# Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets

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Abstract | Members of the CCN family of matricellular proteins are crucial for embryonic development and have important roles in inflammation, wound healing and injury repair in adulthood. Deregulation of CCN protein expression or activities contributes to the pathobiology of various diseases — many of which may arise when inflammation or tissue injury becomes chronic — including fibrosis, atherosclerosis, arthritis and cancer, as well as diabetic nephropathy and retinopathy. Emerging studies indicate that targeting CCN protein expression or signalling pathways holds promise in the development of diagnostics and therapeutics for such diseases. This Review summarizes the biology of CCN proteins, their roles in various pathologies and their potential as therapeutic targets.

Matricellular proteins
Dynamically expressed extracellular matrix proteins with a modular structure that have regulatory roles and are often involved in wound healing.

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Beyond serving as a scaffold for the organization of cells into tissues, the extracellular matrix (ECM) is also a multifunctional regulator of cellular behaviour. ECM proteins can modulate the activity or bioavailability of extracellular signalling molecules such as growth factors, cytokines, chemokines and extracellular enzymes, or they can directly bind to and signal through cell-surface receptors to regulate cellular functions. A subset of ECM proteins, known as matricellular proteins, are dynamically expressed and serve primarily regulatory rather than structural roles[1,2]. Among known matricellular proteins are members of the CCN protein family, a group of highly conserved secreted proteins that have been identified by differential expression screening[2–4]. The synthesis of these proteins is regulated by mitogenic growth factors or oncogenic transformation. The acronym 'CCN' is derived from the first three members of the protein family described, namely cysteine-rich angiogenic inducer 61 (CYR61; also known as CCN1), connective tissue growth factor (CTGF; also known as CCN2) and nephroblastoma overexpressed (NOV; also known as CCN3). Together with a set of three WNT-inducible signalling pathway proteins — WISP1 (also known as CCN4), WISP2 (also known as CCN5) and WISP3 (also known as CCN6) — they comprise a family of six homologous cysteine-rich proteins in mammals that have been renamed CCN1–CCN6 by international consensus[5].

All CCN proteins have a similar modular structure, which consists of an amino-terminal secretory peptide followed by four conserved domains with sequence homologies to insulin-like growth factor-binding proteins, the von Willebrand factor C (VWC) domain, thrombospondin type 1 repeat (TSR) and a carboxy-terminal domain that contains a cysteine-knot motif (BOX 1). A non-conserved, protease-sensitive central hinge region bisects the proteins into two halves that bind to distinct cell-surface receptors. The expression of CCN proteins is exquisitely regulated on transcriptional, post-transcriptional and translational levels in response to changes in environmental stimuli, including those encountered during tissue repair (BOX 1; FIG. 1).

At the cellular level, CCN proteins regulate cell adhesion, migration, proliferation, differentiation, apoptosis, survival, senescence and gene expression. By modulating one or more aspects of these cellular functions in a cell-specific manner, CCN proteins coordinate complex biological processes, including cardiovascular and skeletal development during embryogenesis, as well as inflammation, wound healing and tissue repair in adulthood. Aberrant expression of CCN proteins is associated with a remarkable range of seemingly unrelated diseases. A useful albeit simplistic framework with which to rationalize the roles of CCN proteins in this array of diseases is that many pathological conditions arise when inflammation or tissue injury becomes chronic, and in this process CCN proteins are concomitantly deregulated.

Studies in animal models and in patients have confirmed that CCN proteins are involved in many diseases related to inflammation and tissue repair, including arthritis, atherosclerosis, restenosis after vascular injury,

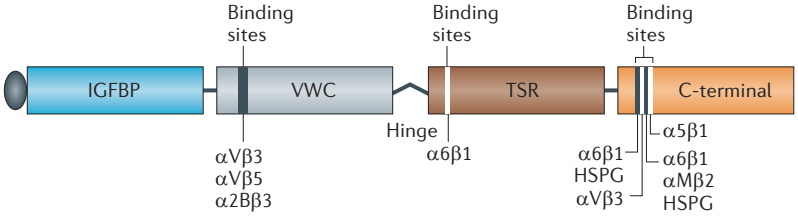
Box 1 | **The CCN family of matricellular proteins**

The CCN matricellular proteins (except for WNT-inducible signalling pathway protein 2 (WISP2; also known as CCN5)) are composed of an amino-terminal secretory peptide and four conserved modular domains: the insulin-like growth factor binding protein (IGFBP) domain, the von Willebrand factor C (VWC) domain, the thrombospondin type 1 repeat (TSR) domain and the carboxy-terminal domain. Each of these domains is encoded by a separate exon. A non-conserved central hinge region divides the protein into two halves with different binding capabilities for extracellular proteins and cell-surface receptors (FIG. 2). The locations of identified binding sites for integrin receptors and heparan sulphate proteoglycans (HSPGs) are indicated in the schematic diagram[2]. With the exception of CCN5 and CCN6 (also known as WISP3), CCN proteins contain 38 conserved cysteine residues located throughout the polypeptide; CCN5 lacks the precise C-terminal domain but otherwise contains all conserved cysteine residues in the remaining three domains, whereas in CCN6 four cysteine residues in the VWC domain are not conserved.

The genes encoding proteins of the CCN protein family are exquisitely sensitive to regulation by mitogenic signals and a range of environmental perturbations, including exposure to growth factors, inflammatory cytokines, steroid hormones, oxygen deprivation, ultraviolet radiation and mechanical forces (FIG. 1). During embryonic development, CCN proteins are broadly expressed in many organs and tissues, especially in endothelial cells throughout the embryo and in the developing cardiovascular, skeletal, renal and neuronal systems[32,177]. During adulthood, expression of CCN proteins is downregulated in many tissues but is upregulated at sites of inflammation, wound healing and tissue repair[2,36].

Identified as immediate-early genes, the genes encoding cysteine-rich angiogenic inducer 61 (CYR61; also known as CCN1) and connective tissue growth factor (CTGF; also known as CCN2) are expressed at a very low level in quiescent fibroblasts but are transcriptionally activated — without requiring *de novo* protein synthesis — within minutes of stimulation by mitogenic growth factors such as platelet-derived growth factor, fibroblast growth factor 2 and transforming growth factor-β1. Analysis of the *Ccn1* and *Ccn2* promoters has revealed that crucial regulatory elements and transcription factors govern their expression[2,3], and the essential promoter for apposite regulation of *Ccn1* in transgenic mice has been identified[37]. *CCN1* and *CCN2* are targets of the transcriptional co-activators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), which interact with the TEAD family of transcription factors to regulate the expression of genes related to cell proliferation and survival[100] and in response to mechanotransduction[178] (FIG. 2). By contrast, the nephroblastoma overexpressed gene (*NOV*; also known as *CCN3*) and *CCN5* are downregulated by mitogenic signals and upregulated under conditions of growth arrest in several cell types[61,113]. In addition, genes that encode CCN proteins are downregulated by specific microRNAs in various cell types; for example, *CCN2* is regulated by miR-133 and miR-30 in cardiomyocytes and miR-18a in chondrocytes[179,180], and downregulation of *CCN1* by miR-155 may contribute to pre-eclampsia[174]. The presence of internal ribosome entry sites facilitates the preferential translation of the *CCN1* mRNA under conditions of cellular stress — for example, after viral infections[181,182].

Structural variants or isoforms of CCN proteins, some of which have been detected in biological fluids and may have different activities, can be generated by differential splicing[183,184], proteolysis[35,185] and post-translational modifications[186]. Alternative splicing typically results in the production of truncated proteins or proteins with either the VWC or TSR domains deleted. These structural variants have the potential to substantially expand the functional complexity of CCN proteins, although it is currently unknown how they are regulated and how they affect the biological functions of CCN proteins *in vivo*. Although CCN proteins are secreted proteins with N-terminal signal peptides, they may be endocytosed after binding to receptors. Several CCN proteins have been detected in the nucleus and, although their nuclear function is unknown, studies have suggested the intriguing possibility that CCN1 and CCN5 might regulate transcription[187,188].




fibrosis and cancer, as well as diabetic nephropathy and retinopathy. Modulating the expression or function of CCN proteins has yielded substantial benefits in animal models of disease, suggesting that CCN proteins are promising therapeutic targets in these pathologies. Clinical trials targeting CCN2 using a humanized monoclonal antibody (FG-3019) have shown encouraging results in several human diseases, including diabetic nephropathy[6]. However, the therapeutic potential of other CCN proteins has yet to be evaluated in a clinical setting. In this Review, we discuss recent insights into the regulation and function of CCN proteins in various biological processes, summarize the evidence for their participation in disease pathologies, explore their potential as diagnostic markers and therapeutic targets, and discuss various targeting strategies as applied to these proteins.

## Functions and mechanisms of action

As members of the CCN protein family serve both distinct and overlapping biological roles, these highly conserved proteins bind many of the same receptors and function through similar mechanisms to regulate a common set of biological processes. However, the specific biological responses to some of the CCN proteins may be similar in some cell types but different in others, and at times diametrically opposed. Nevertheless, some generalizations can be made about how CCN proteins function. First, many activities of CCN proteins are mediated through their direct binding to integrin receptors on the cell surface, with the participation of one or more co-receptors in some contexts. The convergence of signals initiated from multiple receptors in some cases leads to unique biological responses that are not achieved with a single receptor, and the requirement of multiple receptors for certain functions of CCN proteins contributes to target cell specificity. Second, CCN proteins can regulate the expression of biologically active molecules such as growth factors, cytokines and matrix metalloproteinases (MMPs), and physically interact with some of these molecules to modulate their bioavailability and activity (FIG. 2). In addition, CCN proteins can interact with and be tethered to other ECM proteins — including decorin, fibronectin, vitronectin and perlecan — potentially functioning as a local scaffold that coordinates the interaction of specific biologically active molecules or ECM proteins with the target cell (FIG. 2). Third, CCN proteins can profoundly alter the biological activities of cytokines, and possibly growth factors, through signalling crosstalk (FIG. 3). Through these and other mechanisms (BOX 1), CCN proteins can integrate and modulate multiple signalling pathways to elicit cell-specific responses.

