CCN2 (Cellular Communication Network factor 2) in the bone marrow microenvironment, normal and malignant hematopoiesis

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Abstract
CCN2, formerly termed Connective Tissue Growth Factor, is a protein belonging to the Cellular Communication Network (CCN)-family of secreted extracellular matrix-associated proteins. As a matricellular protein it is mainly considered to be active as a modifier of signaling activity of several different signaling pathways and as an orchestrator of their cross-talk. Furthermore, CCN2 and its fragments have been implicated in the regulation of a multitude of biological processes, including cell proliferation, differentiation, adhesion, migration, cell survival, apoptosis and the production of extracellular matrix products, as well as in more complex processes such as embryonic development, angiogenesis, chondrogenesis, osteogenesis, fibrosis, mechanotransduction and inflammation. Its function is complex and context dependent, depending on cell type, state of differentiation and microenvironmental context. CCN2 plays a role in many diseases, especially those associated with fibrosis, but has also been implicated in many different forms of cancer. In the bone marrow (BM), CCN2 is highly expressed in mesenchymal stem/stromal cells (MSCs). CCN2 is important for MSC function, supporting its proliferation, migration and differentiation. In addition, stromal CCN2 supports the maintenance and longtime survival of hematopoietic stem cells, and in the presence of interleukin 7, stimulates the differentiation of pro-B lymphocytes into pre-B lymphocytes. Overexpression of CCN2 is seen in the majority of B-acute lymphoblastic leukemias, especially in certain cytogenetic subgroups associated with poor outcome. In acute myeloid leukemia, CCN2 expression is increased in MSCs, which has been associated with leukemic engraftment in vivo. In this review, the complex function of CCN2 in the BM microenvironment and in normal as well as malignant hematopoiesis is discussed. In addition, an overview is given of data on the remaining CCN family members regarding normal and malignant hematopoiesis, having many similarities and some differences in their function.

Keywords CCN2 · CTGF · Connective tissue growth factor · Bone marrow · Hematopoiesis · Leukemogenesis

Introduction

The bone marrow microenvironment

The bone marrow (BM) is the most important source of blood cells in the adult. It is the primary site where hematopoietic stem cells (HSCs) reside and give rise to more restricted progenitor cells that mature to become the different specialized blood cells. Besides hematopoietic cells, the BM contains mesenchymal stem cells, various more mature mesenchymal cells such as fibroblasts, adipocytes, osteoblasts, endothelial cells and perivascular stromal cells, as well neuronal cells and extracellular matrix (ECM), which together constitute the BM microenvironment. Integrins and selectins on the cell surface of stromal and hematopoietic cells mediate cell–cell and cell–matrix interactions (Anthony and Link 2014; Lindner et al. 2010).
One of the most important functions of the BM microenvironment is the formation of so-called ‘niches’, local tissue microenvironments for the maintenance, regulation and differentiation of the HSCs (the stem cell niche) and other hematopoietic cells (Morrison and Spradling 2008). Different niches are recognized in the BM; the osteoblastic (endosteal) niche localized at the inner surface of the bone cavity where calcium levels are high, the (peri)vascular niche subdivided into a (peri)sinusoidal niche and a (peri)arteriolar niche containing high levels of stem cell factor (SCF) and CXC chemokine ligand-12 (CXCL12), the erythropoietic niche with a central macrophage, and a niche for lymphopoiesis located in the perisinusoidal region where both CXCL12 and interleukin 7 (IL-7) levels are high (Acar et al. 2015; Asada et al. 2017; Aurrand-Lions and Mancini 2018; Chasis and Mohandas 2008; Fujita et al. 2015; Ho and Méndez-Ferrer 2020; Lazzari and Butler 2018; Morrison and Scadden 2014; Pinho and Frenette 2019; Wei and Frenette 2018). Actively cycling, short-term HSCs as well as quiescent long-term HSCs are mainly localized in close proximity to vascular niches, but the different roles of the two vascular niches, arteriolar and sinusoidal, in HSC function are still not fully understood (Acar et al. 2015; Asada et al. 2017; Aurrand-Lions and Mancini 2018; Ho and Méndez-Ferrer 2020; Lazzari and Butler 2018; Wei and Frenette 2018). Mesenchymal cells, which include mesenchymal stem/progenitor cells and more differentiated mesenchymal cells such as perivascular stromal cells and osteoblasts, are important players in these niches as they secrete cytokines and other factors to support and regulate hematopoiesis (Morrison and Scadden 2014). When changed, the BM microenvironment can also support malignant hematopoiesis. Malignant niches differ from normal niches by the interaction with malignant cells. A role for malignant niche characteristics have been implicated in both myelodysplastic and myeloproliferative neoplasms as well as in lymphoid and myeloid leukemias (Arranz et al. 2014; Balderman et al. 2016; Battula et al. 2013; Blau et al. 2007; Boyerinas et al. 2013; Guerrouahen et al. 2011; Korn and Mendez-Ferrer 2017; Lazzari and Butler 2018; Li and Calvi 2017; Sangaletti et al. 2017; Schepers et al. 2013).

Homeostasis of the BM microenvironment is tightly regulated by a complex and not fully elucidated interplay between different cell types, structural ECM components and soluble factors such as cytokines, hormones, growth factors and matricellular proteins. Matricellular proteins are proteins secreted into the extracellular environment, modulating cell function and cell–matrix interactions by binding to cell-surface receptors, structural matrix proteins and other soluble matrix proteins (Bornstein 2009; Bornstein and Sage 2002). The Cellular Communication Network (CCN) family forms an important group of matricellular proteins (Chen and Lau 2009). Bork was the first to conceive the different CCN proteins as members of a (single) family (Bork 1993), that now consists of six structural related proteins.

The CCN proteins act as central mediators of mechanotransduction and play important roles in, amongst others, angiogenesis, inflammation, connective tissue deposition, and a broad range of pathological processes including fibrosis and cancer (Chaqour 2020; Leask 2020). The proteins of the CCN family might play as a team and ideally should be assessed together rather than individually (Peidl et al. 2019; Perbal 2018; Riser et al. 2009, 2010), but for most of them, little is known about their possible involvement in hematopoiesis and the BM microenvironment. Since CCN2 is by far the most studied CCN protein in this field, it will therefore be the focus of this review, and the more limited available knowledge on the other CCN proteins is included at the end.

**CCN2 protein**

Although CCN2 was originally thought to be a classical growth factor and named connective tissue growth factor (CTGF), no high-affinity classical growth factor receptor for CCN2 has been discovered (Lau 2016). Instead, CCN2 has the capacity to interact with a range of cell surface receptors, ECM macromolecules, growth factors and proteases, thereby directly or indirectly regulating cellular function, which are features common to all matricellular proteins (Bornstein 1995; Murphy-Ullrich and Sage 2014). Furthermore, all four structural domains of the CCN proteins are homologous to other ECM-associated proteins (Lau and Lam 1999). Therefore, the protein originally called CTGF is nowadays considered a matricellular protein and has been renamed CCN2 by the HUGO Gene Nomenclature Committee (Brigstock et al. 2003; Perbal et al. 2018).

CCN2 (Fig. 1) is a cysteine-rich 36–38 kDa (depending on the level of N-linked glycosylation) protein (Bradham et al. 1991; Yang et al. 1998), consisting of a secretory signal peptide and four functionally distinct and highly conserved domains/modules (Bork 1993; Holbourn et al. 2008). The N-terminal fragment of CCN2 is made up by an insulin-like growth factor binding protein (IGFBP) module and a von Willebrand factor C (VWC) module (Brigstock 1999; Lau and Lam 1999), which is linked by a hinge region to the C-terminal fragment consisting of a thrombospondin type 1 repeat (TSP1) module and a C-terminal cysteine knot (CT) module (Brigstock 1999; Lau and Lam 1999). Each module interacts with other extracellular proteins and/or proteoglycans (Perbal 2018). CCN2 is mainly secreted as an extracellular protein and has been identified in various human biological fluids (Bradham et al. 1991; Yang et al. 1998). Loss of its 2 kDa N-terminal secretory signal peptide leads to intracellular retention of the protein (Welch et al. 2015).
CCN2 function and expression

CCN2 is expressed in a variety of tissues during embryonic development, with highest levels in vascular tissues and maturing chondrocytes (Hall-Glenn et al. 2012; Ivkovic et al. 2003). In the adult, CCN2 expression can be induced in various cell types, including endothelial cells (Bradham et al. 1991; Lee et al. 2015; Yan et al. 2014), vascular smooth muscle cells (Gao et al. 2007; Ko et al. 2012; Liu et al. 2008; Rodriguez-Vita et al. 2005), chondrocytes (Nakanishi et al. 1997), fibroblasts (Grotendorst 1997; Guo et al. 2011; Holmes et al. 2003; Igarashi et al. 1993) and mesangial cells (Goppel-Streebe et al. 2001). CCN2 acts in an autocrine or paracrine fashion and its regulation and modes of action are complex and context dependent, depending on cell type, state of differentiation, and microenvironmental context (Cicha and Goppel-Streebe 2009; Guo et al. 2011).

As previously reviewed, CCN2 and its fragments have been implicated in the regulation of a multitude of biological phenomena, including cell proliferation, differentiation, adhesion, migration, cell survival, apoptosis and the production of ECM products (de Winter et al. 2008; Jun and Lau 2011; Takigawa 2018), as well as in embryonic development, angiogenesis, chondrogenesis, osteogenesis, fibrosis, mechanotransduction and inflammation (Chaour 2020; Jun and Lau 2011; Kubota and Takigawa 2013; Takigawa 2013, 2018). It should be noted, however, that at least several of these propositions have not been based on robust assays using sufficiently characterized and purified CCN2 and its fragments. As discussed by Leask, adhesion assays are probably the only robust, universally agreed-upon in vitro assays for assessing CCN activity, at least of full-length CCN proteins (Leask 2020).

CCN2 has the ability to interact with a wide variety of proteins and receptors by its different modules, and is considered to be active as a modifier of signaling activity of several different signaling pathways and as an orchestrator of their cross-talk (Leask 2020; Perbal 2018; Ramazani et al. 2018). CCN2 can regulate biological processes in various ways (Fig. 2): (1) It can bind to several cell surface receptors, thereby initiating signal transduction, (2) It can bind to growth factors, modulating their presentation and binding to cell-surface receptors, and subsequent initiation of downstream signaling pathways, (3) It has a modifying role in mediating matrix turnover by binding to (structural) ECM proteins, (4) It is involved in the regulation of the activity of cytokines and growth factors through modulation of crosstalk between signaling pathways, and (5) It has been reported to act intracellularly, after uptake into the cytosol via endocytic pathways and into the nucleus, where it may affect gene transcription (Kawata et al. 2006, 2012; Lau 2016; Ramazani et al. 2018; Takigawa 2018; Wahab et al. 2001).

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**Fig. 1** CCN2 protein structure. Schematic representation of the full length CCN2 protein, which is made up by a signal peptide and 4 protein domains, depicted by the blue cylinders. Domain 1 consists of the insulin-like growth factor binding protein (IGFBP) module and domain 2 contains the von Willebrand factor C (VWC) module, together forming the N-terminal fragment of the protein. Domain 3 consists of the thrombospondin type 1 repeat (TSP1) module and domain 4 contains the C-terminal cysteine knot (CT) module, together forming the C-terminal fragment of the protein. The N- and C-terminal fragments are joint by a hinge region. Between the different domains, multiple cleavage sites are present, were CCN2 is cleaved by proteases, plasmin, chymotrypsin and matrix metalloproteinases. Loss of the signal peptide leads to intracellular retention of the protein. The protein contains 2 glycosylation sites. The functional relevance of glycosylation is, however, still unknown. Other (not depicted) posttranscriptional and posttranslational modifications to which the protein is subject to, are splicing, regulation by miRNAs and multimerisation.
The reported binding partners of CCN2 are summarized in Table 1 and depicted in Fig. 2. It should be noted here that at least some of the interactions of CCN2 with other molecules have been studied only in ex vivo conditions under circumstances that might not be fully representative of in vivo conditions. By binding to cell surface receptors, CCN2 can alter various intracellular signaling pathways, including the ERK pathway (Aoyama et al. 2012; Lee et al.)
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2015; Rayego-Mateos et al. 2013; Yang et al. 2004), WNT pathway (Liu and Leask 2013; Mercurio et al. 2004; Rooney et al. 2011), JNK pathway (Aoyama et al. 2015; Yosimichi et al. 2006), NFkB pathway (Aoyama et al. 2015; Edwards et al. 2011) and others (Wahab et al. 2005; Yosimichi et al. 2006). Its binding to growth factors usually has an inhibitory effect: Binding to bone morphogenetic proteins (BMPs) inhibits BMP signaling (Abreu et al. 2002; Maeda et al. 2009, Nguyen et al. 2008), resulting in reduced phosphorylation of EKR and Smad 1/5/8 (Maeda et al. 2009; Mundy et al. 2014; Nguyen et al. 2008), and binding to vascular endothelial growth factor 165 (VEGF165)

