden on clinical care. CTGF has been associated with numerous diabetic complications, but it is of particular interest to diabetic nephropathy. Soon after its identification, the expression of CTGF *mRNA* was

shown to be strongly upregulated in human mesangial cells cultured under high glucose, and in kidney biopsies of patients with DN [191,192]. These observations were extended in rodent models of both type 1 and type 2 DN [193–195]. Furthermore, severity of DN was aggravated in transgenic mice with specific overexpression of CTGF in glomerular podocytes [196]. In kidney biopsies of patients [191] with type 2 DN, tubular expression of CTGF correlated with proteinuria, serum creatinine, and interstitial fibrosis [197]. Interestingly, overexpression of CTGF in human diabetic glomeruli was also associated with reduction in podocyte markers and decreased signaling activity of BMP [198], both of which are both important elements of DN. These data suggest that CTGF is a pathogenic factor in the development and progression of DN.

Upregulation of CTGF expression was also observed in the diabetic myocardium, liver, retina, and aorta [194,199–201]. In addition, soluble CTGF levels were elevated in the vitreous of patients with diabetic retinopathy [202]. CTGF levels were also increased in plasma and urine of patients with DN and plasma CTGF was shown to be an independent predictor of end-stage renal disease in patients with DN, proving its promising potential as a biomarker in diabetic patients [203,204].

Various studies have investigated the role of CTGF as a target for therapy in diabetes. In mouse models of type 1 and type 2 DN, treatment with CTGF antisense oligodeoxynucleotides proved to be successful in attenuation of proteinuria, and in reduction of genes involved in mesangial matrix expansion [205]. Similar observations were seen in diabetic mice lacking one CTGF allele [206] and in diabetic mice treated with an antibody against CTGF [207]. In these two latter studies, the attenuation of DN by inhibition of CTGF was associated with preserved BMP signaling and decreased β -catenin expression. Moreover, although Dendooven et al. and Patel et al. did not find a correlation between the -945 GC SNP (rs6918698, associated with pulmonary disease in SSc and affecting SP1 binding to the CTGF promoter) with nephropathy or outcome in type 1 diabetic patients, or with cardiac and kidney complications in type 2 diabetic patients [208,209], Wang et al. found that another -20 GC SNP in the promoter of CTGF enhances the relative risk of microalbuminuria in diabetic patients up to three-fold and also increases the risk of developing diabetic nephropathy. This polymorphism is located at the Smad1-binding domain of CTGF promoter and is actively involved in Smad1 regulation of basal CTGF transcription [210]. Altogether this data emphasizes the important direct role of CTGF in DN as well as its regulatory effects on the implicated growth factors and signaling pathways.

Treatment with the monoclonal anti-CTGF antibody FG-3019/pamrevlumab has also been found to be safe for human patients. FG-3019/pamrevlumab therapy may reduce urinary albumin excretion in

patients with diabetes [211]. However, many larger trials would be needed before clinical implication can be considered.

Solid organ transplantation

Allograft fibrosis is a multifactorial phenomenon which limits the graft survival in all solid organ transplantations. Ischemic reperfusion injury, infections, calcineurin inhibitors and acute rejection are some of the initial insults to the allografts leading to fibrosis [212]. Apart from the initial insults, chronic rejection following organ transplantation also induces loss of allograft function. It was shown that TGF β and CTGF were expressed in cardiac allografts of mice with chronic rejection but were not expressed in grafts without it [213]. TGF β transfection of syngeneic grafts did not result in CTGF expression or chronic rejection. This indicates that TGF β alone is not sufficient to induce chronic rejection, and that CTGF is required. The authors suggested that CTGF might serve as a therapeutic target for organ chronic rejection.