***Cell-surface receptors.*** Many activities of CCN proteins are mediated through their direct binding to integrin receptors, with the involvement of co-receptors in some contexts (TABLE 1). At least eight integrins have been shown to bind CCN proteins[2]. As distinct integrins are differentially expressed in various cell types and may mediate disparate activities, CCN proteins can achieve remarkable

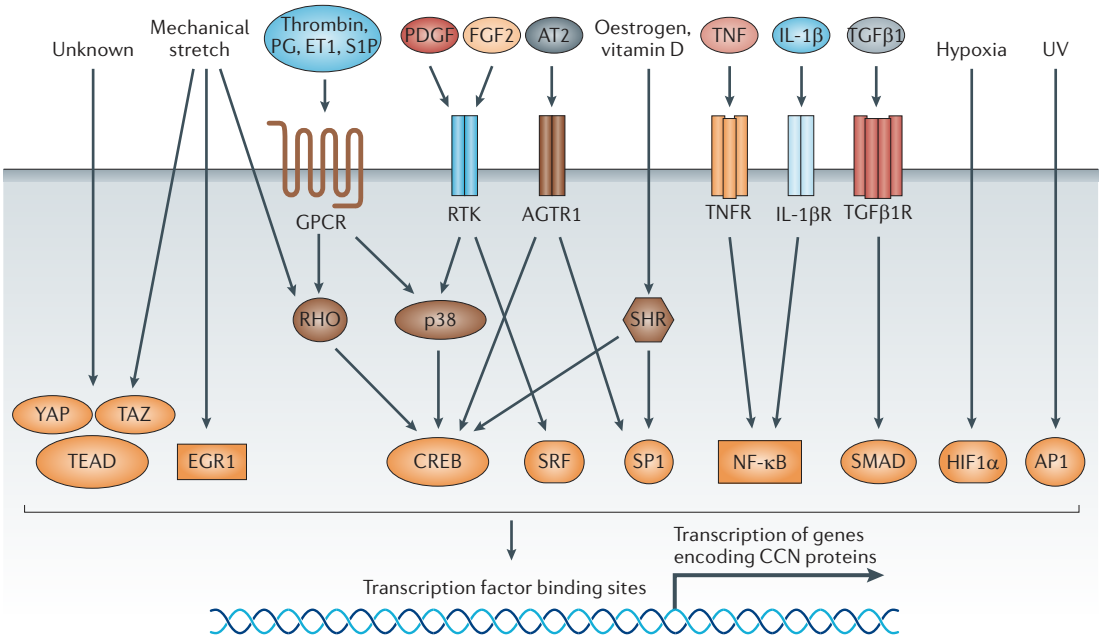



Figure 1 | **Transcriptional regulation of genes encoding CCN proteins*.** Several extracellular and environmental stimuli rapidly induce the expression of genes encoding CCN proteins; this occurs through the activation of various transcription factors, usually without requiring *de novo* protein synthesis. These stimuli include: platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF2), transforming growth factor-β1 (TGFβ1), interleukin-1β (IL-1β), tumour necrosis factor (TNF), angiotensin II (AT2)[2,3], agonists of G protein-coupled receptors (GPCRs)[189] (such as thrombin, prostaglandin (PG), endothelin 1 (ET1) and sphingosine-1-phosphate (S1P)), as well as hormones (such as oestrogen and vitamin D) that bind to steroid hormone receptors (SHRs). Other stimuli include hypoxia, ultraviolet (UV) radiation and mechanical stretch[37,129]. The locations of the binding sites of various transcription factors vary for each gene encoding CCN proteins. AGTR1, angiotensin II receptor type 1; AP1, activator protein 1; CREB, cyclic AMP-responsive element binding protein; EGR1, early growth response protein 1; HIF1α, hypoxia-inducible factor 1α; IL-1βR, IL-1β receptor; NF-κB, nuclear factor-κB; p38, p38 mitogen-activated protein kinase; RTK, receptor tyrosine kinase; SP1, specificity protein 1; SRF, serum response factor; TAZ, transcriptional co-activator with PDZ-binding motif; TGFβ1R, TGFβ1 receptor; TNFR, TNF receptor; YAP, Yes-associated protein. *The 'CCN' acronym is derived from the first three members of the CCN protein family: cysteine-rich angiogenic inducer 61 (CY61; also known as CCN1), connective tissue growth factor (CTGF; also known as CCN2) and nephroblastoma overexpressed (NOV; also known as CCN3).

Matrix metalloproteinases
Members of a family of >20 zinc-dependent endopeptidases that are involved in the degradation of extracellular matrix proteins, cleavage of cell-surface receptors, release of apoptotic ligands, and activation of chemokines and cytokines.

Integrins
Members of a family of heterodimeric cell-surface signalling receptors that mediate cell–cell and cell–matrix interactions; they are composed of 18 α-subunits and 8 β-subunits, which constitute 24 known integrin heterodimers in mammals.

Immediate-early genes
Genes that are transcriptionally activated with rapid kinetics, without requiring *de novo* protein synthesis, by a range of stimuli including viral infection or various extracellular signals.

Low-density lipoprotein receptor-related protein
Members of this family of proteins are cell-surface receptors that bind a broad spectrum of ligands, facilitating endocytosis and the signalling of associated receptors.

functional versatility through their interactions with different integrins in a cell- and context-specific manner. Although CCN proteins do not contain the canonical RGD sequence that binds to several integrins, they interact with integrins through their non-canonical binding sites, many of which have been identified (BOX 1). Site-directed mutations in these binding sites abolish the specific activities for which the cognate integrins are responsible, both *in vitro* and *in vivo*[7,8], providing compelling evidence for the role of these integrins in mediating the functions of CCN proteins.

At least two types of co-receptors have been identified for CCN proteins. The first of these are cell-surface heparan sulphate proteoglycans (HSPGs), of which syndecan 4 has been identified as a crucial co-receptor for the functions of CCN proteins[7,9]. Low-density lipoprotein receptor-related proteins, which are endocytic receptors that cooperate with many growth factor receptors and integrins to modulate cellular responses[10], are the second class of co-receptor to interact with CCN proteins[11]. CCN2 can interact with neurotrophic tyrosine kinase receptor type 1 (NTRK1; also known as TRKA) in human mesangial cells to enhance transforming growth factor-β (TGFβ) signalling[12], and in glioma cells to activate nuclear factor-κB (NF-κB)[13]. CCN2 binds to TRKA in a complex with β1 integrins, indicating that TRKA also functions as a co-receptor with integrins[13]. In addition, CCN3 can bind to Notch and suppress myoblast and osteoblast differentiation[14,15]; whether integrins are involved in these signalling events is unknown.

***Cell adhesion, migration and proliferation.*** As cell-adhesive proteins of the ECM, CCN proteins support cell adhesion and promote cell spreading in many cell types (TABLE 1). The process of cell adhesion may also lead to other cellular responses including cell migration, proliferation and altered gene expression. The adhesion of human skin fibroblasts to CCN1 and CCN2 is mediated through α6β1 integrins and HSPGs, and results in cell-adhesive signalling events including the rapid formation of α6β1 integrin-containing focal adhesion complexes, actin cytoskeleton reorganization, formation of filopodia and lamellipodia, as well as the activation of focal adhesion kinase, paxillin and the small GTPase RAC[16]. The effects of CCN proteins on cell proliferation and migration are cell-specific. In fibroblasts

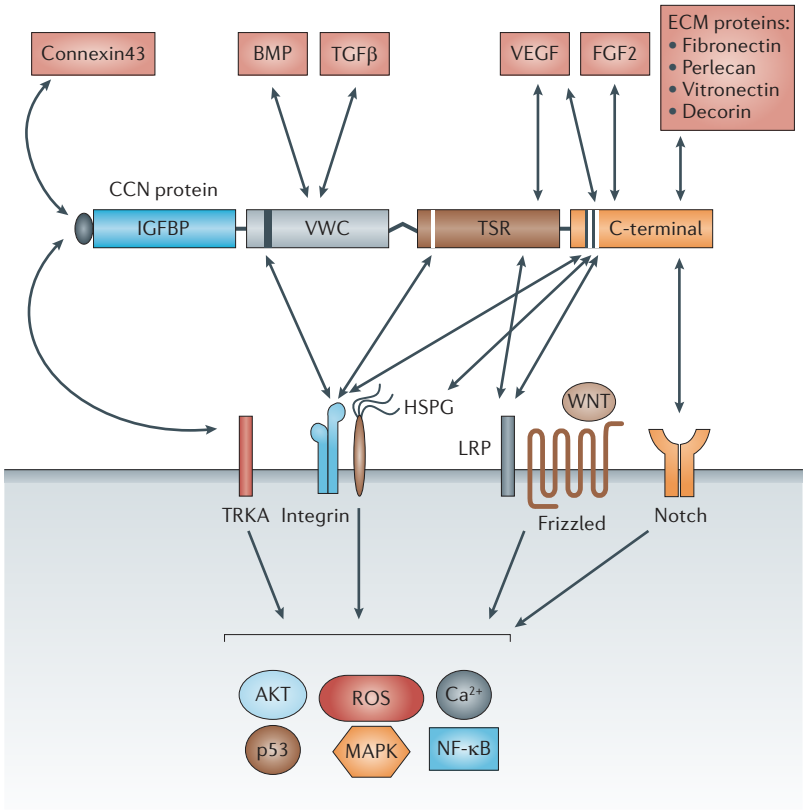



Figure 2 | **Molecular interactions through modular domains of CCN proteins.** CCN proteins physically interact with several extracellular matrix (ECM) proteins (including fibronectin[190], perlecan[191], vitronectin[192] and decorin[168]), and growth factors (including vascular endothelial growth factor (VEGF)[34], fibroblast growth factor 2 (FGF2)[193], transforming growth factor-β (TGFβ)[39] and bone morphogenetic proteins (BMPs)[39]), as well as the gap junction protein connexin43 (REFS 194,195). The specific modular domains mediating the interactions, where elucidated, are indicated. Although fibronectin and perlecan are known to bind to the carboxy-terminal domain, it is not clear whether decorin and vitronectin also bind to this domain. CCN proteins also bind to and signal through several cell-surface receptors including several integrins[2,37], which function in concert with heparan sulphate proteoglycans (HSPGs) or low-density lipoprotein receptor-related proteins (LRPs) as co-receptors in some contexts. Connective tissue growth factor (CTGF; also known as CCN2) also binds to neurotrophic tyrosine kinase receptor type 1 (TRKA) in a complex with β1 integrins[13], both of which act as co-receptors in some contexts, and nephroblastoma overexpressed (NOV; also known as CCN3) can bind to Notch[14]. CCN proteins can modulate WNT signalling, in part by binding to the WNT co-receptor LRP6 (REF. 11). The modular domains of CCN proteins may interact in a combinatorial manner to induce unique activities and functions[4]. For example, synergism of cysteine-rich angiogenic inducer 61 (CYR61; also known as CCN1) with tumour necrosis factor requires its binding to both αVβ5 and α6β1 integrins through the von Willebrand factor C (VWC) and C-terminal domains, respectively, to induce signals from both integrins that converge within the cell[7]; activation of either integrin alone is insufficient. IGFBP, insulin-like growth factor-binding protein; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; p53, cellular tumour antigen p53; ROS, reactive oxygen species; TSR, thrombospondin type 1 repeat.

CCN1, CCN2 and CCN3 have no intrinsic ability to induce mitogenesis on their own but they can enhance DNA synthesis that has been induced by other mitogenic growth factors by acting through αVβ3 integrin[2]. Although CCN2 is capable of promoting DNA synthesis in chondrocytes and osteoblasts[17,18], it also induces a G1 cell cycle arrest in mesangial cells[19]. CCN1, CCN2 and CCN3 stimulate cell migration or chemotaxis in fibroblasts and endothelial cells, and promote the invasiveness of certain cancer cells[2]. For example, CCN3 promotes the migration and invasion of Ewing's sarcoma cells but inhibits their proliferation[20]. CCN1 and CCN2 enhance vascular smooth muscle cell proliferation and migration[21,22], whereas CCN3 and CCN5 inhibit these processes[23,24].