Table 1: Reported binding partners of CCN2

| Factor | Abbreviation | References |
|--------|--------------|------------|
| Cell surface receptors | | |
| Integrins | | Babic et al. (1999), Ball et al. (2003), Chen et al. (2001), Gao and Brigstock (2004), Gao and Brigstock (2005), Jedasadyannama et al. (1999), Kiwanuka et al. (2013), Lau (2016), Liu et al. (2012), Rayego-Mateos et al. (2013), Schober et al. (2002) |
| Lipoprotein receptor-related protein-1 -4, and -6 | LRP-1, LRP-4, LRP-6 | Gao (2003), Kawata et al. (2012), Mercurio et al. (2004), Okhawara et al. (2020), Ren et al. 2013; Rooney et al. (2011), Segarini et al. (2001), Yang et al. (2004) |
| Neurotrophin receptors: Tropomyosin receptor kinase A and P75NTR | TrkA, P75NTR | Edwards et al. (2011), Rayego-Mateos et al. (2013), Wahab et al. (2005), Wang et al. (2010) |
| Insulin-like growth factor 2 receptor/Cation-independent mannose-6-phosphate receptor | IGF-2-R/M6P | Blalock et al. (2012) |
| Fibroblast growth factor receptor 1, 2 and 3 | FGFR-1, FGFR-2, FGFR-3 | Aoyama et al. (2012), Nishida et al. (2011b) |
| Epidermal growth factor receptor | EGFR | Rayego-Mateos et al. (2013) |
| Receptor activator of NF-kB | RANK | Aoyama et al. (2015) |
| Dendritic cell-specific transmembrane protein | DC-STAMP | Nishida et al. (2011a) |
| Osteoprotegerin | OPG | Aoyama et al. (2015) |
| Formyl peptide receptor-like 1 | FPRL1 | Lee et al. (2015) |
| Growth factors | | |
| Transforming growth factor beta | TGF-β | Abreu et al. (2002) and Khattab et al. (2015) |
| Bone morphogenetic protein 2, 4 and 7 | BMP-2, BMP-4, MBP-7 | Abreu et al. (2002), Maeda et al. (2009), Nguyen et al. (2008) |
| Platelet derived growth factor-B and -BB | PDGF-BB, PDGF-B | Khattab et al. (2015), Pi et al. (2012) |
| Vascular endothelial growth factors-A and -C | VEGF-A, VEGF-C | Heroult et al. (2004), Inoki et al. (2002), Khattab et al. (2015), Kimashi et al. (2017) |
| Fibroblast growth factor 2 | FGF2 | Nishida et al. (2011b) |
| Insulin-like growth factors 1 and 2 | IGF1/2 | Kim et al. (1997), Lam et al. (2003) |
| Growth-differentiation factor 5 | GDF-5 | Khattab et al. (2015) |
| Structural matrix proteins | | |
| Perlecan | | Nishida et al. (2003) |
| Aggrecan | | Aoyama et al. (2009) |
| Fibronectin | | Hoshijima et al. (2006), Pi et al. (2008) |
| Decorin | | Vial et al. (2011) |
| Other | | |
| Heparin | | Ball et al. (2003), Brigestock et al. (1997), Frazier et al. (1996), Gao (2003), Kirieva et al. (1997) |
| Heparan sulphate proteoglycans (cell surface and matrix associated) | HSPGs | Ball et al. (2003), Chen et al. (2001), Gao (2003), Gao and Brigestock (2004), Kirieva et al. (1997), Nishida et al. (2003) |
| CCN2 | | Hoshijima et al. (2012) |
| CCN3 | | Hoshijima et al. (2012) |
| Wnt inhibitory factor 1 (Wif-1) | Wif-1 | Surmann-Schmitt et al. (2012) |
| Slit guidance ligand 3 | Slit-3 | Pi et al. (2012) |
| von Willebrand factor | vWF | Pi et al. (2012) |
inhibits its angiogenic activity by interrupting binding to its major receptor VEGFR-2 (Heroult et al. 2004; Inoki et al. 2002). Binding of the CT module, but not full length CCN2, to fibroblast growth factor 2 (FGF2) inhibits its binding to fibroblast growth factor receptor-1 (FGFR1) and thereby its activation (Nishida et al. 2011b). Furthermore, there are indications that CCN2 has an inhibitory effect on insulin-like growth factor (IGF) signaling and WNT signaling (Smerdel-Ramoya et al. 2008). Binding of CCN2 to transforming growth factor beta (TGF-β) has been reported to enhance TGF-β signaling (Abreu et al. 2002). This interesting notion has, however, not been reproduced in more recent literature.

In addition to its direct effects on cell surface receptors and growth factors, CCN2 can increase the level of matrix metalloproteinases (MMPs), a large family of enzymes playing a central role in the ECM by the degradation of specific ECM components and cleavage of growth factors and their binding proteins, by upregulating their gene expression in fibroblasts and endothelial cells (Chen et al. 2001; Fan and Karnovsky 2002; Kondo et al. 2002). On the other hand, CCN2 itself is a substrate of several MMPs, by which it can be cleaved in the hinge region (Dean et al. 2007; Hashimoto et al. 2002; Tam et al. 2004). For an overview and more detail of CCN2 binding partners and intracellular signaling pathways we like to refer to several recent reviews (Chaqour 2020; Lau 2016; Ramazani et al. 2018; Takigawa 2018).

**Regulation of CCN2 expression**

TGF-β is a powerful and well-known inducer of CCN2 trans- 
scription (Brunner et al. 1991; Grotendorst et al. 1996; Holmes et al. 2001; Igarashi et al. 1993; Yang et al. 1998), but many other factors, summarized in Table 2 and depicted in Fig. 2, can also directly or indirectly induce CCN2 mRNA expression through the initiation of signaling pathways and the activation of transcription factors, as previously reviewed (Chaqour 2020; Jun and Lau 2011; Ramazani et al. 2018; Takigawa 2018). For example, on mechanical stress, the transcriptional regulators YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) induce CCN2 gene transcription (Dupont et al. 2011; Nagasawa-Masuda and Terai 2017; Preisser et al. 2016; Raghunathan et al. 2014). FAK (focal adhesion factor) is an important factor in mechanotransduction, which controls the nuclear translocation and activation of YAP and subsequent CCN2 gene expression in response to mechanical activation (Lachowski et al. 2018). In addition, CCN2 can induce its own expression by auto-induction, resulting in a positive feedback loop (Riser et al. 2000; Shimo et al. 2001b). Other factors, summarized in Table 3 and depicted in Fig. 2, can directly or indirectly inhibit CCN2 expression. For involvement of specific regulatory elements in the CCN2 gene we refer to Leask et al. (2009). Furthermore, CCN2 expression can be regulated at the posttranscriptional and posttranslational level by various factors, including VEGF (Kondo et al. 2006), hypoxia (Kondo et al. 2002), tumor necrosis factor α (TNF-α), interferon gamma (IFN-γ) (Cooker et al. 2007; Laug et al. 2012), and a host of different microRNAs (miRNAs) (Cai et al. 2018; Che et al. 2019; Chen et al. 2016, 2019; Ernst et al. 2010; Fox et al. 2013; He et al. 2017; Mu et al. 2016; Qiao et al. 2017; Sun et al. 2016). For example, CCN2 and miRNA-21 are components of a positive feedback loop in pancreatic stellate cells, that may serve as an amplification mechanism for enhanced collagen production (Charrier et al. 2014a). On the other hand, CCN2 can increase the expression of miRNA-302, which targets the TGFβ type II receptor and thereby decreases its expression, constituting a negative feedback loop (Faherty et al. 2012).

**CCN2 and disease**

CCN2 has been implicated in the pathophysiology of many diseases; increased CCN2 expression has been demonstrated in the tissue of a range of diseases that are accompanied by fibrosis such as fibrotic lung diseases, scleroderma, chronic pancreatitis, renal fibrosis, liver cirrhosis, myocardial infarction, and Crohn’s disease (Abou-Shady et al. 2000; di Mola et al. 1999, 2004; Ito et al. 1998; Ohnishi et al. 1998; Pan et al. 2001; Shi-wen et al. 2000), as well as in diabetic retinopathy and in the osteoarthritic cartilage of patients with osteoarthritis (Omoto et al. 2004; Tikellis et al. 2004). In addition, increased levels of CCN2 cleavage products have been demonstrated in human extracellular fluids, including plasma, urine, dermal interstitial fluid and vitreous fluid, of patients with fibrotic diseases, correlating with the severity of fibrosis (Leask et al. 2009). Furthermore, increased CCN2 plasma levels have been associated with cardiac dysfunction and increased risk of cardiovascular events in patients with atherosclerotic disease (Behnes et al. 2014; Gerritsen et al. 2016; Koitabashi et al. 2009), and altered CCN2 expression has been demonstrated in more than 25 different forms of cancer, with deregulation of CCN2 expression usually correlating with worse clinical outcome (Wells et al. 2015). Besides altered CCN2 expression in tumor cells, elevated CCN2 expression in stromal fibroblasts is implicated in the desmoplastic response in various cancer types, and CTGF expression in stromal cells can advance tumor growth or promote invasion as reviewed by Wells et al. (2015).
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### Table 2  Reported (direct or indirect) inducers of CCN2 mRNA expression

| Factor                             | References                                                                 |
|------------------------------------|---------------------------------------------------------------------------|
| **Growth factors**                 |                                                                           |
| TGF-β                              | Gao and Brigstock (2005), Goppelt-Struebe et al. (2001), Grotendorst et al. (1996), Holmes et al. (2003), Igarashi et al. (1993), Nakanishi et al. (1997), Riser et al. (2000), Wunderlich et al. (2000) |
| PDGF                               | Gao and Brigstock (2005)                                                  |
| Epidermal growth factor (EGF)      | Wunderlich et al. (2000)                                                  |
| Basic fibroblast growth factor (bFGF)| Wunderlich et al. (2000)                                                  |
| BMP-2                              | Nakanishi et al. (1997)                                                   |
| VEGF                               | Lee et al. (2015), Suzuma et al. (2000)                                   |
| **Hormones**                       |                                                                           |
| Steroids                           | Dammeier et al. (1998), Kubota et al. (2003), Moritani et al. (2005), Okada et al. (2006), Pereira et al. (2000) |
| Angiotensin II                     | Gao et al. (2007), Gu et al. (2012)                                      |
| Endothelin-1                       | Kemp et al. (2004), Rodriguez-Vita et al. (2005), Shi-Wen et al. (2007) |
| Aldosteron                         | Lee et al. (2004)                                                        |
| **Coagulation factors**            |                                                                           |
| Thrombin                           | Bai et al. (2013), Chambers et al. (2000)                                |
| Factor Xa                          | Chambers et al. (2000)                                                   |
| **Glucose metabolism related**     |                                                                           |
| Glucose                            | Lam et al. (2003), Murphy et al. (1999), Paradis et al. (2001), Riser et al. (2000) |
| Glycolysis—via adenosine triphosphate (ATP) | Akashi et al. (2018)                                                        |
| Advanced glycosylation end products| Twigg et al. (2001)                                                      |
| Insulin                            | Paradis et al. (2001)                                                    |
| **Cytokines**                      |                                                                           |
| Tumor necrosis factor alpha (TNF-α) | Cooker et al. (2007)                                                    |
| **Other**                          |                                                                           |
| Bioactive lipids                   | Chowdhury and Chaqour (2004), Goppelt-Struebe et al. (2001), Muehlich et al. (2004) |
| Ethanol and acetaldehyde           | Charrier et al. (2014b), Gao and Brigstock (2005)                        |
| UV-light                           | Kafi et al. (2004), Quan et al. (2009)                                   |
| Mechanical stress (shear and cell stretch) | Guo et al. (2011), Honjo et al. (2012), Kessler et al. (2001), Riser et al. (2000), Schild and Trueb (2002), Schild and Trueb (2004), Wong et al. (2003), Yamashiro et al. (2001) |
| Hypoxia—via hypoxia-inducible-factor-1 (HIF-1α) | Higgins et al. (2004), Kondo et al. (2002), Shimo et al. (2001a), Valle-Tenney et al. (2020) |
| Nitric oxide (NO)                  | Tsai et al. (2014)                                                       |
| CCN2                               | Bradham et al. (1991), Kawaki et al. (2008), Parada et al. (2013), Riser et al. (2000), Shimo et al. (2001b) |
| Insulin-like growth factor-binding protein 5 (IGFBP-5) | Nguyen et al. (2018)                                                        |

*TNF-α has also been reported to inhibit CCN2 mRNA expression (see Table 3)*

### CCN2 and the normal BM microenvironment (Fig. 3)

#### CCN2 expression and function in mesenchymal stem and stromal cells

Mesenchymal stem cells are essential for the maintenance of the BM microenvironment; they can self-renew and have the capacity to differentiate into other mesenchymal cell types, including chondrocytes, adipocytes, fibroblasts and osteoblasts. They also stimulate the production of the ECM (Huang et al. 2016), and maintain hematopoiesis by the secretion of cytokines that stimulate proliferation of hematopoietic progenitor cells (Huang et al. 2016). Mesenchymal stem cells are rare, constituting only 1 in 3.4×10⁴ nucleated BM cells (Wexler et al. 2003). As it is difficult to identify mesenchymal stem cells on a per cell basis, mesenchymal stem cell-enriched cell populations of both true mesenchymal stem cells and more differentiated mesenchymal stromal cells are used in most experimental settings (Lindner et al. 2016).
Table 3  Reported (direct or indirect) inhibitors of CCN2 mRNA expression

| Factor                                | References                                      |
|---------------------------------------|------------------------------------------------|
| **Growth factors**                    |                                                |
| Hepatocyte growth factor              | Inoue et al. (2003), Kroening et al. (2009)    |
| **Cytokines**                         |                                                |
| Tumor necrosis factor alpha (TNF-α)  | Abraham et al. (2000), Dammeier et al. (1998), |
|                                       | Laug et al. (2012), Lin et al. (1998), Moritani |
|                                       | et al. (2005)                                   |
| Interferon-gamma (IFN-γ)              | Laug et al. (2012)                              |
| Interleukin 1 beta (IL-1β)            | Masuko et al. (2010)                            |
| **Elevators of cyclic adenosine monophosphate (cAMP) levels** |                                        |
| Cholera toxin                         | Duncan et al. (1999), Kothapalli et al. (1998), |
| Prostaglandin E2                      | Masuko et al. (2010), Ricupero et al. (1999)    |
| **Other**                             |                                                |
| CCN3                                  | Kawaki et al. (2008)                            |
| Insulin-like growth factor binding protein-4 (IGFBP-4) | Su et al. (2019)                |

“TNF-α has also been reported to induce CCN2 mRNA expression (see Table 2)

Fig. 3  CCN2 in the bone marrow microenvironment. CCN2 mRNA, depicted by , is present in different bone marrow (BM) mesenchymal cells, including endothelial cells, osteoblasts, adipocytes and fibroblasts, with highest levels ( ) reported in mesenchymal stem/stromal cell (MSC) and CXCL12-abundant reticular (CAR) cells. CCN2 exerts different actions in the BM. a In the presence of interleukin 7 (IL-7), CCN2 promotes pro-B cell to pre-B cell differentiation. b CCN2 produced by MSCs affects the (long-term) qualities of hematopoietic stem cells (HSCs). HSCs, in turn, upregulate CCN2 expression by MSCs. c CCN2 enhances the differentiation of MSCs into endothelial cells, osteoblast and fibroblasts, but has an inhibitory effect on the differentiation of MSCs into adipocytes. d CCN2 might induce the production of the ECM proteins collagen type I and type III, fibronectin, decorin, TGFβ-2 and lysyl oxidase by fibroblast. e CCN2 binds to fibronectin, perlecan and decorin, known constituents of the BM extracellular matrix. The effects hereof in the BM are yet unknown.