Both *in vitro* studies [214] and mouse renal allografts [215] show that CTGF expression by renal tubule epithelial cells is a key mediator of TGF β -dependent interstitial fibrogenesis. Urinary CTGF concentrations were higher in the presence of interstitial fibrosis in concurrent human renal allograft biopsies. It has been demonstrated that urinary CTGF concentrations were higher in the presence of interstitial fibrosis in concurrent human renal allograft biopsies [216]. Very recently, in a retrospective cohort analysis of 160 kidney transplant recipients with paired protocol renal biopsies, tubule-interstitial expression and urinary excretion of CTGF at 3 months after renal transplantation together with donor age predicted progression of interstitial fibrosis and tubular atrophy before any functional deterioration of allografts 5 years after transplantation [217].

Clinical and translational aspects

There is an ongoing quest for identification of biomarkers for fibrosis in different disease contexts. There are some successful studies pointing towards CTGF as a potential biomarker. For instance, the urinary and interstitial CTGF can predict the evolution of histological damage in renal transplant recipients [217]. However, given the complex and context-dependent nature of fibrosis, measuring CTGF in all disease settings might not be so straightforward. CTGF undergoes glomerular filtration, tubular reabsorption (almost complete under normal circumstances) and can be produced by tubular epithelial cells, immune cells and mesangial cells (including fibroblasts). The presence of CTGF in urine can therefore reflect (1) intrarenal production, (2) tubular dysfunction and (3) saturation of tubular reabsorption, resulting from

increased filtration (glomerular damage) or from high plasma concentrations [124]. Therefore, there exists a potential benefit in the identification of markers directly linked to increased CTGF. One possible candidate is autotoxins, which have attracted the attention of researchers and pharmaceutical companies. One of these autotoxins is phospholipid lysophosphatidic acid (LPA), which is involved in liver, kidney and pulmonary fibrosis, and directly induces CTGF expression [242]. For example, in a radiation-induced model of lung fibrosis, LPA stimulated fibroblast proliferation, and inhibiting CTGF reversed this effect. Moreover, VPC12249, a LPA receptor 1/3 antagonist, decreases CTGF and TGF β production in animals and reduces fibroblast pool and matrix deposition [243]. In an animal model of renal fibrosis, the proximal tubule epithelial cell-produced CTGF induced accumulation of fibroblasts and differentiation of myofibroblasts. They also showed that LPA signaling directly induces CTGF in renal proximal tubule cells through signaling pathways that comprise Ga12/13, Rho, Rho-associated protein kinase (ROCK) and actin polymerization [186]. In these pathologies, inhibition of LPA seems to be a valid therapeutic option. Galapagos has developed a first-in class autotoxin inhibitor GLPG1690 [244] which was evaluated in a randomized, double-blind, placebo-controlled study in subjects with IPF (clinical trial number NCT0273880). The results of the study are favorable for treatment of these patients and might suggest a novel therapeutic option in other disease settings where similar pathological pathways are involved.

The most advanced trials with an anti-CTGF agent have been performed with FG-3019/pamrevlumab for IPF, which was discussed earlier, and this compound is currently tested in clinical trials for muscular dystrophy and pancreatic cancer as well. These studies exemplify successful treatments for fibrosis *via* CTGF targeting, and could be extrapolated to other disease contexts by conducting additional clinical trials.

Conclusions and future directions

CTGF is one of the best-studied factors in fibrosis and, across different pathologies, it has strong potential as a diagnostic, prognostic and therapeutic biomarker. However, given the complex nature of tissue fibrosis in different organs and conditions, a composite panel of markers might be needed. The current interest in discovering new pathways also includes epigenetic mechanisms. For example, DNA methylation, histone modifications and *mi-RNA* effects makes them attractive therapeutic targets [245]. For instance the *mi-RNA-29* family is involved in heart, lung, kidney and liver fibrosis and is an interesting target to be studied in different fibrosis models [246]. RXi Pharmaceuticals have launched 2 phase 1 clinical trials (NCT01780077 and NCT01640912) that show

safety, tolerability and proof of concept of their compound that targets CTGF-regulating *mi-RNA* and phase 2 clinical trials for this compound are in progress.