***Cell survival, apoptosis and cellular senescence.*** Cell adhesion to ECM proteins promotes cell survival, whereas detachment from the ECM induces cell death by anoikis in many cell types. Although adhesion of endothelial cells to CCN1, CCN2 or CCN3 through αVβ3 integrin supports cell survival[2], these CCN proteins can also promote apoptosis as cell adhesion substrates in fibroblasts by interacting with α6β1 integrin[9]. The apoptosis-promoting activity may be most relevant in the context of inflammation as CCN1, CCN2 and CCN3 all have the unusual ability to enable the inflammatory cytokine tumour necrosis factor (TNF) to induce apoptosis without inhibiting NF-κB signalling or *de novo* protein synthesis — conditions required for TNF cytotoxicity in normal cells *in vitro*[25] (FIG. 3). These CCN proteins also facilitate the apoptotic activity of lymphotoxin, and enhance the apoptotic activity of other TNF family cytokines such as the FAS ligand and TNF-related apoptosis-inducing ligand[26,27]. Knock-in mice expressing an apoptosis-defective *Ccn1* allele are substantially resistant to TNF- or FAS-mediated hepatic apoptosis *in vivo*, indicating that CCN1 is a physiological regulator of TNF- and FAS ligand-mediated cytotoxicity[7,26]. By contrast, CCN4 inhibits TNF-induced cell death in cardiomyocytes[28], which suggests that the interplay between various CCN proteins and the TNF family of cytokines may profoundly affect the biological outcome during inflammatory responses.

A recent unexpected finding is that CCN1 can induce cellular senescence in fibroblasts by acting as a cell adhesion molecule[8]. By binding to α6β1 integrin and cell-surface HSPGs, CCN1 activates the RAC1-dependent NADPH oxidase 1 to induce a robust and sustained level of reactive oxygen species production, leading to activation of the cellular tumour antigen p53 and the retinoblastoma-associated protein pRb, which results in senescence (FIG. 3). An important feature of senescent cells is the expression of the senescence-associated secretory phenotype, which is characterized by the increased expression of ECM-degrading enzymes such as MMPs, inflammatory cytokines and chemokines, and downregulation of ECM components such as collagen, thus imposing a matrix-degrading phenotype[29]. Consequently, CCN1-induced myofibroblast senescence functions as a mechanism for limiting fibrosis during wound healing, as discussed below[30].

***Angiogenesis, chondrogenesis and osteogenesis.*** CCN1, CCN2 and CCN3 are potent angiogenic inducers, functioning through direct binding to αVβ3 integrin in endothelial cells to promote proliferation and induce

Anoikis
Induction of programmed cell death by detachment of cells from the extracellular matrix.

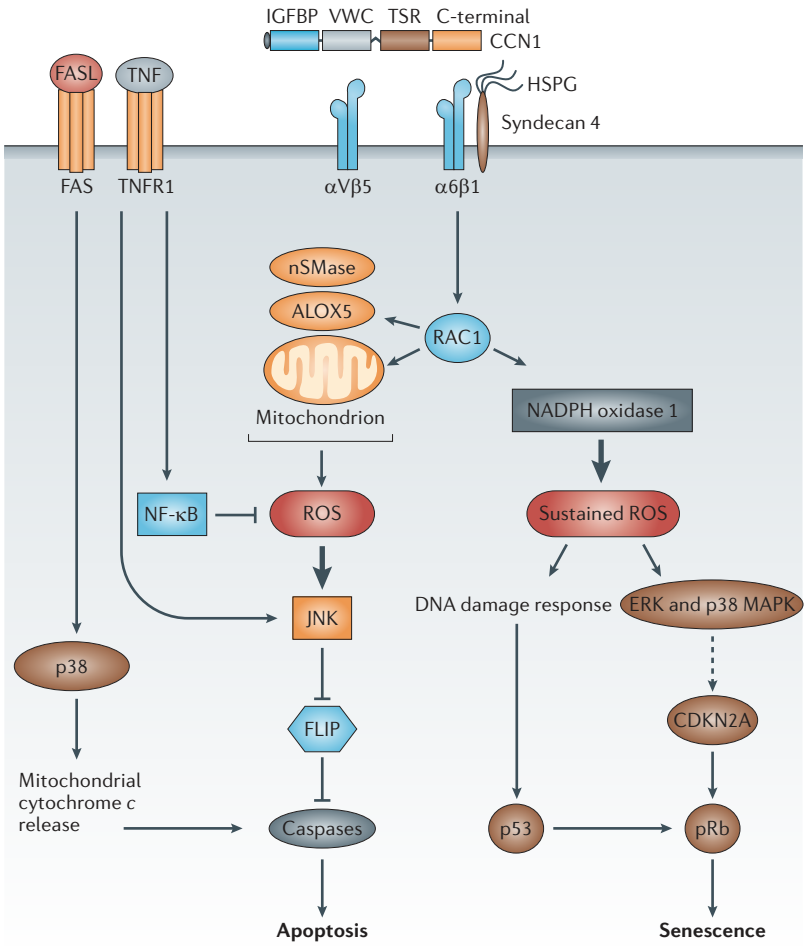



Figure 3 | **Signalling mechanism of CCN1-induced senescence and crosstalk with TNF and FASL.** The binding of cysteine-rich angiogenic inducer 61 (CYR61; also known as CCN1) to α6β1 integrin and heparan sulphate proteoglycans (HSPGs) — for example, syndecan 4 — through the carboxy-terminal domain triggers the activation of RAC1 and NADPH oxidase 1, which leads to substantially more robust and sustained levels of reactive oxygen species (ROS) than via cell adhesion to other extracellular matrix proteins. The sustained levels of ROS induce a DNA damage response as well as the activation of cellular tumour antigen p53, and trigger the activation of extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK), which in turn induces cyclin-dependent kinase inhibitor 2A (CDKN2A) and activates the retinoblastoma-associated protein pRb[8]. Activated p53 and pRb contribute to the induction of cellular senescence. If αVβ5 integrin is also engaged by the von Willebrand factor C (VWC) domain of CCN1, RAC1-dependent ROS accumulation includes contribution from arachidonate 5-lipoxygenase (ALOX5) and the mitochondria[7]; neutral sphingomyelinase (nSMase) also contributes to CCN1-induced ROS production[26]. CCN1-induced ROS production counteracts the effect of nuclear factor-κB (NF-κB), which is strongly activated by tumour necrosis factor (TNF) and induces the expression of antioxidant proteins. The high level of ROS inhibits cysteine phosphatases that can inactivate MAPKs such as JUN N-terminal kinase (JNK), ERK and p38 MAPK, leading to a hyperactivation of these kinases[196]. Activated JNK targets the proteasomal degradation of cellular FLICE-like inhibitory protein (FLIP)[197], an inhibitor of caspase activation, which enables the activation of caspase 8 and caspase 10 by TNF to induce apoptosis without blocking *de novo* protein synthesis or NF-κB signalling[7]. In addition to CCN1, connective tissue growth factor (CTGF; also known as CCN2) and nephroblastoma overexpressed (NOV; also known as CCN3) synergize with TNF to induce apoptosis, presumably through a similar mechanism[25]. In the presence of the FAS ligand (FASL), which can trigger apoptosis on its own, CCN1- or CCN2-induced ROS production leads to the hyperactivation of p38 MAPK, which enhances cytochrome *c* release from the mitochondria and thereby increases apoptosis[26]. IGFBP, insulin-like growth factor-binding protein; TNFR1, TNF receptor 1; TSR, thrombospondin type 1 repeat.

chemotaxis and tubule formation[31,32]. They can also regulate the expression[33] and activities of other angiogenic factors such as vascular endothelial growth factor A and vascular endothelial growth factor C[34,35]. Through their angiogenic activities, these CCN proteins may be involved in embryonic development, inflammatory diseases and tumorigenesis[32,36].

Both CCN1 and CCN2 promote the differentiation of chondrocytes and osteoblasts[37,38]. CCN proteins can interact with members of the bone morphogenetic protein (BMP) and TGFβ family, most probably through the chordin-like homology sequence found in the VWC domain, and modulate the binding affinity of BMPs and TGFβ for their respective receptors[39]. CCN2 and CCN3 can bind BMP2 and inhibit its functions in promoting chondrogenic and osteogenic differentiation[15,40], respectively, whereas CCN4 can bind BMP2 and enhances its function in osteogenesis[41]. CCN2 regulates WNT signalling by interacting with the WNT receptor complex through direct binding to the WNT co-receptor low-density lipoprotein receptor-related protein 6 (REF. 11). CCN1 inhibits osteoclastogenesis[42], whereas CCN3 stimulates it through a process that may involve calcium flux[43]. In transgenic mice, overexpression of CCN2 or CCN3 in osteoblasts antagonizes both BMP and WNT signalling, and results in osteopaenia[44,45].

***Inflammation.*** Accumulating evidence indicates that CCN proteins modulate inflammatory responses[36]. CCN1 and CCN2 are induced by inflammatory cytokines, or following viral or bacterial infection, and CCN proteins in turn regulate the activity and expression of cytokines and chemokines. Examples include the ability of CCN proteins to substantially alter the cytotoxicity of the TNF family of cytokines, both *in vitro* and *in vivo*[7,26], as well as the ability of CCN1 to reprogramme macrophages towards M1 polarization and activate the expression of pro-inflammatory cytokines[46]. CCN1 also regulates immune cell infiltration *in vivo* in an experimental model of autoimmune myocarditis[47]. CCN3 inhibits NF-κB activation in endothelial cells, suggesting that it may regulate endothelial inflammation[48]. Furthermore, CCN proteins are involved in the pathobiology of many inflammatory diseases, as discussed below.

***Stem cell differentiation and self-renewal.*** A recent study has shown that CCN2 activity is sufficient to drive the differentiation of human bone marrow mesenchymal stem cells into α-smooth muscle actin (αSMA)-negative fibroblasts, which are primed for differentiation into αSMA-positive myofibroblasts when they are subsequently treated with TGFβ1 (REF. 49). Both CCN1 and CCN2 are targets of the canonical WNT–β-catenin signalling pathway in mesenchymal stem cells, and they regulate the differentiation of these cells into osteoblasts[50,51]. CCN3 is essential for the self-renewal of $CD34^+$ haematopoietic stem cells from umbilical cord blood, highlighting its potential utility for promoting stem cell engraftment[52]. Consistent with a function in stem cell self-renewal, CCN3 inhibits both myogenic and osteoblastic differentiation of mesenchymal stem cells[53].