The mesenchymal stem and stromal cells from the cited studies are therefore further referred to as mesenchymal stem/stromal cells (MSCs).

BM-derived MSCs show high expression of CCN2 mRNA (Battula et al. 2013, 2017; Cheung et al. 2014; Djouad et al. 2007; Igarashi et al. 2007; Ren et al. 2011;
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Schutze et al. 2005; Shinde et al. 2014), which can be induced in vitro by Wnt3a and BMPs (Luo et al. 2004). CCN2 is important for MSC function as indicated by several in vitro and mouse studies. Although cell numbers and proportions of MSCs isolated from the BM of newborn CCN2-knockout mice were unchanged compared with those from wild-type (WT) mice (Cheung et al. 2014), MSCs from tissues of CCN2 homozygous knockout mice were not able to generate colony-forming unit fibroblast-like cells in vitro (Battula et al. 2013), implying a functional defect. Indeed, a role for CCN2 in MSC cell growth has been demonstrated, as CCN2 is shown to enhance proliferation and inhibit apoptosis of MSCs in vitro (Battula et al. 2013; Wang et al. 2009; Wells et al. 2016). In addition, CCN2 enhances MSC cell migration and recruitment, shown by either the addition of exogenous CCN2 or through plasmid induced expression of CCN2 (Luo et al. 2004; Wang et al. 2009). Furthermore, CCN2 affects MSC differentiation. Although knockdown of endogenous CCN2 expression in MSCs did not affect their capacity to differentiate into osteoblasts or chondrocytes (Battula et al. 2013), increase of CCN2 by either exposure to recombinant CCN2 (rCCN2) or transfection of CCN2-expressing plasmids did enhance the differentiation of human BM MSCs into osteoblasts and chondrocytes as well as fibroblasts in culture (Lee et al. 2010; Nishida et al. 2004; Wang et al. 2009). MSCs can also be differentiated into myofibroblasts by the addition of rCCN2, but only when stimulated subsequently with TGF-β (Lee et al. 2010; Wang et al. 2009). On the other hand, knockdown of CCN2 expression in MSCs enhanced adipocytic differentiation (Battula et al. 2013), suggesting an inhibitory effect of CCN2 on adipogenesis. When MSCs differentiate into these progenitor cells with lineage commitment, CCN2 expression was shown to decrease (Luo et al. 2004; Schutze et al. 2005).

### CCN2 expression and function in endothelial and perivascular cells

Endothelial cells and perivascular stromal cells are part of the (peri)vascular niche and contribute to the microenvironment by the production of SCF and other growth factors, cytokines, chemokines and adhesion molecules such as E-selectin and CXCL12 (Ding and Morrison 2013; Saccchetti et al. 2007; Sipkins et al. 2005; Sugiyama et al. 2006; Winkler et al. 2012; Zhao et al. 2019).

CCN2 has been shown to induce neovascularisation and to promote the adhesion, migration, proliferation and survival of vascular endothelial cells in vitro (Babic et al. 1999; Shimo et al. 1998, 1999). CCN2 mRNA expression in endothelial cells increases in vitro by the addition of bioactive lipids such as sphingosine-1-phosphate and lysophosphatidic acid (Muehlich et al. 2004). Also, addition of freshly isolated platelets to endothelial cells upregulates their CCN2 mRNA expression (Muehlich et al. 2004), possibly due to the release of constituents of lipoproteins, TGF-β and other CCN2-inducing compounds by platelets.

As expected, CCN2 mRNA is present in endothelial cells derived from the BM as well (Cheung et al. 2014). The perivascular region of the BM contains a heterogeneous population of stromal cells characterized by very high CXCL12 expression, including the CXCL12-abundant reticular (CAR) cells, which are mesenchymal progenitor cells important for the maintenance of both HSC and B-cells (Eltoukhy et al. 2016; Sugiyama et al. 2006). These CAR-cells were shown to have the highest expression of CCN2 mRNA of all investigated BM stromal cells (Cheung et al. 2014). The cell numbers and the proportions of endothelial cells and CAR cells isolated from CCN2+/− and CCN2−/− newborn mice are unchanged compared with those from WT mice (Cheung et al. 2014), but their function has not been studied in these knock-out models. The role of CCN2 in the perivascular niche thus remains to be established.

### Osteolineage cells contribute to the BM microenvironment by secreting factors such as granulocyte colony stimulating factor (G-CSF) (Taichman and Emerson 1994), thrombopoietin (TPO) (Yoshihara et al. 2007), and CXCL12 (Jung et al. 2006), although their effect on hematopoiesis is not fully determined (Ho and Méndez-Ferrer 2020). Osteolineage cells also express CCN2 (Luo et al. 2004; Safadi et al. 2003; Xu et al. 2000).

CCN2 is long known for its importance in endochondral ossification, and crucial for normal development, growth and regeneration of bone (Ikovic et al. 2003; Kanyama et al. 2003; Lambi et al. 2012; Xu et al. 2000; Yamada et al. 2005). Its mRNA and protein expression have been detected in normal long bones during the period of growth and (re) modeling, and have been located to osteoblasts lining metaphyseal trabeculae and those lining active, osteogenic surfaces in fracture callus (Safadi et al. 2003). CCN2 null mice show severe skeletal abnormalities involving both cartilage and bone, and die shortly after birth due to respiratory distress and cyanosis caused by severe rib cage malformations as well as by disruption of normal lung development due to reduced proliferation and increased apoptosis of pulmonary cells (Baguma-Nibasheka and Kablar 2008; Cheung et al. 2014; Falke et al. 2020; Ikovic et al. 2003; Lambi et al. 2012; Yamada et al. 2005).

As discussed above, increased CCN2 expression enhances the differentiation of human BM MSCs into osteoblasts (Wang et al. 2009). The osteoblast lineage-specific differentiation of MSCs is at least in part regulated by Wnt signaling and osteogenic BMPs, especially BMP-9 (Luo et al. 2004). Of note, in MSCs stimulated by Wnt3a and...
osteogenic BMPs, CCN2 was among the most significantly up-regulated genes (Luo et al. 2004). Prolonged CCN2 expression, in turn, inhibited both Wnt3a and BMP induced osteogenic differentiation, suggesting a regulatory role for CCN2 in normal osteogenesis (Luo et al. 2004).

The differentiation of pre-osteoblasts into bone forming osteoblasts encompasses the following phases in development: 1. proliferation, 2. maturation and extra-cellular matrix synthesis, and 3. matrix mineralization (Neve et al. 2011). A bimodal pattern of CCN2 mRNA levels is observed in primary osteoblast cultures; high CCN2 levels during the proliferative phase of early osteoblast progenitor cells (pre-osteoblasts), diminished CCN2 expression as the progenitor cells show terminal differentiation towards committed osteoblasts (Luo et al. 2004; Xu et al. 2000), and again high CCN2 mRNA levels during the mineralization phase (Safadi et al. 2003). Several studies showed that addition of rCCN2 increases the proliferation as well as mineralization of osteoblasts (Nishida et al. 2000; Safadi et al. 2003; Wang et al. 2009). Furthermore, delivery of rCCN2 into the femoral marrow cavity induced osteogenesis in vivo, which was associated with increased angiogenesis (Safadi et al. 2003; Wang et al. 2009).

Thus, CCN2 expression is important in the proliferation and differentiation of BM osteoblasts and is thereby at least indirectly of importance for the formation and maintenance of the BM microenvironment. A direct effect of CCN2 expression by osteoblasts on hematopoiesis still needs to be determined.

**CCN2 expression and function in adipocytes**

Several studies indicate a regulatory role of adipocytes in hematopoiesis, although their effect might be context-dependent. Some studies indicate an inhibitory effect of BM adipocytes on hematopoiesis, as adipocyte-rich marrow spaces in mice contain less HSCs and hematopoietic progenitors cells, and adipocytes inhibit hematopoietic recovery (Ambrosi et al. 2017; Naveiras et al. 2009; Zhu et al. 2013). Another study, in contrast, shows that BM adipocytes express high levels of SCF and have a stimulatory effect on hematopoiesis, increasing hematopoietic recovery after irradiation (Zhou et al. 2017). The latter, however, seems location dependent as adipocytes in long bones promote hematopoietic recovery after irradiation, while those in the caudal vertebrae inhibit hematopoietic recovery, despite SCF production (Zhou et al. 2017). The factors responsible for these disparate observations remain to be identified.

The effect of CCN2 on adipogenesis seems inhibitory; when CCN2 is knocked down in MSCs, they differentiate into adipocytes at a six fold higher rate (Battula et al. 2013). Furthermore, when CCN2 knockdown MSCs are used to form humanized extramedullary bone, this contains less cortical bone and more adipose-like marrow tissue when compared with that derived from normal MSCs (Battula et al. 2013). The possible impact hereof on hematopoiesis still remains to be elucidated.

**CCN2 expression and function in the extracellular matrix**

The ECM is a fibrillar basement network that plays a key role in cell proliferation, differentiation and migration, as well as in interactions between cells (Midwood et al. 2004). The BM ECM is composed of structural and non-structural (soluble) proteins. The structural matrix proteins include collagens, proteoglycans and other glycoproteins, the most abundant being collagens I-XI, fibronectin, laminin, tenascin, thrombospondin and elastin (Klamer and Voermans 2014). The soluble ECM proteins include growth factors, cytokines, hormones and matricellular proteins, including those of the CCN family.

CCN2 is expressed by various BM stromal cells, which include mesenchymal stem cells as well as more differentiated mesenchymal cells such as osteoblasts and CAR cells (Battula et al. 2013, 2017; Cheung et al. 2014; Igarashi et al. 2007; Istvanffy et al. 2015; Luo et al. 2004; Ren et al. 2011; Safadi et al. 2003; Schutz et al. 2005; Xu et al. 2000). Furthermore, BM stromal cells show a high level of CCN2 binding through the low density lipoprotein receptor-related protein-1 (LRP1) (Segarini et al. 2001), a well-known binding partner of CCN2. CCN2 is expected to bind many other factors in the BM as it contains many known binding partners of CCN2, such as integrins, heparan sulphate proteoglycans and growth factors. CCN2 might also induce the production of BM ECM proteins, as one study reports that BM stromal cells incubated with recombinant human CCN2 (rhCCN2) show increased expression of genes associated with ECM synthesis, including collagen type I and type III, lysyl oxidase, fibronectin, decorin and TGFβ-2 (Wells et al. 2016). It should be noted here that, typically, it has been difficult to show such activity of rCCN2 per se in other experiments.

Thus, as a matricellular protein, CCN2 can be expected to regulate intercellular signaling in the BM ECM. Furthermore, the relevance of ECM-characteristics for HSC biology and blood cell maturation underscores that CCN2 regulation might indirectly affect hematopoiesis through ECM-modification (Durand et al. 2018; Ho and Méndez-Ferrer 2020; Klamer and Voermans 2014).
CCN2 and normal hematopoiesis

**CCN2 expression and function in hematopoietic stem cells, myelopoiesis and lymphopoiesis**

CCN2 has not been detected in HSCs, and transplant studies showed that HSCs of CCN2−/− or WT neonatal mice transplanted into WT recipients had similar HSC properties after transplantation, indicating that CCN2 indeed has minimal cell-autonomous effects in HSCs (Cheung et al. 2014). HSCs, however, are able to upregulate CCN2 expression in BM stromal cells (Istvanffy et al. 2015) and there is substantial evidence that stromal CCN2 does affect hematopoiesis.

The effects of stromal CCN2 on hematopoiesis have been investigated by in vitro and in vivo studies. Murine HSCs co-cultured on stroma with decreased CCN2 protein content (shCCN2 stroma) showed reduced colony formation with increased number of hematopoietic stem and progenitor cells in G0 phase and senescence, and delayed time to first cell division, indicating that stromal CCN2 supports the growth and proliferation of HSCs and hematopoietic progenitor cells (Istvanffy et al. 2015). Stromal CCN2 seems to regulate the G0/G1 transition in murine HSCs by concerted action on TGF-β and WNT signaling pathways (Istvanffy et al. 2015). Furthermore, HSCs cultured on shCCN2 stroma and subsequently transplanted into recipient WT mice in a competitive setting, showed normal initial engraftment, but at later time points (10 and 16 weeks), there was a significant decline of myeloid and B-lymphoid but not T-lymphoid engraftment (Istvanffy et al. 2015). Adding rCCN2 to these cultures partly compensated for the diminished CCN2 production by stromal cells, significantly enhancing both the number of B- and T-cells, whereas the number of myeloid cells did not change (Istvanffy et al. 2015). In addition, when the donor HSCs isolated from primary recipients were again transplanted in equal numbers into secondary recipients, none of the secondary recipients of HSCs from shCCN2 stromal co-cultures engrafted more than 1% in the peripheral blood, BM, and spleen in contrast to control co-cultures (Istvanffy et al. 2015). Thus, co-culturing HSCs with CCN2-deficient stroma affects HSC properties in mice, with stromal CCN2 supporting HSC maintenance and longtime survival as well as supporting both myelopoiesis and B-lymphopoiesis.