In conclusion, despite existing pitfalls and challenges in the field, fundamental and clinical advances in research have provided a number of promising treatment approaches for tissue fibrosis. However, it is important to remember that future approaches should also address the specific genetic makeups of patients in responding to anti-fibrotic medications. This requires integrated approaches that take into consideration both fundamental and translational research trajectories that collaborate with the aim of discovering new fibrosis pathways and tailoring treatments to patients' specific medical needs.

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Abbreviations used:

AP1, activator protein 1; ASK1, apoptosis signal-regulating kinase 1; AT1R, angiotensin 2 type 1 receptor; AT2R, angiotensin 2 type 2 receptor; BCL-2, B-cell lymphoma 2; BMP4, bone morphogenic protein 4; BMP7, bone morphogenic protein 7; BMPs, bone morphogenic proteins; cAMP, cyclic adenosine monophosphate; CKD, chronic kidney disease; CTGF, connective tissue growth factor; CYR61, cysteine rich angiogenic inducer 61; c-Src, proto-oncogene c; DDR, DNA damage repair; DMD,

Duchenne muscular dystrophy; DN, diabetic nephropathy; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ET-1, endothelin-1; Ets1, ETS proto-oncogene 1; FAK, focal adhesion kinase; FGF-2, fibroblast growth factor 2; FGFB, fibroblast growth factor 2; FOXO1/3a, forkhead box protein O1/forkhead box protein O3a; FZD7, frizzled 7; HIF1 α , hypoxia-inducible factor 1 α ; HSC, hepatic stellate cell; HSPGs, heparan sulfate proteoglycans; IGF, insulin-like growth factor; IL-1R, interleukin 1 receptor; IL1 β , interleukin 1 β ; IPF, idiopathic pulmonary fibrosis; ITGs, integrins; ITG β 1, integrin α 3/ β 1; JAK2, Janus kinase 2; LPA, lysophosphatidic acid; LRP1, low-density lipoprotein receptor-related protein 1; LRP5, lipoprotein receptor related proteins; MAPK, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; MEK/ERK, mitogen-activated protein kinase kinase/extracellular signal-regulated kinase; *Mi-RNA*, micro RNA; MMP2, matrix metalloprotease 2; MMP3, matrix metalloprotease 3; MMPs, matrix metalloproteases; MPK1, mitogen activated protein kinase (MAPK) phosphatase 1; NF κ -B, nuclear factor kappa-light-chain-enhancer of activated B cell; NK, c-Jun N-terminal kinase; NOV, nephroblastoma overexpressed protein; PAR1, protease-activated receptor 1; PDGF, platelet-derived growth factor; PDGFB, platelet-derived growth factor B; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tension homolog; Rac1, Ras-related C3 botulinum toxin substrate; RAS-GTP, RAS-guanosine triphosphate; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; RP6, low-density lipoprotein receptor-related protein 6; RRT, renal replacement therapy; RTK, receptor tyrosine kinase; Sirt1, Sirtuin 1; SP1/SP3, specificity protein 1/specificity protein 3; SRF, serum response factor; SSc, systemic sclerosis; STAT3, signal transducer and activator of transcription 3; TAK1, transforming growth factor beta-activated kinase 1; TAZ, transcriptional coactivator with PDZ-binding motif; TEAD, transcriptional enhancer factor TEF-1; TGF β , transforming growth factor β ; TGF β R, transforming growth factor β receptor; TGs, integrins; TK, tyrosine kinase; TNF, tumor necrosis factor; TNF- α , tumor necrosis factor α ; TNFR, tumor necrosis factor receptor; UUO, unilateral ureteral obstruction; UV, ultra violet; VEGF, vascular endothelial growth factor; VEGFs, vascular endothelial growth factors; VWF, von Willebrand factor type C; YAP, yes-associated protein.

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