## CCN proteins in embryonic development

Targeted disruptions of genes encoding CCN proteins in mice have been accomplished (with the exception of *Ccn4*), and phenotypes of the knockout mice are listed in TABLE 2. Although the phenotypes of each knockout mouse are distinct, skeletal and vascular defects are among the most commonly observed phenotypes. Given the importance of several CCN proteins in ovulation, placentation and development, the potential risks of targeting their expression or function in pregnant women or in women who are intending to get pregnant should be carefully evaluated. *Ccn1*-null mice are embryonic lethal owing to impaired angiogenesis, placental insufficiency and severe atrioventricular septal defects (AVSD)[33,54]. Although *Ccn1*$^{+/-}$ mice are viable, 20% of adult mice display persistent ostium primum atrial septal defects[54]. These defects resemble those observed in patients with mutations in atrioventricular

Table 1 | **Cellular processes modulated by CCN proteins***

| Cellular responses | CCN proteins | Cells types or processes; cell-surface receptors involved | Refs |
|---|---|---|---|
| Cell adhesion | CCN1 and CCN2 (+) | Fibroblasts; α6β1 integrin and HSPGs | 2 |
| | CCN1 and CCN2 (+) | Activated endothelial cells; αVβ3 integrin | 2 |
| | CCN1 (+) | Smooth muscle cells; α6β1 integrin and HSPGs | 2 |
| | CCN1 and CCN2 (+) | Platelets; α2Bβ3 integrin | 2 |
| | CCN1 and CCN2 (+) | Monocytes; αMβ2 integrin | 2 |
| | CCN1 and CCN2 (+) | Macrophages; αMβ2 integrin | 46 |
| | CCN2 (+) | Hepatic stellate cells; αVβ3 integrin and LRPs | 2 |
| | CCN2 (+) | Pancreatic stellate cells; α5β1 integrin and HSPGs | 2 |
| | CCN3 (+) | Endothelial cells; αVβ3, αβ1 and α6β1 integrins, as well as HSPGs | 2 |
| Migration and/or invasiveness | CCN1 (+) | Fibroblasts; αVβ5 integrin | 2 |
| | CCN1, CCN2 and CCN3 (+) | Microvascular endothelial cell chemotaxis; αVβ3 integrin | 2 |
| | CCN1 (+) | Smooth muscle cell chemotaxis; α6β1 integrin and HSPGs | 2 |
| | CCN3 (+) | Endothelial cells; αVβ3 and α5β1 integrins | 2 |
| | CCN3 and CCN5 (–) | Inhibition of vascular smooth muscle cell migration | 23, 24 |
| | CCN4 (–) | Lung cancer cell invasiveness | 111 |
| DNA synthesis and/or proliferation | CCN1 (+) | Fibroblasts; αVβ3 integrin | 2 |
| | CCN1 (+) | Astrocytoma cells; α5, α6 and β1 integrins | 189 |
| | CCN3 and CCN5 (–) | Smooth muscle cells | 23 |
| Survival | CCN1, CCN2 and CCN3 (+) | Endothelial cells; αVβ3 integrin | 2 |
| | CCN1 (+) | Breast cancer cells; αVβ3 integrin | 98 |
| Apoptosis | CCN1 (+) | Fibroblasts; α6β1 integrin and syndecan 4 | 9 |
| | CCN1, CCN2 and CCN3 (+) | Synergism with TNF in fibroblasts; α6β1 and αVβ5 integrins, as well as syndecan 4 | 7 |
| | CCN1 and CCN2 (+) | Synergism with FASL in fibroblasts; α6β1 integrin and HSPGs | 26 |
| Differentiation | CCN1 (+) | Osteoblastic differentiation; αVβ3 integrin | 198 |
| Angiogenesis | CCN1, CCN2 and CCN3 (+) | Endothelial tubule formation; αVβ3 integrin | 2 |
| Inflammation | CCN1 (+) | Macrophages, NF-κB activation and M1 macrophage polarization; αMβ2 integrin | 46 |
| | CCN1 (+) | MCF7 breast cancer cells and NF-κB activation; αvβ3 integrin | 98 |
| | CCN1 (–) | Immune cell infiltration in autoimmune myocarditis | 47 |
| | CCN2 (+) | Mesangial cells and chemokine expression; TRKA and HSPGs | 199 |
| | CCN3 (+) | Endothelial cells, NF-κB activation | 48 |
| Senescence | CCN1 (+) | Fibroblasts; α6β1 integrin and HSPGs | 8 |

CCN1, cysteine-rich angiogenic inducer 61 (also known as CYR61); CCN2, connective tissue growth factor (also known as CTGF); CCN3, nephroblastoma overexpressed (also known as NOV); CCN4, WNT-inducible signalling pathway protein 1 (also known as WISP1); CCN5, WNT-inducible signalling pathway protein 2 (also known as WISP2); FASL, FAS ligand; HSPG, heparan sulphate proteoglycan; LRP, low-density lipoprotein receptor-related protein; NF-κB, nuclear factor-κB; TNF, tumour necrosis factor; TRKA, neurotrophic tyrosine kinase receptor type 1. *The table shows selected cellular processes that are stimulated (+) or inhibited (–) by CCN proteins in specific cell types, and lists the cell-surface receptors involved.

**Cellular senescence**
A state of irreversible cell cycle arrest induced by various forms of cellular stresses including DNA damage, oncogene activation, oxidative stress, chromatin disruption and telomere erosion.

**Senescence-associated secretory phenotype**
A phenotype that is one of the characteristics of senescent cells and includes increased secretion of inflammatory cytokines and matrix-degrading enzymes, as well as downregulation of extracellular matrix proteins such as collagen.

**Chondrocytes**
Connective tissue cells that produce the cartilaginous matrix, including type II collagen and proteoglycans, and reside within the lacuna of the cartilage matrix.

**Osteoblasts**
Mononucleate cells that are responsible for bone formation. They produce osteoid, which is composed mainly of type I collagen, and are responsible for mineralization of the osteoid matrix.

septal defect 1 (*AVSD1*), a susceptibility locus for non-syndromic AVSD identified by linkage analysis[55]. As the human *CCN1* gene and *AVSD1* map to the same chromosomal location, these findings suggest that CCN1 may be a candidate gene for AVSD in humans[37].

*Ccn2*-null mice are perinatal lethal owing to respiratory defects that occur as a secondary consequence of severe skeletal malformations[56]; observations from these mice are consistent with a wealth of data showing the roles of CCN2 in chondrogenesis and endochondral ossification[38]. *Ccn2*-null embryos also exhibit pulmonary hypoplasia[57] and decreased numbers of β-cells in the pancreas[58]. Cell-specific deletion of *Ccn2* in the ovary and uterus of mice resulted in disrupted follicle development and steroidogenesis, decreased ovulation and increased numbers of corpus lutea[59]. By contrast, *Ccn3*-knockout mice are viable and largely normal, exhibiting only modest and transient sexually dimorphic skeletal abnormalities[60]. Although *Ccn5*-null mice suffer early embryonic death[61], *Ccn6*-knockout mice display no observable phenotype[62].

## CCN proteins in diseases and targeting studies

As CCN proteins are highly expressed and have important roles at sites of inflammation and tissue repair, they are often deregulated when these processes become chronic and progress to pathological conditions. For this reason, numerous gene profiling studies have found that CCN proteins are aberrantly expressed in a plethora of diseases, many of which are associated with chronic inflammation and tissue repair. Studies in animal models have established that deregulated CCN proteins indeed contribute to the pathologies of many of these diseases, although their mechanisms of action are not completely understood (TABLE 3). Nevertheless, proof-of-principle studies have shown that targeting CCN proteins can yield substantial benefits in animal models. Moreover, the levels of CCN proteins in biological fluids may serve as non-invasive diagnostic or prognostic biomarkers for certain pathologies. Clinical trials targeting CCN2 are already underway, and initial results are encouraging; as discussed below, these results reinforce the notion that CCN proteins are emerging as attractive therapeutic targets for a broad spectrum of diseases.

***Wound healing and fibrotic diseases.*** Mammalian wound healing and tissue repair occur in a similar way in virtually all organ systems, in three distinct but overlapping phases: first, inflammation occurs, which is followed by the formation of granulation tissue and ECM deposition, and concludes with matrix remodelling and resolution of the granulation tissue[63]. At the site of injury, activated resident fibroblasts and recruited fibrocytes differentiate into αSMA-positive myofibroblasts, which proliferate and deposit ECM proteins to promote healing and support tissue integrity while the damaged tissue is being repaired and remodelled. However, excessive ECM deposition may occur in wound repair, which can lead to fibrosis, scarring and loss of tissue function.

Fibrosis is substantially exacerbated when inflammation or tissue injury becomes chronic, and it contributes to the pathologies of chronic diseases in many organ systems[63,64], including the liver (as a result of viral infections or alcoholism), the kidneys (as a result of diabetes) and the heart (as a result of tissue remodelling following myocardial infarction). These pathologies can lead to organ failure and death, yet there is currently no US Food and Drug Administration-approved drug available for treating fibrosis. Emerging studies indicate that various CCN proteins are involved in promoting or inhibiting fibrosis in association with tissue repair, and provide unique opportunities for the development of novel therapeutics and diagnostics for this pathology.

Following wound formation, platelet α-granules release abundant amounts of CCN2 (REFS 65,66) and other growth factors that can induce the expression of CCN proteins at the site of injury. CCN2 acts synergistically with TGFβ1, a potent pro-fibrotic regulator, to promote matrix protein deposition and fibrogenesis both *in vitro* and *in vivo*[67]. Subcutaneous or intraperitoneal injections of either TGFβ or CCN2 individually do not induce persistent fibrosis, whereas co-injection of both proteins together results in sustained fibrosis[68].

CCN2 is overexpressed in human fibrotic diseases of virtually every organ or tissue. Correspondingly, levels of CCN2 in the serum and biological fluids of patients correlate with the severity of systemic fibrosis (known as scleroderma) as well as fibrosis of the heart, kidneys, liver and lungs, which suggests that CCN2 levels in biological fluids may be useful as a non-invasive biomarker for many fibrotic diseases[69,70]. However, despite this close association with fibrosis, overexpression of *Ccn2* in parenchymal cells of most organs does not induce fibrosis in mice but the fibrotic response is exacerbated when these mice are challenged with injury[67]. However, overexpression of *Ccn2* specifically in fibroblasts — the major cell type responsible for matrix deposition — is sufficient to drive fibrosis in lung, skin and kidney tissues, as well as arteries[71]. Furthermore, *Ccn2* deletion in fibroblasts of mice confers resistance to bleomycin-induced skin fibrosis[72].

These observations suggest that the action of CCN2 in fibroblasts may be strongly autocrine. Consistent with its pro-fibrotic function, inhibition or downregulation of CCN2 ameliorates fibrosis in many organ systems. Knockdown of *Ccn2* by antisense oligonucleotides or small interfering RNAs (siRNAs) reduces carbon tetrachloride-induced[73,74] and *N*-nitrosodimethylamine-induced[75] liver fibrosis, ureteral obstruction-induced renal fibrosis[76], fibrotic scarring in cutaneous wounds[77] and renal interstitial fibrogenesis following partial nephrectomy[78]. Although less is known about the functions of CCN4, it has been shown that *CCN4* is upregulated in patients with idiopathic pulmonary fibrosis, as well as in a mouse model of bleomycin-induced lung fibrosis; in addition, recombinant CCN4 enhances ECM deposition in human fibroblasts[79]. Correspondingly, orotracheal application of CCN4-neutralizing antibodies to the lung ameliorates bleomycin-induced lung fibrosis in mice[79].

In contrast to the pro-fibrotic activity of CCN2 and CCN4, CCN1 functions to limit fibrosis through a novel mechanism that invokes the induction of cellular

Atrioventricular septal defects
A form of congenital heart defect in which the heart chambers are poorly formed as a result of impairments in the endocardial cushions, which contribute to the formation of the atrial and ventricular septa, as well as the mitral and tricuspid valves.

Platelet α-granules
Cytoplasmic granules containing many protein factors that are involved in inflammation and wound repair. They are synthesized predominantly in megakaryocytes and include cell-adhesive proteins, growth and coagulation factors, and protease inhibitors, which are released following platelet activation at sites of vessel injury.