CCN2 involvement in myelopoiesis and B-lymphopoiesis is also demonstrated by another study, showing that myeloid cell numbers in newborn CCN2−/− mice were decreased in the liver, although unaltered in BM and spleen, whereas B-cell numbers in the same mice were increased in the liver, while decreased in BM and spleen (Cheung et al. 2014). T-cells were the same in all the compartments compared with WT mice (Cheung et al. 2014). The opposite effects of CCN2 on myelopoiesis and B-lymphopoiesis in BM and spleen versus liver might relate to differences in microenvironmental context in these compartments, although this has not been further explored. In the associated transplant study, the authors found similar results for B-cells; mice receiving liver-derived cells containing high numbers of HSCs, showed significantly decreased numbers of B-cells in both BM and spleen when these cells were derived from CCN2−/− neonatal mice compared with that from WT mice, indicating that presence of CCN2 in the microenvironment of developing HSCs supports B-lymphopoiesis in BM and spleen (Cheung et al. 2014). As for the lower numbers of B-cells in the BM of mice receiving CCN2−/− cells, pre-B and later differentiation stages were herein most affected, while pro-B populations remained unchanged and overall B-cell function was not affected (Cheung et al. 2014). This effect on B-lymphopoiesis is supported by in vitro studies, showing that CCN2, in the presence of IL-7, could potentiate B-cell proliferation and promote pro-B to pre-B cell differentiation, but not the further differentiation into slgM + B cells (Cheung et al. 2014). In contrast to B-cells, the myeloid population in the BM of recipients transplanted with cells from CCN2−/− mice was more abundant, which points to a possible inhibitory effect of CCN2 on myelopoiesis in this compartment (Cheung et al. 2014). The apparent discrepancy with the aforementioned study by Istvanffy et al., reporting that HSCs cultured on shCCN2 stroma transplanted into recipient WT mice showed a decline of myeloid engraftment, suggesting a supportive effect of stromal CCN2 on myelopoiesis (Istvanffy et al. 2015), might relate to the differences in experimental set up.

In all, it can be concluded that stromal/environmental CCN2 is important for maintenance and longtime survival of HSCs and affects both myelopoiesis and B-lymphopoiesis. The effect on the latter two is likely to be highly dependent on the local microenvironment of the different tissue compartments and the precise effect of CCN2 on especially the myelopoiesis needs to be further determined.

**CCN2 expression in peripheral blood, megakaryopoiesis and erythropoiesis**

Normal mononuclear cells derived from peripheral blood show very low to undetectable CCN2 mRNA levels (Kim et al. 1997; Sala-Torra et al. 2007; Vorwerk et al. 2000). Plasma and serum mainly contain the N-terminal fragment of CCN2, while the full-length protein is abundant in platelets and released upon platelet stimulation (Cicha et al. 2004; Kubota et al. 2004; Miyazaki et al. 2010; Roestenberg et al. 2015).
increased CCN2 mRNA expression compared with control leukemia. Lymphoblast from both pediatric and adult B-acute et al. 2010), although immunohistochemical staining indicated strong expression of CCN2 in megakaryocytes in vivo (Astrom et al. 2015; Cicha et al. 2004; Sumiyoshi et al. 2010), although immunohistochemical staining indicated strong expression of CCN2 in megakaryocytes of primary myelofibrosis and in a subpopulation of megakaryocytes in patients with X-linked thrombocytopenia with thalassemia (Astrom et al. 2015). Thus, platelets are likely to take up CCN2 from the environment via endocytosis, similar to other molecules (Escolar et al. 2008), which is supported by the observation that human platelets are able to absorb exogenous CCN2 in vitro (Sumiyoshi et al. 2010). Possible receptors for endocytosis of CCN2 by platelets are LRPI and integrin αIIbβ3, which are both known to bind CCN2 and to mediate endocytosis of their ligands (Jedsadayanmata et al. 1999; Kawata et al. 2006, 2012). The source of this platelet CCN2 is hypothesized to come from mesenchymal cells in the microenvironment, including chondrocytes and stromal fibroblasts. The transcription factor Myeloid Zinc Finger 1 (MZF1) can directly regulate CCN2 gene expression of BM stromal cells by binding to its promoter (Piszczatowski et al. 2015), and in vitro treatment of stromal fibroblasts with either vitamin A or D activates the MZF1 pathway, which increases CCN2 production and results in enhanced loading of CCN2 into developing platelets (Rozado et al. 2014). Whether other cells and factors are involved in CCN2 loading of platelets is undetermined.

To our knowledge, no data are available on the role of CCN2 in erythropoiesis.

CCN2 and malignant hematopoiesis

CCN2 has been implicated in more than 25 different forms of cancer, mostly based on correlations (either positively or negatively) with clinical outcome (Wells et al. 2015). Altered CCN2 expression has been reported in tumor cells as well as in supporting stromal cells (Wells et al. 2015).

Several studies have investigated the role of CCN2 in leukemia. Lymphoblast from both pediatric and adult B-acute lymphoblastic leukemias (B-ALL) show moderate to highly increased CCN2 mRNA expression compared with control cells (often CD34 positive cells, CD19+igM- cells or mononuclear cells) in the majority (60–80%) of cases (Boag et al. 2007; Sala-Torra et al. 2007; Vorwerk et al. 2000, 2002). MSCs isolated from the BM of acute myeloid leukemia (AML)-bearing mice showed increased CCN2 expression compared with MSCs from control mice (Battula et al. 2017). CCN2 expression has occasionally been described in chronic myeloid leukemia (CML) cells (Vorwerk et al. 2000), although this has not been confirmed by later studies. CCN2 gene amplification or mutations have not been reported.

CCN2 expression, effect and regulation in acute lymphoblastic leukemias

CCN2 expression and prognostic effect in ALL

High CCN2 mRNA expression is frequently observed in the lymphoblasts of B-ALL, but rarely in T-ALL (Advani et al. 2010; Boag et al. 2007; Gandemer et al. 2007; Kang et al. 2010; Lu et al. 2014; Sala-Torra et al. 2007; Tesfai et al. 2012; Vorwerk et al. 2002, 2000; Welch et al. 2013, 2015). It could be hypothesized that this is related to the fact that CCN2 plays a role in normal B-cell development while no effects in T-cell development have been reported (Cheung et al. 2014).

Several studies investigated the prognostic effect of CCN2 expression in pediatric and adult B-ALL. In pediatric B-ALL, increased CCN2 expression has been associated with certain cytogenetic subgroups; B-ALL with BCR-ABL, ETV6-RUNX1 (TEL-AML1) or translocations of MLL showed high CCN2 expression (Boag et al. 2007; Gandemer et al. 2007; Tesfai et al. 2012), while those with hypodiploidy showed low CCN2 expression, and B-ALL with an E2A-PBX1 translocation showed hardly any CCN2 expression (Boag et al. 2007). Thus, with the exception of ETV6-RUNX1, high CCN2 expression is associated with poor prognostic cytogenetics, and low/no CCN2 expression with favorable cytogenetics. In addition, high CCN2 gene expression was part of the high risk profile in a study on pediatric ALL patients with high risk features (Kang et al. 2010). This study used a 38-gene expression classifier predictive of relapse-free survival (RFS) to distinguish 2 groups, one with low relapse risk (81% 4-year RFS) and one with high relapse risk (50% 4-year RFS). Patients with very high-risk features (BCR-ABL1 or hypodiploidy) were excluded, as well as those with low-risk features (trisomies of chromosomes 4 or 10; t[12;21](ETV6-RUNX1)) unless they had central nervous system disease or testicular localization. In an earlier study on pediatric B-ALL, CCN2 expression of lymphoblasts at diagnosis was not found to be predictive of relapse, as the same number of patients with a relapse had CCN2 expressing lymphoblasts at diagnosis.
as those in continued remission (Vorwerk et al. 2002). This study, however, differed from the later published study in that it did not select for high-risk patients. Furthermore, it merely looked at absence or presence of CCN2 expression without taking the level of CCN2 expression into account. As discussed above, low CCN2 expression has been associated with favorable cytogenetics. Therefore, the level of CCN2 expression, and not merely its presence or absence, seems to be important for its prognostic value.

In adult B-ALL, higher CCN2 expression levels have also been associated with worsening of overall survival (Advani et al. 2010; Sala-Torra et al. 2007). In a study on 79 adult ALL specimens, a higher CCN2 expression level in blood or BM lymphoblasts was an independent negative predictor of survival in a multivariate proportional hazards model and correlated with the percentage of CD34 expressing blasts, although there was no correlation between CCN2 expression levels and rate of complete remission or resistant disease (Sala-Torra et al. 2007). There were also no significant differences in CCN2 expression when analyzed according to sex, age, French–American–British (FAB) classification, SWOG performance status, white blood cell counts, and number of blasts in peripheral blood or BM (Sala-Torra et al. 2007). Similar findings were reported in a smaller study on 33 adult ALL patients with relapsed or refractory disease; CCN2 expression was not predictive for complete remission rate nor for resistant disease, but there was a trend for patients with higher expression of CCN2 in circulating lymphoblasts to have an inferior overall survival (Advani et al. 2010).

Several in vitro and in vivo studies support the notion that CCN2 has a pro-leukemic effect in B-ALL. In vitro, knockdown of CCN2 in B-ALL cell lines reduces leukemia cell growth due to reduced proliferation as well as increased apoptosis (Lu et al. 2014; Wells et al. 2016). The reduced proliferation is likely due to inhibition of the G_{1}/S transition, associated with decreased levels of phospho-AKT and increased levels of p27, whereas the increased apoptosis is associated with increased levels of the pro-apoptotic BCL-2 family protein BIM (Lu et al. 2014). In vivo, mice injected with genetically engineered B-ALL cells with overexpressed CCN2 showed reduced survival (Wells et al. 2016). Mice injected with B-ALL with knocked down CCN2 showed less engraftment in the BM compared with mice transplanted with control cells (B-ALL cells with empty vector) (Wells et al. 2016). Thus, reduced survival associated with elevated CCN2 expression seems related to increased engraftment, proliferation, and apoptosis resistance (Wells et al. 2016).

B-ALL cells can also secrete CCN2 (Boag et al. 2007; Welch et al. 2015; Wells et al. 2016), and addition of rhCCN2 promotes adhesion of B-ALL cells to stromal cells in vitro, which induces them to overexpress genes associated with cell cycle, intracellular transport and ECM synthesis (Wells et al. 2016). Therefore, CCN2 secreted by B-ALL cells might also enhance leukemia engraftment due to its modifying effects on the microenvironment and ECM interactions (Wells et al. 2016).

Seemingly in contrast to the above, one study showed increased leukemic engraftment when CCN2 was knocked down in MSCs, which suggests a protective effect of stromal CCN2, diminishing leukemic outgrowth (Battula et al. 2013). This study used CCN2 knockdown MSCs to form humanized extramedullary BM (EXM-BM) in WT mice. Increased leukemic engraftment of ALL cells was observed in this EXM-BM compared with that in the control EXM-BM derived from normal MSCs (Battula et al. 2013). This disparity suggests that not only the effects of CCN2 can be cell-type dependent, but also that the source of CCN2 might be critical (leukemic blast versus stromal cell). Another explanation might relate to the fact that actions of CCN proteins are context dependent. In the latter study, the CCN2 knockdown MSCs are used to induce newly formed bone and BM when injected together with human endothelial colony-forming cells (Battula et al. 2013). The EXM-BM derived from these CCN2 knockdown MSCs proved to be more adipocyte-rich, attributed to an inhibitory effect of CCN2 on adipogenesis, and expressed significantly higher levels of CXCL12 and of the adipocyte growth factor leptin than the EXM-BM derived from normal MSCs (Battula et al. 2013). Thus, there is not merely a down-regulation of CCN2 expression in the knockdown MSCs, but a profound change in the microenvironment they contribute to, which can be attributed to the effects of CCN2 on MSC differentiation. In particular, the enhanced leukemic engraftment in this setting might be due to the enhanced fat content of the BM with increased leptin and CXCL12 expression, possibly overriding a negative effect of CCN2 deficiency, as leptin enhances leukemia cell growth (Konopleva et al. 1999; Tabe et al. 2004) and CXCL12 is a known homing factor for leukemia cells (Möhle et al. 2000).

**Epigenetic and post-transcriptional regulation of CCN2 in acute lymphoblastic leukemia**

Both epigenetic regulation and post-transcriptional regulation of CCN2 might play a role in the pathophysiology of ALL.

DNA methylation is the only epigenetic modification of CCN2 studied in ALL. The CCN2 locus contains a dense CpG island at the 5′ end of the coding region and demethylation of this region was shown to be a common feature of pediatric B-ALL; mononuclear cells extracted from BM of these patients showed this locus to be largely unmethylated, regardless of the level of CCN2 gene expression (Welch...
et al. 2013). Remarkably, CD34 + cells from normal BM also showed extensive hypomethylation of the CCN2 locus, while BM lymphoblasts from T-ALL patients, not expressing detectable levels of CCN2 mRNA, showed hypermethylation focused at either end of the CCN2 CpG island (Welch et al. 2013). In B-ALL cell lines, an inverse correlation between the methylation state of the CCN2 locus and CCN2 gene expression was found: B-ALL cell lines with unmethylated CCN2 CpG islands showed high levels of CCN2 expression, while those with methylated CCN2 CpG islands showed no measurable CCN2 expression (Welch et al. 2013). In conclusion, while in B-ALL cell lines, demethylation of the CCN2 locus was associated with increased CCN2 expression, demethylation in ALL BM samples was commonly present but not related to CCN2 gene expression. Further studies are needed to elucidate the exact role of the CCN2 methylation status in ALL.

Alternative splicing of CCN2 in B-ALL is described by one study, which showed several novel short CCN2 mRNA isoforms (alternative splice forms) in B-ALL cell lines and B-ALL specimens (Welch et al. 2015). The splice forms all exhibited variable loss of sequences corresponding to exons 1–3, and in some cases loss of exon 4, but always full retention of exon 5 containing the CT domain (Welch et al. 2015). The short isoform encoding only the CT domain was the most frequently observed CCN2 alternative splice form, being present in 70% of the investigated B-ALL specimens expressing full length CCN2 (Welch et al. 2015). The shorter transcripts (but not the full length transcript) showed higher expression during the most active phase of cell growth, suggesting that they may be associated with the proliferation of B-lineage ALL cells (Welch et al. 2015). The truncated CCN2 protein with only the CT domain can still strongly bind to heparin, mediate cell adhesion and induce proliferation (Ball et al. 2003; Brigstock et al. 1997; Holbourn et al. 2009), but differences in biological activity of alternative splice products, compared to those of full length CCN2, largely remain to be elucidated.

MiRNAs play an important regulatory role in hematopoiesis and leukemogenesis (Fernandes 2017; Grobbelaar and Ford 2019; Schotte et al. 2009; Yeh et al. 2016; Yendamuri and Calin 2009; Zhang et al. 2018). The miRNA-17–92 cluster is essential for B-cell development, regulating pro-B to pre-B cell development and apoptosis of B-cells, and amplification of the miR-17–92 coding region has been associated with lymphoproliferative disease (Koralov et al. 2008; Ventura et al. 2008; Xiao et al. 2008). CCN2 is a predicted target of several miRNAs, including the miRNA-17–92 cluster (Chen et al. 2016; Ernst et al. 2010; Fox et al. 2013). It is, however, still unknown if and how miRNAs might relate CCN2 expression to ALL biology.

**CCN2 expression and effect in acute myeloid leukemia**

CCN2 expression has not been demonstrated in AML blast cells. But similar to normal HSCs, AML cells can induce CCN2 expression in MSCs, which relies on BMP-mediated signaling (Battula et al. 2017; Li et al. 2019). The CCN2 gene in MSCs from AML-bearing mice is upregulated 12- to 33-fold across various AML genotypes compared with that in MSCs from non-AML bearing control mice (Battula et al. 2017).

The effect of stromal CCN2 on AML engraftment is not fully established. One study indicates a pro-leukemic effect; transgenic mice overexpressing CCN2 in stromal cells, injected with AML cells, show: (1) a fourfold (time-dependent) enhancement of leukemia engraftment, (2) a higher percentage of leukemia cells in the peripheral blood, and (3) more leukemia engraftment in spleens compared with WT mice (Battula et al. 2017). Another study, however, suggests an opposite effect of stromal CCN2. Increased leukemic engraftment was observed in humanized extramedullary BM (EXM-BM) in mice when this BM was formed from CCN2 knockdown MSCs (Battula et al. 2013). As has been discussed above in more detail in the section on CCN2 and ALL, the EXM-BM derived from CCN2 knockdown MSCs proved to be more adipocyte-rich, which can be attributed to the inhibitory effect of CCN2 on the adipogenic differentiation of MSCs, and expressed higher levels of leptin and CXCL12 than the EXM-BM derived from normal MSCs (Battula et al. 2013). Thus, there is not merely a down-regulation of CCN2 expression in MSCs, but a complete change in microenvironment, with more adipose tissue and higher levels of leptin and CXCL-12, factors known to enhance leukemic growth and homing, possibly overruling a negative effect of CCN2.

**CCN2 expression and effect in myeloid neoplasms with fibrosis**

As described above, CCN2 enhances differentiation of cultured human BM MSCs into (myo)fibroblasts when stimulated subsequently with TGF-β (Lee et al. 2010). CCN2 is required for the differentiation of progenitor cells into contractile myofibroblasts through the regulation of extracellular matrix, cytoskeleton, cell adhesion, and cell migration genes, at least in dermal fibroblasts, as well as for the recruitment of progenitor cells to the fibrotic lesion in response to bleomycin, as has been shown by two different mouse models (Liu et al. 2014; Tsang et al. 2020; Liu et al. 2014). Another mouse fibrosis model has shown that either CCN2 mRNA or an application of exogenous CCN2 protein seems required for the development of persistent fibrosis (Mori et al. 1999). Although initially there has been some misconception that
CCN2 would be merely a down-stream mediator of TGF-β, it has now been shown that CCN2 rather acts as cofactor mediating and amplifying the profibrotic actions of TGF-β through domain-specific interactions with TGF-β and its receptor (Holmes et al. 2001; Khankan et al. 2011; Mori et al. 2008), and that TGF-β and CCN2 have overlapping and distinct fibrogenic effects (Gore-Hyer et al. 2002). In a wide variety of in vivo systems, CCN2 is required for experimental fibrosis. CCN2 is important as a key central mediator of the feed-forward system that both initiates and perpetuates fibrosis since adhesive signaling/mechanotransduction, mediated by FAK and YAP/TAZ, is required for fibrosis and CCN2 activates this pathway (Dupont et al. 2011; Lachowski et al. 2018).

Higher mRNA levels of TGF-β and CCN2 have been demonstrated in BM of myelodysplastic syndrome (MDS) with fibrosis than in MDS without fibrosis (Hussein et al. 2018), suggesting a role for both in its pathophysiology. The TGF-β pathway has already been implicated in the pathogenesis of many BM disorders, including myeloid neoplasms (Bataller et al. 2019), and TGF-β is known to play a central role in the induction of BM fibrosis in myeloproliferative neoplasms (Agarwal et al. 2016). The role of CCN2 in myeloproliferative neoplasms or other BM diseases with fibrosis still needs to be elucidated.

**CCN2 expression and effect in plasma cell neoplasia**

Only 1 study investigated the role of CCN2 in plasma cell neoplasia. This study reported significantly lower plasma levels of whole CCN2 in multiple myeloma (MM) patients compared with healthy controls, and in MM patients with bone disease compared with those without (Munemasa et al. 2007). Therefore, lowered CCN2 might be an indicator of bone disease in MM patients.

**Other CCN protein family members**

CCN2 is just one representative of the family of closely related CCN genes, which also includes CCN1 (Cysteine rich 61/Cyr61), CCN3 (Nephroblastoma overexpressed/NOV), CCN4 (Wnt-inducible-secreted protein (WISP)-1), CCN5 (WISP-2) and CCN6 (WISP-3) (Brigstock et al. 1997; Perbal 2018). The CCN family members can work in concert to orchestrate a multitude of biological processes in similar but also partly opposite ways (Peidl et al. 2019; Perbal 2018). The CCN family genes should ideally be studied together rather than separate. Unfortunately, the limited availability of well characterized tools including purified individual CCN proteins and the high complexity of their individual biologies have largely prevented such studies thus far.

As discussed in the reviews on CCN proteins in cancers and their tumor microenvironment, the role of a particular CCN protein in either potentiating or inhibiting tumor progression is related to the tumor type, and altered protein expression can be observed in either tumor cells or in tumor associated stromal cells (Wells et al. 2016; Yeger and Perbal 2016). With respect to their role in the BM microenvironment and in normal or malignant hematopoiesis, we have found no integrative studies involving multiple CCN genes. The available data on the individual non-CCN2 protein family members are discussed below and their expression in hematologic malignancies of the BM is summarized in Table 4.

### CCN1

High levels of CCN1 mRNA and protein have been demonstrated in human BM stromal cells (Djouad et al. 2007; Johnson et al. 2014; Li et al. 2012; Long et al. 2015; Schutze et al. 2005). The highest CCN1 expression is observed in MSCs, which decreases during osteogenic, adipogenic and chondrogenic differentiation (Djouad et al. 2007; Schutze et al. 2005).

In (T- and B-) ALL and CML, CCN1 expression has been detected in cell lines, and increased CCN1 levels have been demonstrated in BM-derived mononuclear cells, BM aspirate supernatant, and plasma samples of ALL and CML patients (Cao et al. 2019; Song et al. 2019; Zhu et al. 2016). In ALL, BM and plasma CCN1 levels correlate with the percentage

| Table 4 Expression of CCN proteins in hematologic malignancies of the bone marrow |
|------------------------------------------|-----|-----|-----|-----|
| B-ALL                                    | CCN1 | CCN2 | CCN3 | CCN4 |
| Tumor cell                               | ↑   | ↑   | n/r | ↑   |
| Stromal cell                             | n/r | n/r | n/r | n/r |
| T-ALL                                    |     |     |     |     |
| Tumor cell                               | ↑   |  -  | n/r | n/r |
| Stromal cell                             | n/r | n/r | n/r | n/r |
| CML                                      |     |     |     |     |
| Tumor cell                               | ↑   | n/r | ↓   | n/r |
| Stromal cell                             | n/r | n/r | n/r | n/r |
| AML                                      |     |     |     |     |
| Tumor cell                               | ↑   | n/r | n/r | n/r |
| Stromal cell                             | ↑   | ↑   | n/r | n/r |
| MM                                       |     |     |     |     |
| Tumor cell                               | –/↑ | ↑   | n/r | n/r |
| Stromal cell                             | ↑   | n/r | n/r | ↓   |

Increased (↑), decreased (↓), or unaltered (–) expression of CCN proteins in tumor cells and tumor associated stromal cells as far as reported in literature

N/R not reported, ALL acute lymphoblastic leukemia, CML chronic myeloid leukemia, AML acute myeloid leukemia, MM multiple myeloma
of blasts (Zhu et al. 2016). In both ALL and CML, CCN1 enhances leukemic cell survival in vitro by decreasing apoptosis through enhanced BCL2 expression via the NF-κB pathway (Song et al. 2019; Zhu et al. 2016). In ALL, AKT but not ERK1/2 affects in vitro NF-κB signaling by CCN1 (Zhu et al. 2016), whereas in CML, AKT nor ERK1/2 are involved in in vitro NF-κB signaling by CCN1 (Song et al. 2019). Direct and indirect effects of CCN1 in CML and ALL are depicted in Fig. 4a, b, respectively. CCN1 inhibition increases Imatinib-induced apoptosis of CML cells in vitro.

**Figure 4** a Direct and indirect intracellular effects of CCN proteins in chronic myeloid leukemia (CML). CCN1 expression is increased in CML cells, inhibiting apoptosis through enhanced expression of the anti-apoptotic protein BCL2 via the NF-κB pathway, without involvement of AKT or ERK1/2. CCN3 expression is decreased in CML cells, decreasing its inhibitory effect of CCN3 on ERK and AKT phosphorylation, resulting in elevated levels of phosphorylated ERK and AKT. This leads to less apoptosis, presumably via the NF-κB pathway. In addition, decreased CCN3 levels result in less caspase 3 cleavage, thereby also reducing apoptosis. Furthermore, decreased CCN3 levels lead to less inhibition of NOTCH1 signaling, resulting in higher levels of NOTCH. This results in decreased expression of p27, disrupting cell cycle regulation.

b. acute lymphoblastic leukemia (ALL): CCN1 (T-ALL), CCN2 (B-ALL), CCN4 (T-ALL)
and restores the sensitivity of CML cells to Imatinib in vivo (Song et al. 2019). Similarly, CCN1 decreases Cytarabine chemosensitivity in ALL cells via NF-κB pathway activation, and inhibition of CCN1 can restore ALL cell response to Cytarabine in vitro (Cao et al. 2019). Thus, CCN1 expression of leukemic cells seems to enhance tumor cell growth as well as drug resistance in both CML and ALL.

In AML, CCN1 expression has been demonstrated in leukemic cells as well as in stromal cells (Long et al. 2015; Niu et al. 2014). CCN1 expression in AML cells was upregulated by co-culturing them with BM stromal cells (Long et al. 2015). The amount of CCN1 expression in AML cells varies, with some BM samples and cell lines showing high expression, while others having low or no detectable expression (Niu et al. 2014). CCN1 promotes growth and survival of AML cells through the MEK/ERK pathway, with up-regulation of c-Myc and Bcl-xL, an anti-apoptotic protein of the Bcl2-family, and down-regulation of Bax, a pro-apoptotic member of the Bcl-2 family (Niu et al. 2014). The β-catenin/survivin pathway does not seem to be involved (Niu et al. 2014). Inhibition of stromal CCN1 partially reverses the stroma-induced resistance to mitoxantrone by increasing the mitoxantrone-induced apoptosis by AML cells (Long et al. 2015), suggesting a role for CCN1 in stroma-mediated chemoresistance. Spleen tyrosine kinase (SYK) is involved in this CCN1 signaling (Long et al. 2015). Thus, in AML, CCN1 seems to have a pro-leukemic effect in both tumor and stromal cells. CCN1 gene polymorphisms have been associated with either a lowered or increased risk of AML (Niu et al. 2014).

Increased CCN1 protein and gene expression have also been demonstrated in BM of patients with a plasma cell neoplasia (Johnson et al. 2014; Liu et al. 2017). Contradicting results, however, have been reported regarding cell type (plasma cells versus stromal cells) expressing CCN1 and its effect on tumor cell growth. Johnson et al. found high CCN1 gene expression in multiple myeloma (MM) associated BM stromal cells, but no CCN1 expression in cultured (normal or malignant) plasma cells (Johnson et al. 2014), whereas Dotterweich et al. did demonstrated CCN1 mRNA and protein in the cells of their MM cell line, which could be markedly increased by MSC contact or addition of recombinant CCN1 (Dotterweich et al. 2014). And whereas Johnson et al. showed recombinant CCN1 to inhibit growth of MM cells (Johnson et al. 2014), the viability of primary myeloma cells in the study of Dotterweich et al. increased significantly after CCN1 incubation, implying a pro-myeloma effect of CCN1 (Dotterweich et al. 2014). The contradicting results of CCN1 on MM cells might be explained by the use of different cell lines (H929 versus INA-6 MM cell line), difference between cell lines versus patient samples, and differences in experimental conditions as the effects of CCN proteins are known to be dependent on the micro-environment including the presence of certain cytokines.

Two studies imply that CCN1 has a favorable clinical effect in plasma cell neoplasms; Johnson et al. showed elevated serum CCN1 levels to be associated with a longer time to progression of monoclonal gammopathy of undetermined significance (MGUS) to overt MM and as such with a superior overall survival (Johnson et al. 2014), and Liu et al. showed increased CCN1 protein levels in the BM to be inversely associated with the severity of myeloma associated bone lesions (Liu et al. 2017). In addition, both studies confirmed in mouse models that overexpressed CCN1 in engrafted MM cells results in reduced bone disease (Johnson et al. 2014; Liu et al. 2017). This effect seems opposite to that of CCN2, showing decreased levels in MM patients with bone disease (Munemasa et al. 2007).

**CCN3**

CCN3 gene expression has been demonstrated in HSCs and hematopoietic progenitor cells (Bruno et al. 2004; Gupta et al. 2007; Ishihara et al. 2014; Kimura et al. 2010) as well as in MSCs (Djouad et al. 2007). In MSCs, CCN3 expression increases two-to-threefold after chondrogenesis (Djouad et al. 2007). In HSCs, CCN3 is essential for self-renewal, which seems to be at least in part due to its effect on cell cycling and NOTCH signaling (Gupta et al. 2007).