Table 2 | **Phenotypes of knockout and knock-in mice**

| Mouse model | Mouse phenotypes | Other information | Refs |
|---|---|---|---|
| *Ccn1*-knockout | • Embryonic lethality<br>• Failure in chorioallantoic fusion in 30% of embryos<br>• Placental insufficiency; deficient vessel bifurcation at the chorioallantoic junction<br>• Haemorrhage; loss of large-vessel integrity<br>• Severe atrioventricular septal defects | • Aberrant apoptosis in vascular cells and cardiac cushion mesenchymal cells<br>• Haploinsufficiency leads to persistent ostium primum atrial septal defects in 20% of adult mice | 33,54 |
| *Ccn2*-knockout | • Perinatal lethality<br>• Severe skeletal dysplasia, enlarged hypertrophic zones, impaired endochondral ossification, decreased numbers of β-cells and increased numbers of glucagon-positive cells in pancreas<br>• Pulmonary hypoplasia | • Premature exit of chondrocytes from the cell cycle<br>• Delayed replacement with osteoblasts<br>• Decreased expression of VEGF in hypertrophic zones | 56–58 |
| *Ccn3*-knockout | • Mice are viable and largely appear to be normal<br>• Modest, transient and sexually dimorphic skeletal abnormalities | • Enhanced neointimal thickening after photochemically induced thrombosis | 24,60 |
| *Ccn5*-knockout | • Early embryonic lethality | - | 61 |
| *Ccn6*-knockout | • No difference to wild-type<br>• No *Ccn6* mRNA detected by northern blotting or *in situ* hybridization | • Mutations in human CCN6 cause the autosomal recessive disorder progressive pseudorheumatoid dysplasia | 62,160 |
| *Ccn1*-knock-in (*Ccn1*$^{dm/dm}$)* | • Mice are viable, fertile and developmentally normal<br>• Mice are significantly resistant to TNF- or FAS-mediated hepatocyte apoptosis<br>• Mice lack senescent cells in skin wound healing, leading to exacerbated fibrosis | • Mice express CCN1 that is unable to bind fibroblast receptors (α6β1 integrin-HSPG) | 7,8,26 |
| Floxed *Ccn2*‡ | • Deletion in early limb bud mesenchyma results in transient osteopaenia at 1 month<br>• Deletion in osteoblasts results in osteopaenia in 6-month-old male mice<br>• Deletion in fibroblasts renders mice resistant to bleomycin-induced skin fibrosis<br>• Deletion in ovary and uterus results in impaired follicle development, steroidogenesis and ovulation | - | 59,72, 200 |
| *Ccn2* (high to low expression) | • Ninefold overexpression of CCN2 causes embryonic death at embryonic day 10 to day 12<br>• Mice expressing 30% of normal level of CCN2 are viable and normal | - | 201 |

CCN1, cysteine-rich angiogenic inducer 61 (also known as CYR61); CCN2, connective tissue growth factor (also known as CTGF); CCN3, nephroblastoma overexpressed (also known as NOV); CCN5, WNT-inducible signalling pathway protein 2 (also known as WISP2); CCN6, WNT-inducible signalling pathway protein 3 (also known as WISP3); FASL, FAS ligand; HSPG, heparan sulphate proteoglycan; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. *Homozygous mice in which both *Ccn1* genomic loci have been replaced by the *Ccn1-dm* allele, which encodes a CCN1 protein that is unable to bind α6β1 integrin-HSPG. ‡Homozygous mice in which both *Ccn2* genomic loci are flanked by the LoxP sequence of bacteriophage P1, allowing specific deletion of the *Ccn2* gene in the presence of the Cre recombinase, which is usually expressed under the control of a cell-specific or conditionally activated promoter.

senescence in myofibroblasts[8]. Accumulation of senescent cells is an integral part of the normal wound healing response that functions to curb fibrosis, and this process is controlled by CCN1 (REF. 30) (FIG. 4). CCN1 gradually accumulates in granulation tissue in excisional cutaneous wounds, reaching a level sufficient to trigger senescence in myofibroblasts as the healing process enters the resolution phase, at which point senescent cells express the senescence-associated secretory phenotype that involves the secretion of MMPs and the downregulation of collagen I and TGFβ1 (REF. 8). Thus, CCN1 acts as an antifibrotic senescence switch, converting the ECM-synthesizing myofibroblasts into ECM-degrading senescent cells.

This mechanism of controlling fibrosis achieves molecular parsimony by simultaneously halting further ECM production in myofibroblasts, accelerating ECM degradation and facilitating granulation tissue resolution as senescent cells are cleared by natural killer cells[30,80]. In knock-in mice expressing senescence-defective CCN1, senescent cells do not accumulate in the granulation tissue, and wound healing proceeds with exacerbated fibrosis[8]. Topical treatment of these wounds with purified CCN1 reverses this defect and reduces fibrosis by increasing the number of senescent cells, suggesting a therapeutic potential for CCN1 in the treatment of fibrosis. Interestingly, hepatic stellate cells also undergo senescence to limit fibrosis in a mouse model of chemically induced liver injury[80], indicating that this mechanism of controlling fibrosis may be a general feature of wound repair in different organs and diverse modes of injuries.

Although CCN1 inhibits fibrosis by inducing cellular senescence[8], CCN3 and CCN5 appear to exert antifibrotic activities through alternative mechanisms. CCN3 suppresses CCN2 and collagen I expression in mesangial cells *in vitro*[81], whereas overexpression of CCN5 reduces cardiac hypertrophy and fibrosis in transgenic mice, possibly by inhibiting TGFβ–SMAD signalling[82]. As CCN2 and CCN4 are pro-fibrotic (and CCN1, CCN3 and CCN5 are antifibrotic, at least in certain biological contexts), it is crucial that reagents that target members of the CCN protein family have a high level of specificity. Approaches in this regard are discussed below.

***CCN proteins in cancer.*** It has long been recognized that chronic injuries and deregulated wound healing are risk factors in the development of cancer[83]. Recent studies have further underscored the important role of inflammation in tumorigenesis and tumour progression[84]. Inflammation occurring at early stages of neoplastic transformation can accelerate the development of incipient neoplasias into full-blown cancer by releasing mutagenic chemicals such as reactive oxygen species. Moreover, inflammation is associated with most — if not all — tumours, and contributes to cancer progression by providing a rich arsenal of biologically active molecules to the tumour microenvironment, including factors that promote cell growth, survival, motility, invasion, angiogenesis and matrix degradation. Aberrant expression of CCN proteins has been identified in a range of tumour types[85]. With some notable exceptions[86,87] CCN1, CCN2 and CCN4 have generally been associated with the promotion of cell proliferation and tumour growth, whereas CCN3, CCN5 and CCN6 have been associated with inhibition of these processes (TABLE 4).

CCN1 and CCN2 may enhance tumour growth through their potent angiogenic activity[31,88]. Accordingly, forced expression of *CCN1* in breast cancer cells promotes tumour growth in xenografts with increased vascularization[89,90], and expression of *CCN2* in tumour-reactive stroma enhances the density of microvessels in xenograft tumours in nude mice[91,92]. Consequently, xenograft tumour growth is inhibited by silencing the expression of either *CCN1* or *CCN2* in cancer cells of the prostate[93], pancreas[94,95] and ovary[96]. Both CCN1 and CCN2 can promote epithelial-to-mesenchymal transition[95,97], enhance the survival of some cancer cells through the induction of anti-apoptotic proteins[98,99] and mediate resistance to paclitaxel in breast cancer[100]. Direct intratumoural delivery of CCN1 siRNA into pre-established glioma xenografts decreases tumour growth by up to 40% in a dose-dependent manner, suggesting that targeting CCN1 has therapeutic potential[101]. Furthermore, *CCN2* is a key component of the gene signature that facilitates metastasis to bone[102], and treatment of nude mice with a CCN2-specific murine monoclonal antibody markedly decreased osteolytic bone metastasis of human breast cancer cell xenografts[103]. Notably, administration of FG-3019, a CCN2-specific humanized monoclonal antibody, inhibits tumour growth and metastases in xenograft and orthotopic mouse models of pancreatic cancer[104,105]. As human pancreatic cancers are largely recalcitrant to therapy, the possibility of treating this form of cancer by targeting CCN2 is particularly encouraging.

Overexpression of *CCN4* in normal rat kidney fibroblasts confers the ability to form tumours in nude mice[106], and elevated *CCN4* expression has been observed in advanced breast and colon cancers[107,108] as well as adenosquamous lung carcinomas[109]. However, CCN4 appears to inhibit metastasis and is preferentially expressed in melanoma cell lines with a low metastastic potential[110]. Lung cancer cells overexpressing *CCN4* are less invasive and exhibit reduced metastasis in nude mice, possibly owing to the integrin-mediated inhibition of RAC by CCN4 (REF. 111). A splice variant of *CCN4* lacking the VWC domain has been detected in scirrhous gastric carcinomas, and transfectants expressing this variant enhance the invasive characteristic of co-cultured gastric carcinoma cells[112]. Therefore, it is possible that CCN4 isoforms expressed as a result of differential splicing might underlie the pro- and anti-invasive activities of CCN4, although this possibility has not been thoroughly investigated.

CCN3, CCN5 and CCN6 have a negative effect on cell proliferation. Although CCN3 inhibits the proliferation of cancer cells[113], it appears to promote metastasis[20]. Overexpression of *CCN3* results in reduced tumour size in glioma cell xenografts[114] but enhances metastatic potential in xenotransplanted melanoma cells[115]. *CCN3* expression is associated with a higher risk of metastasis and worse prognosis in patients with cancers such as Ewing's sarcoma[116], melanoma[115] and breast cancer[117]. In addition to solid tumours, CCN3 is downregulated in chronic myeloid leukaemia as a consequence of the activity of breakpoint cluster region (BCR)-ABL kinase, a chimeric protein generated through the chromosomal translocation between chromosome 9 and chromosome 22 (REF. 118). CCN3 expression is increased in patients who respond to imatinib therapy. Forced expression of CCN3 inhibits proliferation and restores growth control in chronic myeloid leukaemia cells, and sensitizes them to imatinib-induced apoptosis, suggesting that CCN3 may be an alternative target for novel therapeutics against chronic myeloid leukaemia[119].

CCN5 inhibits the growth of vascular smooth muscle cells, uterine myometrial cells, leiomyoma cells and oestrogen receptor-negative breast cancer cells[61]. CCN5 expression is significantly decreased in leiomyomas, pancreatic cancers and salivary gland tumours. *CCN6* is expressed in the normal mammary epithelium but its expression is lost or downregulated in 60% of inflammatory breast carcinomas, and restoration of *CCN6* expression reduces tumour growth in nude mice[120]. It has been suggested that CCN6 acts by interfering with the insulin-like growth factor 1 signalling pathway, which promotes the proliferation, survival and metastasis of breast cancer cells[121].

CCN proteins can therefore act both positively and negatively in tumorigenesis and tumour progression, depending on the tumour type. Although CCN1 and CCN2 are known inducers of angiogenesis, and may promote the growth of some tumour types, they can also

Epithelial-to-mesenchymal transition
A process — typically involving repression of E-cadherin expression — in which epithelial cells lose their adhesive properties and gain mobility. This transition is essential for many developmental processes and occurs during oncogenic transformation.