Similar to the other CCN family members, expression of CCN3 is strongly influenced by cytokines from the microenvironment. Without stimulation, HSCs show low levels of CCN3, but its expression can increase over 100-fold upon stimulation (Kimura et al. 2010). Interleukin 3 (IL-3) is the key cytokine for CCN3 induction in HSCs, directly increasing CCN3 expression by inducing STAT5A/B binding to a γ-interferon-activated sequences site in the CCN3 gene promoter (Kimura et al. 2010). Furthermore, exogenous CCN3 can induce endogenous expression of CCN3 in HSCs by binding to integrin αvβ3 on their cell surface, thereby affecting the long-term repopulating activity of HSCs (Ishihara et al. 2014). This process is context dependent; TPO mediates CCN3 binding to integrin αvβ3, inducing CCN3 expression likely through STAT5 activation, thereby supporting the long-term repopulating activity of HSCs (Ishihara et al. 2014). IFN-γ, however, impairs this TPO-induced expression of CCN3, likely through the activation of STAT1, inhibiting the long-term repopulating activity of HSCs (Ishihara et al. 2014).

In the transplant setting, exposure of umbilical cord blood to CCN3 can enhance engraftment by increased recruitment of cells with the highest long-term HSCs function, as these are preferentially bound to CCN3 through integrin α6 (a.k.a. CD49f) (Gupta et al. 2007). In CML, the CCN3 gene is down-regulated and its protein expression decreased as a direct consequence of the BCR-ABL tyrosine kinase activity, mediated at least in
part by microRNAs 130a/b (McCallum et al. 2006; Suresh et al. 2011). The decreased cellular CCN3 protein content was shown to correlate with increased CCN3 secretion (McCallum et al. 2006). Treatment with Imatinib increases CCN3 expression (McCallum et al. 2006). On the other hand, overexpression of CCN3 by transfection or treatment with the recombinant protein, significantly reduces tumor cell growth by enhancing Imatinib induced apoptosis (McCallum et al. 2009, 2012). CCN3 induced reduced cell growth and enhanced apoptosis is associated with enhanced caspase 3 cleavage and reduced phosphorylation of ERK and AKT (McCallum et al. 2009, 2012). Furthermore, CCN3 upregulates the expression of β4 integrin, which might affect the re-installation of growth control mechanisms (McCallum et al. 2012). In addition, CCN3 inhibits NOTCH1 signaling in CML, which is associated with increased expression of p27, thereby contributing to the restoration of cell cycle regulation (Suresh et al. 2013). Thus, the oncogenic BCR-ABL protein in CML enhances cell survival at least in part through its inhibitory effect on CCN3, resulting in reduced apoptosis and enhanced cell growth. These direct and indirect effects of CCN3 in CML are depicted in Fig. 4a.

A possible pathogenic role for the increased amount of secreted CCN3 needs to be determined. Furthermore, methylation of the CCN3 promoter was significantly increased in peripheral blood samples of CML patients compared with healthy controls, but this was unaffected by Imatinib treatment (Vatanmakanian et al. 2019). Whether hypermethylation of CCN3 is important in the pathophysiology in CML needs to be further investigated.

**CCN4–6**

CCN4, CCN5 and CCN6 expression has been demonstrated in MSCs (Djouad et al. 2007; Schutze et al. 2005). Like CCN3, CCN4 expression increases after chondrogenesis, whereas CCN6 expression decreases (Djouad et al. 2007; Schutze et al. 2005). CCN5 expression declines during adipogenic differentiation (Schutze et al. 2005).

Two studies investigated CCN4 in malignant hematopoiesis. The first describes a threefold decrease in mRNA expression of CCN4 in BM MSCs in MM compared with healthy age-matched controls, which meaning remains to be established (Corre et al. 2007). The other study shows variable and sometimes high mRNA and protein expression of CCN4 in T-ALL cell lines (Zhang et al. 2015). Knockdown of CCN4 in the cell line with the highest expression inhibited proliferation and induced apoptosis by down-regulating expression of, amongst others, p-AKT, p-ERK and Bcl-2, and upregulation of Bax, suggesting a possible pro-leukemic effect of CCN4 in T-ALL (Fig. 4b) (Zhang et al. 2015). We found no studies on CCN5 or CCN6 with regard to normal or malignant hematopoiesis.

**Summary of CCN protein family members**

In summary, the collected data on CCN1-6 with respect to BM microenvironment and normal or malignant hematopoiesis show, as far as the different experimental approaches allow such a comparison, many similarities and some differences between the CCN family members. In normal BM, expression of all CCN proteins have been demonstrated in MSCs, often at high levels and declining during lineage commitment and further differentiation, whereas only CCN3 expression has been found in HSCs. CCN3 directly affects the maintenance of HSCs, while CCN2 indirectly supports it through its secretion by MSCs. In hematologic malignancies of the BM, the CCN family members are often altered in either tumor cells or in the stromal cells (Table 4), usually, but not always, displaying pro-tumor effects. Many aspects of the CCN proteins in the various hematologic malignancies, however, remain to be elucidated.

**CCN2 therapeutic options**

Targeting CCN2 may be a therapeutic option for diseases associated with increased CCN2 expression, malignant as well as non-malignant. CCN2 can be inhibited by:

1. The use of an anti-CCN2 antibody (Aikawa et al. 2006; Alapati et al. 2011; Barbe et al. 2020a, b; Bickelhaupt et al. 2017; Dornhofer et al. 2006; Finger et al. 2014; Makino et al. 2017; Moran-Jones et al. 2015; Neesse et al. 2013; Ohara et al. 2018; Raghu et al. 2016; Richeldi et al. 2020; Sakai et al. 2017),
2. Gene expression silencing by antisense oligonucleotides (ASOs) or small interfering RNAs (siRNAs) (Chen et al. 2014; Gale et al. 2018; Gibson et al. 2017; Jensen et al. 2018; Kang et al. 2020; Li et al. 2006; Okada et al. 2005; Sisco et al. 2008; Sung et al. 2013; Yoko et al. 2004; Yoon et al. 2016),
3. Drugs that indirectly (and less specifically) inhibit CCN2 expression, for example by targeting Sirtuin 1 (Sirt1) (Ren et al. 2017), peroxisome proliferator-activated receptor gamma (PPARγ) (Sun et al. 2006; Zhao et al. 2006), the CCN2 transcriptional regulators YAP and TAZ (Ji et al. 2018), or proteins involved in signaling pathways affecting CCN2 transcription such FAK (Peidl et al. 2019),
4. The use of CCN3, as it can antagonize the effects of CCN2 (Peidl et al. 2019; Riser et al. 2009, 2010, 2014).
Of the above, only the use of an anti-CCN2 antibody and the use of ASOs and siRNAs have made it to clinical trials for their direct effect on CCN2 (Gale et al. 2018; Jensen et al. 2018; https://www.prnewswire.com/news-releases/rxi-pharmaceuticals-announces-positive-results-from-phase-12-trial-with-rxi-109-for-retinal-scarring-300690078.html; http://www.sciencedirect.com/science/article/pii/S0190962214008147). The PPARγ agonist Rosiglitazone is a FDA approved drug for the treatment of type 2 diabetes mellitus and has anti-fibrotic effects with in vitro and in vivo reduction of CCN2 expression in different organs (Gao et al. 2007; Guo et al. 2012; Ihm et al. 2010; Jeon et al. 2015). The FAK inhibitor GSK2256098 is and has been tested in phase 1 and 2 studies for pulmonary hypertension and solid tumors, respectively (http://www.clinicaltrials.gov), whereas the FAK inhibitor PF573228 blocks CCN2 expression in vitro (Peidl et al. 2019). Several Sirt1 activators have been tested in clinical trials as reviewed by Dai et al. (2018), although their suppressive effects on CCN2 expression have only been demonstrated in animal models (Ren et al. 2017). The Hippo pathway is important in oncogenesis and many currently used drugs restrict YAP/TAZ activities, whereas several novel YAP/TAZ inhibitors are under development, as is reviewed by Pobbati and Hong (2020). The use of CCN3 as a counter-regulator and a potential therapeutic agent has thus far been tested in experimental models (Riser et al. 2014).

The only therapeutic option for targeting CCN2 that has been studied in BM diseases, is the use of the humanized monoclonal anti-CCN2 antibody FG-3019 (Pamrevlumab), which, in combination with conventional chemotherapy, significantly prolonged the survival of mice injected with a primary xenograft of B-ALL cells (Lu et al. 2014). Pamrevlumab showed a very favorable clinical safety profile in a phase 2 study of idiopathic pulmonary fibrosis (Raghu et al. 2016; Richeldi et al. 2020). Two other phase 2 studies, on Pamrevlumab in Duchenne muscular dystrophy [https://clinicaltrials.gov/ct2/show/NCT02606136] and on Pamrevlumab in hospitalized patients with acute COVID-19 disease [https://clinicaltrials.gov/show/NCT04432298], are ongoing. Furthermore, three phase 3 studies, including one on locally advanced pancreatic cancer, have started [https://clinicaltrials.gov/ct2/show/NCT03955146; https://clinicaltrials.gov/ct2/show/NCT04371666 and https://clinicaltrials.gov/ct2/show/NCT03941093], while a fourth one is announced [https://clinicaltrials.gov/ct2/show/NCT04419558]. Pamrevlumab has neither been tested in clinical trials on ALL, nor in other BM diseases.

Concluding remarks

The BM microenvironment is a complex and not fully unraveled milieu made up of many different cell populations, structural matrix proteins and soluble factors communicating with each other and forming different niches essential for normal hematopoiesis. It is subject to modulation by many different factors. One of these is CCN2, a matricellular protein with a wide variety of functions, known to be important in ECM for both the production of ECM proteins and the coordination of signaling pathways. CCN2 is produced by MSCs and other mesenchymal cells of the BM. It is involved in many different aspects of MSC biology, including proliferation, migration and differentiation. In addition, it plays a role in normal B-cell development and has been implicated in the maintenance and longtime survival of HSCs. Furthermore, CCN2 is shown to be overexpressed in leukemic cells of B-ALL, the most studied BM disease in this regard, in which it is associated with reduced overall survival. In AML samples, increased CCN2 expression is demonstrated in MSCs, which also affects leukemic engraftment, but in a way that still needs to be determined.

All other CCN family members are expressed in MSCs as well, whereas only CCN3 expression has been demonstrated in HSCs. Except for CCN5 and CCN6, which have not been studied in this context, all CCN protein family members have been associated with hematologic malignancies and often, but not always, their expression is increased in either tumor cells or in stromal cells, mostly displaying pro-tumor effects.

With this review, the authors hope to increase awareness of the CCN proteins, especially CCN2, as important players in the BM and as attractive subject for further studies on the BM microenvironment and BM diseases. These studies should include the use of anti-CCN therapies for their effect in neoplastic diseases such as ALL, but also for their possible disease modifying activities, for example on BM fibrosis.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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References

Abd El Kader T, Kubota S, Nishida T, Hattori T, Aoyama E, Janune D, Hara ES, Ono M, Tabata Y, Kuboki T, Takigawa M (2014)
The regenerative effects of CCN2 independent modules on chondrocytes in vitro and osteochondrosis models in vivo. Bone 59:180–188. https://doi.org/10.1016/j.bone.2013.11.010

Abreu-Shady M, Friess H, Zimmermann A, Di Mola FF, Guo XZ, Baer HU, Büchler MW (2000) Connective tissue growth factor in human liver cirrhosis. Liver 20:296–304. https://doi.org/10.1034/j.1600-0676.2000.200004296.x

Abraham DJ, Shiwen X, Black CM, Sa S, Xu Y, Leask A (2000) Tumor necrosis factor alpha suppresses the induction of connective tissue growth factor by transforming growth factor-beta in normal and scleroderma fibroblasts. J Biol Chem 275:15220–15225. https://doi.org/10.1074/jbc.275.20.15220

Abreu JG, Ketpura NI, Reversade B, De Robertis EM (2002) Connective-tissue growth factor (CTGF) modulates cell signaling by BMP and TGF-beta. Nat Cell Biol 4:599–604. https://doi.org/10.1038/ncb826

Acar M, Kocberlarakota KS, Murphy MM, Peyer JG, Oguro H, Inra CN, Jaieyela C, Zhao Z, Luby-Phelps K, Morrison SJ (2015) Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. Nature 526:126–130. https://doi.org/10.1038/nature15200

Advanis AS, Gundaeker HM, Sala-Torra O, Radich JP, Lai R, Lovak ML, Lancet JE, Coutre SE, Stuart RK, Mims MP, Stiff PJ, Appelbaum FR (2010) Southwest Oncology Group Study S0530: a phase 2 trial of clafobamine and cytapharine for relapsed or refractory acute lymphocytic leukemia. Br J Haematol 151:430–434

Agarwal A, Morrone K, Barretstein M, Zhao ZJ, Verma A, Goel S (2016) Bone marrow fibrosis in primary myelofibrosis: pathogenic mechanisms and the role of TGF-β-beta. Stem Cell Investig 3:5. https://doi.org/10.3978/j.issn.2306-9759.2016.02.03

Aikawa T, Gunn J, Spong SM, Kaus SJ, Korc M (2006) Connective tissue growth factor specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. Mol Cancer Ther 5:1108–1116. https://doi.org/10.1158/1535-7163.MCT-05-0156

Akashi S, Nishida T, El-Seoudi A, Takigawa M, Iida S, Kubota S (2018) Metabolic regulation of the CCN family genes by glycolysis in chondrocytes. J Cell Commun Signal 12:245–252. https://doi.org/10.1007/s12079-017-0420-8

Alapati D, Rong M, Chen S, Hehre D, Rodriguez MM, Lipson KE, Wu S (2011) Connective tissue growth factor antibody therapy attenuates hyperoxia-induced lung injury in neonatal rats. Am J Respir Cell Mol Biol 45:1169–1177. https://doi.org/10.1165/rcmb.2011-0023OC

Ambrosi TH, Scialdone A, Graja A, Gohlke S, Jank AM, Bocian C, Alapati D, Rong M, Chen S, Hehre D, Rodriguez MM, Lipson KE, Akashi S, Nishida T, El-Seoudi A, Takigawa M, Iida S, Kubota S (2012) CCN2/CTGF binds to aggrecan. Biochem J 444:R. J. Leguit et al.