Table 3 | **Targeting CCN proteins in animal models of disease**

| CCN proteins | Animal model | Phenotypes and/or intervention | Refs |
|---|---|---|---|
| ***Fibrosis*** | | | |
| CCN1 | Wound healing in α6β1 integrin-binding defective *Ccn1*-knock-in mice | Enhanced fibrosis in cutaneous wounds; topical application of CCN1 reverses effect | 8 |
| CCN2 | Transgenic mice overexpressing CCN2 in hepatocytes | Exacerbated liver fibrosis induced by CCN4 or bile duct ligation | 202 |
| | Mouse liver fibrosis (induced by $CCl_4$) | CCN2 siRNAs reduce fibrosis | 74 |
| | *N*-nitrosodimethylamine-induced liver fibrosis | CCN2 siRNAs reduce fibrosis | 75 |
| | Streptozotocin-induced diabetes in podocyte-specific *Ccn2*-transgenic mice | Exacerbated renal hypertrophy, proteinuria and mesangial matrix expansion | 134 |
| | Tubulointerstitial fibrosis induced by unilateral ureteral obstruction in rats | Reduction of ECM induction by tail vein injection of CCN2 antisense oligonucleotide | 76 |
| | Subtotal nephrectomy in TGFβ1-transgenic mice | Injection of CCN2 antisense oligonucleotide blocks interstitial fibrosis in remnant kidney | 78 |
| | Cardiomyocyte-specific *Ccn2*-transgenic rodents; angiotensin II-induced pressure overload resulting in heart failure | No cardiac fibrosis; promotes cardiac hypertrophy and protects against pressure overload-induced heart failure | 149 |
| | Deletion of *Ccn2* in fibroblasts and smooth muscle cells in mice | Resistance to bleomycin-induced skin fibrosis | 72 |
| | Fibroblast-specific *Ccn2* overexpression in transgenic mice | Fibrosis in skin, lungs, kidneys and vasculature | 71 |
| CCN4 | Bleomycin-induced pulmonary fibrosis in mice | Neutralizing antibodies attenuate lung fibrosis | 79 |
| CCN5 | Transverse aortic constriction in *Ccn5*-transgenic mice | Inhibition of pressure overload-induced cardiac hypertrophy and fibrosis | 82 |
| ***Cancer*** | | | |
| CCN1 | Glioma xenografts | siRNA inhibits pre-established glioma growth | 101 |
| | Pancreatic cancer cells | shRNA reverses characteristics of EMT and reduces tumorigenicity | 95 |
| CCN2 | Pancreatic cancer xenografts | CCN2 shRNAs decrease tumour growth | 94 |
| | Mouse models of orthotopic and xenografted pancreatic cancer | FG-3019 attenuates tumour growth and metastasis | 104, 105 |
| ***Cardiovascular disease*** | | | |
| CCN1 | Rat carotid artery (balloon injury model) | CCN1 siRNAs suppress neointimal hyperplasia | 145 |
| CCN1 | Autoimmune myocarditis in mice | Adenoviral expression of CCN1 attenuates myocarditis | 47 |
| CCN2 | Rat carotid artery angioplasty with viral perfusion | Recombinant CCN2 application increases neointimal thickening | 143 |
| ***Nephropathy and retinopathy*** | | | |
| CCN1 | Oxygen-induced retinopathy in mice | Intravitreal injection of CCN1-specific antibody reduces retinal neovascularization | 138 |
| CCN2 | Streptozotocin-induced diabetes in mice | Subcutaneous injection of CCN2 antisense oligonucleotide decreases proteinuria and albuminuria | 133, 140 |
| | | Reduced basal lamina thickening of retinal capillaries in $Ccn2^{+/-}$ mice* | |
| ***Wound healing*** | | | |
| CCN1 | Bone fracture (transverse osteotomy) | Intraperitoneal injection of CCN1-specific antibody impaired fracture healing | 154 |
| CCN2 | Rabbit punch wound (on the ears) | Intradermal CCN2 antisense oligonucleotide injection reduces hyperptrophic scarring | 77 |

$CCl_4$, carbon tetrachloride; CCN1, cysteine-rich angiogenic inducer 61 (also known as CYR61); CCN2, connective tissue growth factor (also known as CTGF); CCN4, WNT-inducible signalling pathway protein 1 (also known as WISP1); CCN5, WNT-inducible signalling pathway protein 2 (also known as WISP2); ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; siRNA, small interfering RNA; shRNA, short hairpin RNA. *In $Ccn2^{+/-}$ mice in which only half of the CCN2 is produced, there is reduced pathology (retinal capillary thickening) under diabetic conditions.

suppress tumour growth in some cancers[86,87]. CCN1, CCN2 and CCN3 can synergize with cytokines of the TNF family — particularly TNF-related apoptosis-inducing ligand, which preferentially kills tumour cells[27] — to induce apoptosis, and CCN1 can induce cellular senescence[8]. Both apoptosis and senescence are well-established mechanisms of tumour suppression if they are triggered in damaged cells that are at risk of neoplastic transformation[122], which suggests that CCN proteins may inhibit the development of incipient tumours by promoting apoptosis or senescence in damaged cells.

It is important to note that most experimental studies on the functions of CCN proteins in cancer have used xenograft models and established tumour cell lines, and thus focus on tumour growth and progression rather than the initial stages of tumorigenesis. Whether CCN proteins exert a positive or negative effect on tumour growth may depend on the type of integrin that is preferentially expressed on the tumour cells (for example, αVβ3 integrin is growth-promoting, whereas α6β1 integrin is growth-inhibitory when CCN1 is used as a ligand)[37]. In addition, the positive or negative effect of CCN proteins on tumour growth depends on whether angiogenic factors are limiting, and whether conditions that favour apoptosis or senescence (that is, the accumulation of reactive oxygen species) prevail. Despite these uncertainties regarding how CCN proteins function in tumorigenesis, targeting CCN proteins has shown therapeutic promise in specific cancers (as described above), and these approaches warrant further translational studies.

***Diabetic nephropathy and retinopathy.*** Diabetes is associated with a multitude of metabolic and homeostatic abnormalities, including hyperglycaemia as well as systemic and intraglomerular hypertension, which contribute to microvascular pathologies such as diabetic nephropathy and retinopathy. Substantial evidence from recent studies supports the involvement of chronic inflammation in the pathogenesis of diabetic nephropathy and retinopathy[123,124]. Chronic hyperglycaemia — the hallmark of diabetes — is a pro-inflammatory condition that leads to multiple biochemical alterations including the formation of advanced glycation end products, which are thought to trigger or amplify many aspects of diabetes pathology[125]. Exposure to high levels of glucose stimulates *CCN2* expression in human mesangial cells[126], and the expression of both *CCN1* and *CCN2* is induced by advanced glycation end products *in vitro* and *in vivo*[127,128]. In addition, mechanical stretch and haemodynamic forces simulating hypertension induce the expression of *CCN1* and *CCN2* in vascular endothelial cells and smooth muscle cells, and the expression of *CCN2* in renal mesangial cells[129]. Thus, hyperglycaemia and hypertension are both factors that contribute to the progression of diabetic disease that is associated with the induction of *CCN1* and *CCN2* expression.

Diabetic nephropathy is characterized by hypertrophy of glomeruli and tubules, thickening of their associated basement membrane, and glomerular and interstitial fibrosis; all of these factors compromise kidney function[130]. Microalbuminuria at the incipient stage of diabetic nephropathy progresses to overt proteinuria as glomerular filtration deteriorates. In patients with diabetes, *CCN2* expression is increased in both glomerular podocytes and mesangial cells[130], whereas *CCN1* expression in podocytes is downregulated[131]. CCN2 induces pro-fibrotic responses in cultured human mesangial cells and promotes the infiltration of inflammatory cells into the renal interstitium[130,132]. Treatment of diabetic rats with aminoguanidine to inhibit the formation of advanced glycation end products concomitantly blocks the induction of *Ccn2* expression and albuminuria[127]. Knockdown of *Ccn2* expression by antisense oligonucleotides attenuates the progression of nephropathy in mouse models of diabetes[133], whereas transgenic overexpression of *CCN2* in podocytes enhances urinary albuminuria[134]. Current methods of treating diabetic nephropathy focus on the control of renal hypertension using angiotensin-converting enzyme inhibitors or angiotensin receptor blockers[135]; targeting CCN2 may therefore provide an alternative or complementary form of therapy. Moreover, plasma and urinary levels of CCN2 in patients with diabetes correlate with clinical parameters associated with the severity of diabetic nephropathy, suggesting that CCN2 can be a useful biomarker for this disease[136,137].

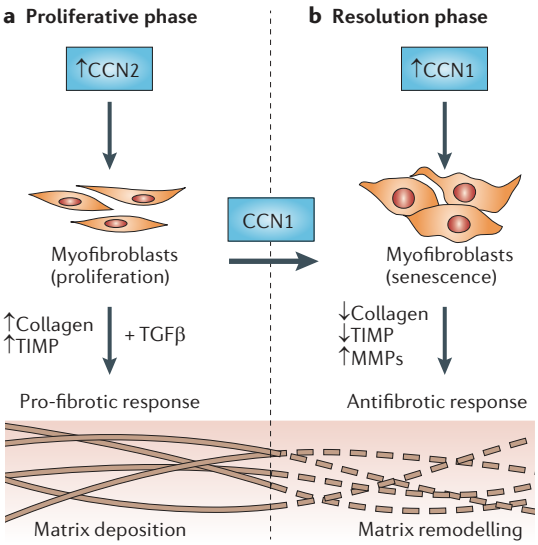



Figure 4 | **Role of CCN proteins in wound healing.** Cysteine-rich angiogenic inducer 61 (CYR61; also known as CCN1) and connective tissue growth factor (CTGF; also known as CCN2) have distinct roles in wound healing. CCN2 functions in the granulation tissue during the proliferative phase and interacts with transforming growth factor-β (TGFβ) to promote the synthesis of the extracellular matrix (ECM), leading to a pro-fibrotic response. As wound healing progresses, CCN1 accumulates to a sufficiently high level to induce an antifibrotic senescence switch in myofibroblasts, thereby limiting fibrosis by converting the ECM-synthesizing myofibroblasts into ECM-degrading senescent cells. MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

Microalbuminuria
A condition in which small amounts of albumin leak into the urine following injury or damage to the kidney, which is indicative of abnormal permeability in the renal glomerulus.