Aoyama E, Hattori T, Hoshijima M, Araki D, Nishida T, Kubota S, Takigawa M (2009) N-terminal domains of CCN family 2/ connective tissue growth factor bind to aggrecan. Biochem J 420:413–420. https://doi.org/10.1042/BJ20081991

Aoyama E, Kubota S, Takigawa M (2012) CCN2/CTGF binds to fibroblast growth factor receptor 2 and modulates its signaling. FEBS Lett 586:4270–4275. https://doi.org/10.1016/j.febslet.2012.10.038

Aoyama E, Kubota S, Khattab HM, Nishida T, Takigawa M (2015) CCN2 enhances RANKL-induced osteoclast differentiation via direct binding to RANK and OPG. Bone 73:242–248. https://doi.org/10.1016/j.bone.2014.12.058

Arranz L, Sanchez-Aguilera A, Martin-Perez D, Isern J, Langa X, Tzankov A, Lundberg P, Muntion S, Tzeng YS, Lai DM, Schwaller J, Skoda RC, Mendez-Ferrer S (2014) Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. Nature 512:78–81. https://doi.org/10.1038/nature13383

Asada N, Kunisaki Y, Pierce H, Wang Z, Fernandez NF, Birbrair A, Ma’ayan A, Frenette PS (2017) Differential cytokine contributions of perivascular haematopoietic stem cell niches. Nat Cell Biol 19:214–223. https://doi.org/10.1038/nccb3475

Astrom M, Hahn-Stromberg V, Zetterberg E, Vedin I, Merup M, Palmblad J (2015) X-linked thrombocytopenia with thalassemia displays bone marrow reticulin fibrosis and enhanced angiogenesis: comparisons with primary myelofibrosis. Am J Hematol 90:E44–E48. https://doi.org/10.1002/ajh.23907

Aurrand-Lions M, Mancini SJ (2018) Murine bone marrow niches from hematopoietic stem cells to B cells. Int J Mol Sci. https://doi.org/10.3390/ijms19082353

Babic AM, Chen C-C, Lau LF (1999) Fisp12/mouse connective tissue growth factor mediates endothelial cell adhesion and migration through integrin alphavbeta3, promotes endothelial cell survival, and induces angiogenesis in vivo. Mol Cell Biol 19:2958–2966

Baguma-Nibasheka M, Kablar B (2008) Pulmonary hypoplasia in the connective tissue growth factor (CTgf) null mouse. Dev Dyn 237:485–493. https://doi.org/10.1002/dvdy.21433

Bai K, Chen BC, Pai HC, Weng CM, Yu CC, Hsu MJ, Yu MC, Ma HP, Wu CH, Hong CY, Kuo ML, Lin CH (2013) Thrombin-induced CCN2 expression in human lung fibroblasts requires the c-Src/JAK2/STAT3 pathway. J Leukoc Biol 93:101–112. https://doi.org/10.1189/jlb.0911449

Balderman SR, Li AJ, Hofman CM, Frisch BJ, Goodman AN, LaMere MW, George AR, Evans AG, Liesveld JL, Becker MW, Calvi LM (2016) Targeting of the bone marrow microenvironment improves outcome in a murine model of myelodysplastic syndrome. Blood 127:616–625. https://doi.org/10.1182/blood-2015-06-565113

Ball DK, Rachfal AW, Kemper SA, Brigstock DR (2003) The heparin-binding 10 kDa fragment of connective tissue growth factor (CTGF) containing module 4 alone stimulates cell adhesion. J Endocrinol 176:R1–R7. https://doi.org/10.1677/joe.0.176r001

Barfe MF, Hilliard BA, Amin M, Harris MY, Hobson LJ, Cruz GE, Dorotan JT, Paul RW, Klyne DM, Popoff SN (2020a) Blocking CTGF/CCN2 reverses neural fibrosis and sensorimotor declines in a rat model of overuse-induced median mononeuropathy. J Orthop Res. https://doi.org/10.1002/jor.24709

Barfe MF, Hilliard BA, Amin M, Harris MY, Hobson LJ, cruz GE, Popoff SN (2020b) Blocking CTGF/CCN2 reduces established skeletal muscle fibrosis in a rat model of overuse injury. FASEB J 34:6554–6569. https://doi.org/10.1096/fj.202000240RR

Bataller A, Montalban-Bravo G, Soltysska IA, Garcia-Manero G (2019) The role of TGFbeta in hematopoiesis and myeloid disorders. Leukemia 33:1076–1089. https://doi.org/10.1038/s41375-019-0420-1

Batulla VL, Chen Y, Cabreira Mda G, Ruvolo V, Wang Z, Ma W, Konoplev S, Shpall E, Lyons K, Strunk D, Bueso-Ramos C, Davis RE, Konopleva M, Andreff M (2013) Connective tissue growth factor regulates adipocyte differentiation of mesenchymal stromal cells and facilitates leukemia bone marrow engraftment. Blood 122:357–366. https://doi.org/10.1182/blood -2012-06-437988

Batulla VL, Le PM, Sun JC, Nguyen K, Yuan B, Zhou X, Sonny lal S, Mc Queen T, Ruvolo V, Michel KA, Ling X, Jacamo R, Shpall E, Wang Z, Rao A, Al-Atrash G, Konopleva M, Davis RE, Harrington MA, Cahill CW, Bueso-Ramos C, Andreff M (2017) AML-induced osteogenic differentiation in mesenchymal...
stromal cells supports leukemia growth. JCI Insight. https://doi.org/10.1172/jci.insight.90036

Behnes M, Brueckmann M, Lang S, Weiss C, Ahmad-nejad P, Neumaier M, Borggreve M, Hoffmann U (2014) Connective tissue growth factor (CTGF/CCN2): diagnostic and prognostic value in acute heart failure. Clin Res Cardiol 103:107–116. https://doi.org/10.1007/s00392-013-0626-6

Bickelhaupt S, Erbel C, Timke C, Winkler U, Dadrich M, Fleischg P, Tietz A, Pföhler J, Gross W, Peschke P, Hoelgen L, Katus HA, Grone HJ, Nicolay NH, Saffrich R, Debuc J, Sternlich MD, Seeley TW, Lipson KE, Huber PE (2017) Effects of CTGF blockade on attenuation and reversal of radiation-induced pulmonary fibrosis. J Natl Cancer Inst. https://doi.org/10.1093/jnci/djw339

Blalock TD, Gibson DJ, Duncan MR, Tulis SS, Grotendorst GR, Schultz CCN2 (Cellular Communication Network factor 2) in the bone marrow microenvironment, normal…

Blau O, Hofmann WK, Baldus CD, Thiel G, Serbent V, Schumann E, Thielsch E, Blau I (2007) Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. Exp Hematol 35:221–229. https://doi.org/10.1016/j.exphem.2006.10.012

Boag JM, Beesley AH, Firth MJ, Freitas JR, Ford J, Brigstock DR, de Klerk NH, Kees UR (2007) High expression of connective tissue growth factor in pre-B acute lymphoblastic leukemia. Br J Haematol 138:740–748. https://doi.org/10.1111/j.1365-2141.2007.07396.x

Bork P (1993) The modular architecture of a new family of growth regulators related to connective tissue growth factor. FEBS Lett 327:125–130. https://doi.org/10.1016/0014-5793(93)80155-a

Bornstein P (1995) Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin I. J Cell Biol 130:503–506. https://doi.org/10.1083/jcb.130.3.503

Bornstein P (2009) Matricellular proteins: an overview. J Cell Commun Signal 3:163–165. https://doi.org/10.1007/s12079-009-0069-z

Bornstein P, Sage EH (2002) Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol 14:608–616

Boyerinas B, Zafrir M, Yesilkanal AE, Price TT, Hyjek EM, Sipkins DA (2013) Adhesion to osteopontin in the bone marrow niche regulates lymphoblastic leukemia cell dormancy. Blood 121:4821–4831. https://doi.org/10.1182/blood-2012-12-475483

Bradham DM, Igarashi A, Potter RL, Grotendorst GR (1991) Connective tissue growth factor: a cysteine-rich mitogen secreted by pancreatic stellate cells (PSC) during chronic pancreatitis and are exported in PSC-derived exosomes. J Cell Commun Signal 5:87–103. https://doi.org/10.1007/s41598-016-0120-3

Chambers RC, Leoni P, Blanc-Brude OP, Wembidge DE, Laurent GJ (2000) Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. J Biol Chem 275:35584–35591. https://doi.org/10.1074/jbc.M003188200

Chaoqur B (2020) Caught between a “Rho” and a hard place: are CCN1/CYR61 and CCN2/CTGF the arbiters of microvascular stiffness? J Cell Commun Signal 14:21–29. https:/doi.org/10.1007/s12079-019-00529-3

Charrier A, Chen R, Chen L, Kemper S, Hattori T, Takigawa M, Brigstock DR (2014a) Connective tissue growth factor (CCN2) and microRNA-21 are components of a positive feedback loop in pancreatic stellate cells (PSC) during chronic pancreatitis and are exported in PSC-derived exosomes. J Cell Commun Signal 8:147–156. https://doi.org/10.1007/s12079-014-0220-3

Charrier A, Chen R, Kemper S, Brigstock DR (2014b) Regulation of pancreatic inflammation by connective tissue growth factor (CTGF/CCN2). Immunology 141:564–576. https://doi.org/10.1111/imn.12215

Chasis JA, Mohandas N (2008) Erythroblastic islands: niches for erythropoiesis. Blood 112:470–478. https://doi.org/10.1182/blood-2008-03-077883

Che H, Wang Y, Li Y, Lv J, Li H, Liu Y, Dong R, Sun Y, Xu X, Zhao J, Wang L (2019) Inhibition of microRNA-150–5p alleviates cardiac inflammation and fibrosis via targeting Smad7 in high glucose-treated cardiac fibroblasts. J Cell Physiol. https://doi.org/10.1002/jcp.29386

Chen CC, Lau LF (2009) Functions and mechanisms of action of CCN matricellular proteins. Int J Biochem Cell Biol 41:771–783. https://doi.org/10.1016/j.biocel.2008.07.025

Chen CC, Chen N, Lau LF (2001) The angiogenic factors Cyr61 and CCN2 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. J Biol Chem 276:10443–10452. https://doi.org/10.1074/jbc.M008087200

Chen L, Charrier A, Zhou Y, Chen R, Yu B, Agarwal K, Tsukamoto H, Lee LJ, Paulatis ME, Brigstock DR (2014) Epigenetic regulation of connective tissue growth factor by MicroRNA-214 delivery in exosomes from mouse or human hepatic stellate cells. Hepatol Int 8:147–156. https://doi.org/10.1016/j.heparcancer.2014.01.011

Chen TC, Ou YL, Huang ZP, Hong YG, Tao YP, Wang ZG, Ni JS, Hsu LJ, Lin H (2019) MicroRNA-214 Mediate CTGF expression and pulmonary fibroblast differentiation. J Cell Physiol 234:2236–2248. https://doi.org/10.1002/jcp.25341

Chen YC, Chen BC, Yu CC, Lin SH, Lin CH (2016) miR-19a, -19b, and -26b Mediate CTGF expression and pulmonary fibroblast differentiation. J Cell Physiol 231:2236–2248. https://doi.org/10.1002/jcp.25341

Cheng LC, Strickland DH, Howlett M, Ford J, Charles AK, Lyons KM, Brigstock DR, Goldschmeding R, Cole CH, Alexander WS, Kees UR (2014) Connective tissue growth factor is expressed in bone marrow stromal cells and promotes interleukin-7-dependent B lymphopoiesis. Haematologica 99:1149–1156. https://doi.org/10.3324/haematol.2013.102327

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CCN2 (Cellular Communication Network factor 2) in the bone marrow microenvironment, normal…
Chowdhury I, Chaqour B (2004) Regulation of connective tissue growth factor (CTGF/CCN2) gene transcription and mRNA stability in smooth muscle cells. Involvement of RhoA GTPase and p38 MAP kinase and sensitivity to actin dynamics. Eur J Biochem 271:4436–4450. https://doi.org/10.111/j.1432-1033.2004.04382.x

Cicha I, Goppelt-Strebe M (2009) Connective tissue growth factor: context-dependent functions and mechanisms of regulation. BioFactors 35:200–208. https://doi.org/10.1002/biof.30

Cicha I, Garlichs CD, Daniel WG, Goppelt-Struebe M (2004) Activated human platelets release connective tissue growth factor. Thromb Haemost 91:755–760. https://doi.org/10.1160/TH03-09-0602

Cooker LA, Peterson D, Rambow J, Riser ML, Riser RE, Najmabadi F, Dammeier J, Beer HD, Brauchle M, Werner S (1998) Dexamethasone-induced down-regulation by cAMP. PASEB J 13:1774–1786

Ducot B, Moriset L, Aragona M, Enzo E, Giuliani S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Biccari S, Elvassore N, Piccolo S (2011) Role of YAP/TAZ in mechanotransduction. Nature 474:179–183. https://doi.org/10.1038/nature10137

Duran C, Charbord P, Jaffredo T (2018) The crosstalk between hematopoietic stem cells and their niches. Curr Opin Hematol 25:285–289. https://doi.org/10.1097/MOH.0000000000000438

Edwards LA, Woolard K, Son MJ, Li A, Lee J, Ene C, Mantey SA, Maric D, Song H, Belova G, Jensen RT, Zhang W, Fine HA (2011) Effect of brain- and tumor-derived connective tissue growth factor on glioma invasion. J Natl Cancer Inst 103:1162–1178. https://doi.org/10.1093/jnci/djr224

Eltoukhhy HS, Sinha G, Moore C, Guirao K, Rameshwar P (2016) CXCL12-abundant ecticular (CAR) cells: a review of the literature with relevance to cancer stem cell survival. J Cancer Stem Cell Res 4:e1004. https://doi.org/10.1433/JSCTR.2016.e1004

Ernst A, Campos B, Meier J, Devens F, Liesenberg F, Wolter M, Reifenberger G, Herold-Mende C, Lichter P, Radlwimmer B (2010) De-repression of CTGF via the miR-17–92 cluster upon differentiation of human glioblastoma spheroid cultures. Oncogene 29:3411–3422. https://doi.org/10.1038/onc.2010.83