Retinopathy is a common microvascular complication of diabetes, which is caused by enhanced angiogenesis and fibrosis in the vitreous cavity of the eye, and results in loss of vision. CCN1 appears to be involved in retinal angiogenesis in rodent models of streptozotocin-induced diabetes or oxygen-induced retinopathy, and in proliferative diabetic retinopathy in humans[128,138]. Intravitreal injection of a neutralizing CCN1-specific antibody in the oxygen-induced retinopathy mouse model significantly reduces retinal neovascularization with no apparent toxicity or inflammation[138]. *CCN2* is highly expressed in microvascular pericytes of the human diabetic retina, and

Table 4 | **CCN proteins in cancers**

| Tumour or cancer | CCN proteins | Expression level | Clinical characterization and/or experimental observations | Refs |
|---|---|---|---|---|
| Breast cancer | CCN1 | Higher | Associated with poor prognosis, tumour stage, metastasis and mortality | 107 |
| | | | Overexpression of CCN1 promotes tumorigenesis in xenografts | 90 |
| | | | Mediates resistance to paclitaxel | 100 |
| | CCN2 | Higher | Associated with poor prognosis and metastasis; mediates resistance to paclitaxel | 100,107 |
| | | | Mediates metastasis to bone | 102 |
| | CCN3 | Higher | Associated with therapy-resistant oestrogen receptor-positive breast cancer | 117 |
| | CCN4 | Higher | Associated with older patients who have advanced tumours | 107 |
| | CCN5 | Higher | Associated with breast cancers with a low metastatic potential that are more differentiated; inversely correlated with estradiol-independency and invasive phenotype | 61 |
| | CCN6 | Lower | Associated with poor prognosis in inflammatory breast cancer | 120 |
| Endometrial cancer | CCN1 | Higher | Poor survival of patients with a specific endometrioid subtype | 203 |
| | | | CCN1 inhibits endometrial cancer cell growth | 204 |
| Ovarian cancer | CCN1 | Higher | Associated with advanced tumour stage; enhanced tumorigenesis in xenografts of CCN1-overexpressing cells | 96 |
| | CCN2 | Lower | Promoter hypermethylation; associated with advanced tumour stage | 205 |
| Prostate cancer | CCN1 | Higher | Associated with high-grade prostate cancer and lower risk of recurrence after surgery | 176 |
| | | | Decreased size of xenografted tumours of DU145 cells is associated with decreased CCN1 expression | 93 |
| | CCN2 | Not known | Xenografts of CCN2-overexpressing prostate cancer cells have increased microvessel density and tumour growth | 91 |
| Glioma | CCN1 and CCN2 | Higher | Associated with tumour grade, pathology, gender and age at diagnosis | 101 |
| | CCN3 | Not known | Reduced tumorigenicity in CCN3-overexpressing cells | 114 |
| Lung cancer | CCN1 and CCN2 | Lower | Associated with tumour stage, tumour histology, metastasis, smoking and family history; expression level correlated with survival of patients Overexpression of CCN1 results in decreased tumour growth of xenografts | 109,206 |
| | CCN4 | Higher | Higher expression in adenosquamous lung carcinoma | 109 |
| Pancreatic cancer | CCN1 | Higher | Expression increases with progression of pancreatic adenocarcinoma | 95 |
| | CCN2 | Higher | Highly expressed in pancreatic cancer | 104 |
| | CCN5 | Lower | Inversely correlated with tumour grade and p53 status | 207 |
| Colon cancer | CCN1 and CCN2 | Higher | Associated with tumour stage | 208 |
| | CCN4 | Higher | Associated with tumour stage, histological grade and lymph node status | 108 |
| Musculoskeletal tumour | CCN2 | Higher | Associated with survival of rhabdomyosarcoma cells | 209 |
| | CCN3 | Higher | Higher risk of metastasis and worse prognosis in Ewing's sarcoma | 116 |
| Melanoma | CCN3 | Higher | Associated with higher degree of metastasis of xenografted CCN3-overexpressing cells | 115 |
| Leukaemia | CCN2 | Higher | Associated with pre-B cell lineage in ALL | 210 |
| | CCN3 | Lower | Restoring CCN3 expression sensitizes CML cells to imatimib therapy | 118 |

ALL, acute lymphoblastic leukaemia; CCN1, cysteine-rich angiogenic inducer 61 (also known as CYR61); CCN2, connective tissue growth factor (also known as CTGF); CCN3, nephroblastoma overexpressed (also known as NOV); CCN4, WNT-inducible signalling pathway protein 1 (also known as WISP1); CCN5, WNT-inducible signalling pathway protein 2 (also known as WISP2); CCN6, WNT-inducible signalling pathway protein 3 (also known as WISP3); CML, chronic myloid leukaemia; p53, cellular tumour antigen p53.

its levels correlate with the degree of fibrosis in vitreoretinal disorders[139]. Heterozygous *Ccn2*$^{+/-}$ mice subjected to streptozotocin-induced diabetes exhibit significantly lower basal membrane thickening of retinal capillaries than wild-type mice[140]. These results are consistent with the notion that targeting CCN proteins may help to ameliorate the pathology of diabetic retinopathy.

***Cardiovascular diseases.*** CCN proteins have been implicated in several vascular pathologies, including atherosclerosis, restenosis, thrombosis and hypertension. Atherosclerosis is a chronic inflammatory disease of the arterial wall that is triggered by the prolonged elevation of lipids in the bloodstream (known as hyperlipidaemia), whereas proliferative restenosis is a common response to vascular injury after balloon angioplasty. CCN1 and CCN2 promote neointimal hyperplasia after vascular injury, whereas CCN3 and CCN5 inhibit it. High levels of both CCN1 and CCN2 have been detected in rupture-prone atherosclerotic plaques — particularly in areas of neovascularization or in areas that are infiltrated with inflammatory cells[141,142] — as well as in the neointima in restenosis after balloon injuries in rats[22,143]. Inhibiting *Ccn1* expression using either its negative regulator forkhead box protein O3A[144] or siRNA[145] effectively reduced neointimal hyperplasia following balloon injury in a rat carotid artery. As neointimal hyperplasia is a common complication in the treatment of atherosclerosis, targeting CCN1 may be beneficial. By contrast, *Ccn3* expression is substantially reduced following balloon injury. CCN3 inhibits smooth muscle cell proliferation in culture, and *Ccn3*-null mice suffer from enhanced neointimal thickening when challenged with vascular injury, which indicates that CCN3 inhibits neointimal hyperplasia[24]. Likewise, CCN5 inhibits vascular smooth muscle cell proliferation and motility, and its expression is substantially reduced in arteries after balloon-induced injury[23].

*CCN1* is upregulated in inflammatory cardiomyopathy in humans, and *CCN1* gene transfer in animal models attenuates autoimmune myocarditis by inhibiting the infiltration of spleen macrophages and lymphocytes into the myocardium[47]. The effects of CCN1 on immune cells are partially mimicked by cyclic RGD peptides, which bind to αV integrins and are in clinical trials as cancer therapeutics[146]. Both *CCN1* and *CCN2* are substantially upregulated in cardiomyocytes of patients with ischaemic cardiomyopathy, and during cardiac remodelling after myocardial infarction[147,148]. Cardiomyocyte-specific expression of *CCN2* alone in transgenic mice did not induce fibrosis but it did exacerbate pressure overload-induced cardiac fibrosis[82] and led to cardiomyocyte hypertrophy by the time the mice were 7 months old[149]. By contrast, CCN5 inhibits the CCN2-induced hypertrophic responses in cardiomyocytes, and transgenic expression of *CCN5* in cardiomyocytes reduces pressure overload-induced cardiac fibrosis[82]. These results indicate that CCN2 and CCN5 have opposing roles in cardiac hypertrophy and fibrosis. CCN5 is the only member of the CCN protein family that lacks the C-terminal domain. Deleting the C-terminal domain of CCN2 renders it CCN5-like in inhibiting hypertrophic responses in cardiomyocytes *in vitro*, and fusing the C-terminal domain of CCN2 to CCN5 transforms the latter protein into a CCN2-like pro-hypertrophic molecule[82]; this indicates that the C-terminal domain of CCN2 has a pro-fibrotic function.

***Arthritis and other inflammatory diseases.*** Osteoarthritis is a common and debilitating degenerative disease affecting the joints, for which the principal forms of treatment provide only temporary pain management, and surgery may be required if joint degeneration is severe. Both CCN2 and CCN4 are strongly upregulated in the cartilage of patients with osteoarthritis[150,151]. CCN4 contributes to cartilage damage by inducing the production of MMPs and aggrecanase in macrophages and chondrocytes[151]. CCN2 promotes the proliferation and differentiation of chondrocytes without inducing calcification of articular cartilage[38]. Direct injection of CCN2 into the joint cavity stimulates cartilage repair in a rat model of osteoarthritis, which suggests that CCN2 may have therapeutic potential in treating osteoarthritis[152]. *CCN1* and *CCN2* are highly expressed in hypertrophic and proliferative chondrocytes during fracture healing, and blockade of CCN1 function by neutralizing antibodies inhibits bone fracture healing in mice[153,154].

*CCN1* and *CCN2* are both highly expressed in rheumatoid arthritis, which is an inflammatory joint disorder that leads to the destruction of articular cartilage[155,156]. CCN1 may contribute to hyperplasia of the synovial lining, resulting in joint destruction in rheumatoid arthritis. CCN2 synergizes with macrophage colony-stimulating factor and receptor activator of NF-κB ligand to enhance osteoclastogenesis, and contributes to bone destruction in the disorder[155]. Current therapies for recalcitrant rheumatoid arthritis include monoclonal antibodies (such as infliximab, golimumab, adalimumab and certolizumab pegol) that target TNF, which can regulate the expression of the *CCN* gene family and functionally interact with CCN proteins[7,36]. In this regard, it is interesting to note that TNF antagonists are also effective therapies in inflammatory bowel disease, and *CCN1* is upregulated 10–20-fold in patients with Crohn's disease or ulcerative colitis[157], suggesting that CCN1 and TNF may functionally interact in these diseases as well.

In the central nervous system, chronic inflammation-like responses of glial cells cause neurodegenerative events including plaque formation, dystrophic neurite growth and excessive phosphorylation of the microtubule-associated protein tau. Thus, neuroinflammation has been implicated in several pathological conditions affecting the central nervous system, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. Elevated CCN2 expression is observed in brain neurons and astrocytes from patients with Alzheimer's disease, and its expression levels correlate with the progression of clinical dementia and the deposition of neuritic plaques and neurofibrillary tangles[158]. Moreover, CCN2 promotes the processing of amyloid precursor protein and the subsequent generation of amyloid-β peptide *in vitro*[159], which suggests that

Balloon angioplasty
A method of mechanically widening a blood vessel that has typically been obstructed as a result of atherosclerosis. The method involves passing a collapsed balloon catheter through the obstructed area and inflating the balloon to open up the blood vessel as the catheter is withdrawn.

CCN2 may contribute to the progression of astrocytes by enhancing β-amyloidosis. However, the normal functions of CCN proteins in the central nervous system are currently unclear.

***Genetic mutations and polymorphisms in human diseases.*** Out of the six members of the *CCN* gene family, only *CCN6* has been associated with a human disease that has Mendelian inheritance. Mutations in *CCN6* cause the autosomal recessive skeletal disease progressive pseudorheumatoid dysplasia, a juvenile-onset degenerative disease of the joints[160]. Alleles that cause progressive pseudorheumatoid dysplasia harbour loss-of-function mutations including deletions as well as frameshift, nonsense and missense mutations[160]. The development of DNA-based prenatal diagnosis strategies to target *CCN6* may be useful for identifying families who are at risk of developing progressive pseudorheumatoid dysplasia, as symptoms of this disease are seen from 3 to 8 years of age. Genetic disruption of *Ccn6* in mice fails to recapitulate the human disease and presents no discernible phenotype[62], although in zebrafish *ccn6* modulates the canonical BMP and WNT signalling pathways that are crucial for cartilage homeostasis[161].

Polymorphisms have been identified in the DNA sequence of genes encoding CCN proteins that are associated with various diseases (TABLE 5). Notably, polymorphisms in the human *CCN2* gene have been found to be significantly associated with scleroderma[162–164], although some of these results may be controversial[165]. Other polymorphisms in the *CCN* gene family have been associated with susceptibility to hypertension, type 1 diabetes, colorectal cancer, hepatic fibrosis and so on. How these polymorphisms affect the expression or function of genes encoding CCN proteins is largely unknown and warrants further studies.

## Therapeutic approaches and clinical trials

Much of the clinical potential of therapeutically targeting CCN proteins is still largely unexplored, as translational studies are still in early stages. As CCN proteins are secreted, humanized monoclonal antibodies are particularly well suited as blocking agents for their activities and thus hold promise as potential therapeutics. In this regard, the hinge region of the protein — which is encoded by the same exon as the VWC domain (BOX 1) — may be a good choice of antigen, as this region is unique to each member of the CCN protein family and contains an abundance of charged amino acids, thus enhancing its antigenicity. Other targeting strategies, such as the use of siRNA or antisense oligonucleotides to downregulate the expression of genes encoding specific CCN proteins, have been successful in animal models[166]. These approaches can also afford a high degree of target gene specificity if possible off-target effects are minimized through careful selection of oligonucleotide sequences.

The use of synthetic peptides as targeting agents for CCN proteins has not been explored in as much detail but nevertheless has some therapeutic potential. For example, recent studies show that decorin can bind CCN2 directly through its leucine-rich repeat domain and inhibit the pro-fibrotic activity of CCN2 (REF. 167), suggesting the possibility of targeting CCN2 using a binding peptide. However, decorin is also known to bind other members of the CCN protein family and may not interact specifically with CCN2 (REF. 168). As CCN proteins interact with αVβ3 integrins to mediate angiogenic functions[31,169], peptides that bind αVβ3 integrin and inhibit αVβ3 integrin-mediated signalling may thwart CCN1-promoted angiogenesis in tumour growth. However, this strategy — targeting αVβ3 integrin — is not specific to CCN proteins. Other peptides have been identified that block the binding of CCN proteins to α6β1 integrin and HSPGs, and inhibit activities mediated through these receptors[170,171], although the utility of these peptides in blocking the functions of CCN proteins has not been investigated *in vivo*.

Several clinical trials targeting CCN2 have either been carried out or are underway. A Phase I study to evaluate the safety and tolerability of FG-3019 (REF. 172) was performed in patients with mild to moderate idiopathic pulmonary fibrosis; results showed that the humanized antibody was safe and well tolerated[173]. A follow-up Phase II study is currently underway (ClinicalTrials.gov identifier: NCT01262001). Another Phase Ib study showed that FG-3019 was well tolerated in the treatment of patients with type 1 or type 2 diabetes and microalbuminuria[6]. The observation that the urinary albumin/creatinine ratio was reduced by more than 50% by the end of the treatment is particularly encouraging as it suggests that the blockade of CCN2 is associated with a decrease in albuminuria.

A similar Phase I study has also been completed in patients with type 1 or type 2 diabetes who had a more severe form of microalbuminuria and were receiving background treatment for hypertension (ClinicalTrials.gov identifier: NCT00754143). On the strength of these observations, a Phase II study was initiated in patients with type 2 diabetes and advanced kidney disease to evaluate the effect of FG-3019 on renal function and associated cardiovascular comorbidities (ClinicalTrials.gov identifier: NCT00913393). Another ongoing Phase I study is evaluating FG-3019 therapy in combination with gemcitabine (a nucleoside analogue chemotherapeutic drug) and erlotinib (a tyrosine kinase inhibitor) for patients with locally advanced or metastatic pancreatic cancer (ClinicalTrials.gov identifier: NCT01181245). In addition, a Phase II study has been initiated in patients with liver fibrosis caused by chronic hepatitis B infection in which the efficacy of FG-3019 for reversing fibrosis is being evaluated (ClinicalTrials.gov identifier: NCT01217632).

Aside from monoclonal antibodies, a 2-*O*-methoxyethyl-modified antisense oligonucleotide (EXC-001) designed to inhibit CCN2 expression has been used in several Phase II clinical trials to study its effects in controlling scarring after breast surgery (ClinicalTrials.gov identifier: NCT01037413) or abdominoplasty surgery (ClinicalTrials.gov identifiers: NCT01037985; NCT01038297). Results show a reduction in scarring with no significant drug-related adverse effects (see the 10 January 2011 press release on the Excaliard

Table 5 | **Mutations and polymorphisms associated with human diseases**

| CCN protein | Identified mutations and SNPs | Associated human diseases | Refs |
|---|---|---|---|
| CCN1 | rs3753794, rs3753793, rs2297140, rs2297141 and polymorphism found in intervening sequence 2, at the +50 position in intron 2 | Plasma HDL cholesterol levels in obese individuals | 211 |
| | rs954353 | Colorectal cancer | 212 |
| CCN2 | –945 (G→C) substitution | Susceptibility to systemic sclerosis | 162 |
| | rs9399005 | Susceptibility to systemic sclerosis | 164 |
| | rs6918698 | Susceptibility to systemic sclerosis | 163 |
| | rs9402373 and rs12526196 | Severity of hepatic fibrosis | 213 |
| | –945 GG genotype | Cardiovascular events of stroke and myocardial infarction in surviving patients who have undergone haemodialysis | 214 |
| | –447C | Higher degree of calcification in patients with symptomatic aortic stenosis | 215 |
| | –20 GG genotype | Susceptibility to nephropathy in type 1 diabetes | 216 |
| CCN3 | ND | ND | |
| CCN4 | 2364 (A→G) substitution | Prevalence of hypertension | 217 |
| | 2365 AA genotype | Spinal osteoarthritis with high endplate sclerosis | 218 |
| CCN5 | ND | ND | |
| CCN6 | Germline mutations (likely to be loss-of-function mutations) | Autosomal recessive disorder progressive pseudorheumatoid dysplasia | 160 |
| | Somatic mutations (frameshift mutations at a polyadenosine tract) | Gastrointestinal tumours from patients with a mutation in the mismatch repair pathway | 219 |
| | 84 AA genotype | Susceptibility to juvenile idiopathic arthritis | 220 |

CCN1, cysteine-rich angiogenic inducer 61 (also known as CYR61); CCN2, connective tissue growth factor (also known as CTGF); CCN3, nephroblastoma overexpressed (also known as NOV); CCN4, WNT-inducible signalling pathway protein 1 (also known as WISP1); CCN5, WNT-inducible signalling pathway protein 2 (also known as WISP2); CCN6, WNT-inducible signalling pathway protein 3 (also known as WISP3); HDL, high-density lipoprotein; ND, not determined; rs, reference SNP ID number; SNP, single nucleotide polymorphism.

Pharmaceuticals website). In addition, RXI-109, a self-delivering RNA interference compound that reduces CCN2 expression, is due to enter clinical trials (see the 7 September 2011 press release on the Galena Biopharma website). It is encouraging that no side effects have been observed in Phase I trials involving humanized monoclonal antibodies or antisense oligonucleotides targeting *CCN2*. As CCN proteins are generally downregulated in many tissues in adulthood and are highly expressed only at sites of inflammation and tissue repair, it is likely that therapeutic targeting of CCN proteins will preferentially affect the sites of pathology and have minimal impact on the normal functions of organs and tissues. Pregnancy might be an exception, as systemic targeting of certain CCN proteins may increase the risk of pre-eclampsia[174] or inhibit placentation[33].

## Conclusions and future directions

The CCN matricellular proteins have important roles in modulating inflammation and tissue repair, and they are often deregulated in a broad spectrum of pathologies that develop when these processes become chronic. Emerging studies have identified opportunities in which levels of CCN proteins in biological fluids may serve as non-invasive diagnostic or prognostic tools — for example, systemic fibrosis[69,175], diabetic nephropathy[137] and renal hypertension[136], as well as some forms of cancer[87,116,176]. The potential of monitoring CCN proteins for the non-invasive assessment of fibrosis is of particular clinical interest. Studies in various animal models have also demonstrated that targeting relevant genes encoding CCN proteins in several diseases — such as cancer, diabetic nephropathy and fibrosis of the liver, kidney and skin — could be therapeutically beneficial. Clinical trials targeting CCN2 have been initiated, and preliminary results are encouraging. We surmise that as studies on CCN proteins progress, more pathologies will be identified for which CCN proteins could serve as potential diagnostic or therapeutic targets.

Among the key challenges in future research is the ability to elucidate the specific role of CCN proteins in disease aetiology. In only a few cases has there been limited functional insight into how CCN proteins might be triggering or contributing to disease pathology. Understanding the crucial pathological role of CCN proteins and their mechanisms of action will help to identify relevant targets for therapeutic intervention as the key signalling steps in these processes are identified. As some members of the CCN protein family often have opposing roles, the expression of a specific CCN protein may be either deleterious or beneficial in a particular pathological context. Therefore, the specificity of reagents targeting members of the CCN protein family is of paramount importance. Furthermore, it is currently unclear how various CCN proteins interact to provoke antithetical effects in some contexts. Future studies that clarify how CCN proteins with opposing functions operate mechanistically could present further prospects for combinatorial therapies that simultaneously downregulate and upregulate the expression or function of rival members of the CCN protein family, thus potentially magnifying the therapeutic impact.

Although CCN proteins represent unusual opportunities as therapeutic targets in a broad spectrum of diseases (as discussed above), their therapeutic potential has so far remained relatively underexplored. Clinical trials that are currently being carried out and planned are investigating the efficacy of targeting only CCN2; other members of the CCN protein family have not yet been investigated, despite having apparent roles in many of the same pathologies as CCN2. This may be due in part to the lack of coordinated efforts to develop humanized neutralizing monoclonal antibodies targeting other members of the CCN protein family, although studies using other targeting strategies can be initiated. As the relative efficacies of targeting distinct members of the CCN protein family in the same pathologies remain to be determined, future studies that investigate the full range of CCN proteins as therapeutic targets will be of considerable interest.

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### Competing interests statement
The authors declare no competing financial interests.

### FURTHER INFORMATION
**Lester F. Lau's homepage:** http://www.uic.edu/com/bcmg/lau.htm
**ClinicalTrials.gov website:** http://clinicaltrials.gov
**Excaliard Pharmaceuticals — 10 January 2011 press release:** http://www.excaliard.com/news/PressReleaseExcaliardJanuary2011.pdf
**Galena BioPharma — 7 September 2011 press release:** http://investors.galenabiopharma.com/releasedetail.cfm?ReleaseID=603651
**The International CCN Society website:** http://www.ccnsociety.com/index.html

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**ERRATUM**

## Perspectives and opportunities for nanomedicine in the management of atherosclerosis

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The following sentence was inadvertently duplicated on page 835:

Subsequently, several other nanomedicinal therapeutics have been approved for clinical use, including an albumin-bound nanoparticle delivering paclitaxel for the treatment of breast cancer (Abraxane; Abraxis BioScience) and liposomal amphotericin B for the treatment of fungal infections (AmBisome; Astellas Pharma).

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