Escolar G, Lopez-Vilchez I, Diaz-Ricart M, White JG, Galan AM (2008) Internalization of tissue factor by platelets. Thromb Res 122:S37–S41. https://doi.org/10.1016/j.thromres.2008.07.0017-3

Faherty N, Curran SP, O’Donovan H, Martin F, Godson C, Brazil DP, Crean JK (2012) CCN2/CTGF increases expression of miR-302 microRNAs, which target the TGFbeta type II receptor with implications for nephropathic cell phenotypes. J Cell Sci 125:5621–5629. https://doi.org/10.1242/jcs.105528

Falke LL, He N, de Sousa C, Lopes SM, Broekhuizen R, Lyons K, Nguyen TQ, Goldschmeding R (2020) FoxD1-driven CCN2 deletion causes axial skeletal deformities, pulmonary hypoplasia, and neonatal asphyctic death. J Cell Commun Signal. https://doi.org/10.1007/s11899-020-00549-4

Fan WH, Karovszky MJ (2012) Increased MMP-2 expression in connective tissue growth factor over-expression vascular smooth muscle cells. J Biol Chem 277:9800–9805. https://doi.org/10.1074/jbc.M1112123200

Fernandes Q (2017) MicroRNA: defining a new niche in leukemogenesis. Blood Rev 31:129–138. https://doi.org/10.1016/j.bled.2016.11.003

Finger EC, Cheng CF, Williams TR, Rankin EB, Bedogni B, Tachiki L, Spong S, Giaccia AJ, Powell MB (2014) CTGF is a therapeutic target for metastatic melanoma. Oncogene 33:1093–1100. https://doi.org/10.1038/onc.2013.47

Fox JL, Dews M, Minn AJ, Thomas-Tikhonenko A (2013) Targeting of TGFbeta signature and its essential component CTGF by miR-18 correlates with improved survival in glioblastoma. RNA 19:177–190. https://doi.org/10.1261/rna.036467.112

Frazier K, Williams S, Kothapalli D, Klappler H, Grotendorst GR (1996) Stimulation of fibroblast cell growth, matrix production, and granulation tissue formation by connective tissue growth factor. J Invest Dermatol 107:404–411. https://doi.org/10.1111/1523-1747.ep12363389

Fujita N, Ichii M, Maeda T, Saitoh N, Yokota T, Yamawaki K, Kakitani M, Tomizuka K, Oritani K, Kanakura Y (2015) Identification of osteoblast stimulating factor 5 as a negative regulator in the
B-lymphopoietic niche. Exp Hematol 43(963–973):e964. https://doi.org/10.1016/j.exphem.2015.07.002

Gale JD, Jensen J, Berman G, Freimuth W, Li G, Pteil A, Kutty M, Rosenthal A, Boswell CB, Noah VEM, Young L (2018) A Placebo-controlled Study of PF-06473871 (anti-connective tissue growth factor antisense oligonucleotide) in reducing hyper- trophic skin scarring. Plast Reconstr Surg Glob Open 6:1861. https://doi.org/10.1097/GOX.0000000000001861

Gandemer V, Rio AG, de Tayrac M, Sibut V, Mottier S, Ly Sunnaram B, Henry C, Monnier A, Berthou C, Le Gall E, Le Treut A, Schmitt C, Le Gall JY, Mosser J, Galibert MD (2007) Five distinct biological processes and 14 differentially expressed genes characterize TEL/AML1-positive leukemia. BMC Genom 8:385. https://doi.org/10.1186/1471-2164-8-385

Gao R (2003) Low density lipoprotein receptor-related protein (LRP) is a heparin-dependent adhesion receptor for connective tissue growth factor (CTGF) in rat activated hepatic stellate cells. Hepatol Res 27:214–220. https://doi.org/10.1016/s1386-6346(03)00241-9

Gao R, Brigstock DR (2004) Connective tissue growth factor (CCN2) induces adhesion of rat activated hepatic stellate cells by binding of its C-terminal domain to integrin alphavbeta3 (and heparan sulfate proteoglycan. J Biol Chem 279:8848–8855. https://doi.org/10.1074/jbc.M412040200

Gao R, Brigstock DR (2005) Connective tissue growth factor (CCN2) in rat pancreatic stellate cell function: integrin alpha5beta1 as a novel CCN2 receptor. Gastroenterology 129:1019–1030. https://doi.org/10.1053/j.gastro.2005.06.067

Gao DF, Niu XL, Hao GH, Peng N, Wei J, Ning N, Wang NP (2007) Rosiglitazone inhibits angiostatin II-induced CTGF expression in vascular smooth muscle cells—role of PPAR-gamma in vascular fibrosis. Biochem Pharmacol 73:185–197. https://doi.org/10.1016/j.bcp.2006.09.019

Gerritsen KG, Falke LL, van Vuuren SH, Leeuwis JW, Broekhuizen R, Nguyen TQ, de Borst GJ, Nathoe HM, Verhaar MC, Kok RJ, Goldschmeding R, Visseren FL, Group SS (2016) Plasma CTGF is independently related to an increased risk of cardiovascular events and mortality in patients with atherosclerotic disease: the SMART study. Growth Factors 34:149–158. https://doi.org/10.1080/08977194.2016.1210142

Gibson DJ, Tuli SS, Schultz GS (2017) Dual-phase iophoresis for the delivery of antisense oligonucleotides. Nucleic Acid Ther 33:461–473. https://doi.org/10.1080/08977194.2016.1163031

Gnatenko DV, Dunn JJ, McCorkle SR, Weissmann D, Perrotta PL, Bahou WF (2003) Transcript profiling of human platelets using microarray and serial analysis of gene expression. Blood 101:2285–2293. https://doi.org/10.1182/blood-2002-09.277

Groppelt-Stuebe M, Hahn A, Iwanciw D, Rehm M, Banas B (2001) Regulation of connective tissue growth factor (ccn2; ctgf) gene expression in human mesangial cells: modulation by HMG CoA reductase inhibitors (statins). Mol Pathol 54:176–179. https://doi.org/10.1136/mp.54.3.176

Gore-Hyer E, Shegogue D, Markiewicz M, Lo S, Hazen-Martin D, Greene EL, Grotendorst GR, Trojanowska M (2002) TGF-beta and CTGF have overlapping and distinct fibrogenic effects on human renal cells. Am J Physiol Renal Physiol 283:F707-716. https://doi.org/10.1152/ajprenal.00007.2002

Grobbelaar C, Ford AM (2019) The role of microRNA in paediatric acute lymphoblastic leukaemia: challenges for diagnosis and therapy. J Oncol 2019:8941471. https://doi.org/10.1155/2019/8941471

Grotendorst GR (1997) Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. Cytok Growth Factor Rev 8:171–179

Grotendorst GR, Duncan MR (2005) Individual domains of connective tissue growth factor regulate fibroblast proliferation and myofibroblast differentiation. FASEB J 19:729–738. https://doi.org/10.1096/fj.04-3217com

Gu J, Liu X, Wang QX, Tan HW, Guo M, Jiang WF, Zhou L (2012) Angiostatin II increases CTGF expression via MAPKs/TFG-beta1/TRA6 pathway in atrial fibroblasts. Exp Cell Res 318:2105–2115. https://doi.org/10.1016/j.yexcr.2012.06.015

Guerruoaen BS, Al-Hijji I, Tabrizi AR (2011) Osteoblastic and vascular endothelial niches, their control on normal hematopoietic stem cells, and their consequences on the development of leukemia. Stem Cells Int 2011:375857. https://doi.org/10.4061/2011/375857

Guo F, Carter DE, Leask A (2011) Mechanical tension increases CCN2/CTGF expression and proliferation in gingival fibroblasts via a TGFbeta-dependent mechanism. PLoS ONE 6:e19756. https://doi.org/10.1371/journal.pone.0019756

Guo Z, Qin Z, Zhang R, Li J, Yin Y (2012) Effect of rosiglitazone on the expression of cardiac adipoenecit receptors and NADPH oxidase in type 2 diabetic rats. Eur J Pharmacol 685:116–125. https://doi.org/10.1016/j.ejphar.2012.04.010

Gupta R, Hong D, Iborra F, Sarno S, Enver T (2007) NOV (CCN3) functions as a regulator of human hematopoietic stem or progenitor cells. Science. https://doi.org/10.1126/science.1136031

Hall-Glen F, De Young RA, Huang BL, van Handel B, Hofmann JJ, Chen TT, Choi A, Ong JR, Benya PD, Mikkola H, Iruela-Arispe ML, Lyons KM (2012) CCN2/connective tissue growth factor is essential for pericyte adhesion and endothelial basement membrane formation during angiogenesis. PLoS ONE 7:e30562. https://doi.org/10.1371/journal.pone.0030562

Hashimoto G, Inoki I, Fujiy I, Aoki T, Ikeda E, Okada Y (2002) Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor. Circ Res 90:633–638. https://doi.org/10.1161/01.RES.0000030562

He M, Chen Z, Martin M, Zhang J, Sangwung P, Woo B, Tremoulet AH, Shimizu C, Jain MK, Burns JC, Shy JY (2017) miR-483 Targeting of CTGF suppresses endothelial-to-mesenchymal transition: therapeutic implications in Kawasaki disease. Circ Res 120:354–365. https://doi.org/10.1161/CIRCRESAHA.116.310235

Heroult M, Bernard-Pierrot I, Delbe J, Hamma-Kourbali Y, Katsoris P, Barriot D, Papadimitriou E, Plouet J, Courtay J (2004) Heparin affin regulatory peptide binds to vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. Oncogene 23:1745–1753. https://doi.org/10.1038/sj.onc.1206879

Higgins DF, Biju MP, Akai Y, Wutz A, Johnson RS, Haase VH (2004) Hypoxic induction of Ctgf is directly mediated by Hif-1. Am J Physiol Renal Physiol 287:F1223–F1232. https://doi.org/10.1152/ajprenal.00245.2004

Ho YH, Méndez-Ferrer S (2020) Microenvironmental contributions to hematopoietic stem cell aging. Haematologica 105:38–46. https://doi.org/10.3324/haematol.2018.211334

Holbourn KP, Acharya KR, Perbal B (2008) The CCN family of proteins: structure-function relationships. Trends Biochem Sci 33:461–473. https://doi.org/10.1016/j.tibs.2008.07.006

Holbourn KP, Perbal B, Ravi Acharya K (2009) Proteins on the catwalk: modelling the structural domains of the CCN family of proteins. J Cell Commun Signal 3:25–41. https://doi.org/10.1007/s12079-009-0048-4

Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A (2001) CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. J Biol Chem 276:10594–10601. https://doi.org/10.1074/jbc.M010149200
Holmes A, Abraham DJ, Chen Y, Denton C, Shi-wen X, Black CM, Leask A (2003) Constitutive connective tissue growth factor expression in sclerodema fibroblasts is dependent on Sp1. J Biol Chem 278:41728–41733. https://doi.org/10.1074/jbc.M305019200

Honjo T, Kubota S, Kamioka H, Sugawara Y, Ishihara Y, Yamashiro T, Takigawa M, Takano-Yamamoto T (2012) Promotion of Ccn2 expression and osteoblastic differentiation by actin polymerization, which is induced by laminar fluid flow stress. J Cell Commun Signal 6:225–232. https://doi.org/10.1007/s12079-012-0177-4

Hoshijima M, Hattori T, Inoue M, Araki D, Hanagata H, Miyauichi A, Takigawa M (2006) CT domain of CCN2/CTGF directly interacts with fibronectin and enhances cell adhesion of chondrocytes through integrin alpha5beta1. FEBS Lett 580:1376–1382. https://doi.org/10.1016/j.febslet.2006.01.061

Hoshijima M, Hattori T, Aoyama E, Nishida T, Yamashiro T, Takigawa M (2012) Roles of heterotopic CCN2/CTGF-CCN3/NOV and homotypic CCN2-CCN2 interactions in expression of the differentiated phenotype of chondrocytes. FEBS J 279:3584–3597. https://doi.org/10.1111/j.1742-4658.2012.08717.x

Huang X, Zhu B, Wang X, Xiao R, Wang C (2016) Three-dimen-
sional co-culture of mesenchymal stem cells and differentiated osteoblasts on human bio-derived bone scaffolds supports active multi-lineage hematopoiesis in vitro: functional implication of the biomimetic HSC niche. Int J Mol Med 38:1141–1151. https://doi.org/10.3892/ijmm.2016.2712

Hussein K, Stucki-Koch A, Kreipe H (2018) Profile of fibrosis-
related gene transcripts and megakaryocytic changes in the bone marrow of myelodysplastic syndromes with fibrosis. Ann Hematol 97:2099–2106. https://doi.org/10.1007/s00277-018-3411-9

Igarashi A, Okochi H, Bradham DM, Grotendorst GR (1993) Regu-
lation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. Mol Biol Cell 4:637–645

Igarashi A, Segoshi K, Sakai Y, Pan H, Kanawa M, Higashi Y, Sugiyama M, Nakamura K, Kurihara H, Yamaguchi S, Tsuji K, Kawamoto T, Kato Y (2007) Selection of common markers for bone marrow stromal cells from various bones using real-time RT-PCR: effects of passage number and donor age. Tissue Eng 13:2405–2417. https://doi.org/10.1089/teng.2006.0340

Ihm SH, Chang K, Kim HY, Baek SH, Youn HJ, Seung KB, Kim JH (2010) Peroxisome proliferator-activated receptor-gamma activation attenuates cardiac fibrosis in type 2 diabetic rats: the effect of rosiglitazone on myocardial expression of receptor for advanced glycation end products and of connective tissue growth factor. Basic Res Cardiol 105:399–407. https://doi.org/10.1007/s00398-009-0071-x

Inoki I, Shiomi T, Hashimoto G, Enomoto H, Nakamura H, Makino K-i, Ikeda E, Takata S, Kobayashi K-i, Okada Y (2002) Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. FASEB J 16:219–221

Inoue T, Okada H, Kobayashi T, Watanabe Y, Kanno M, Kopp JB, Nishida T, Takigawa M, Uneo M, Nakamura T, Suzuki H (2003) Hepatocyte growth factor counteracts transforming growth factor-beta1, through attenuation of connective tissue growth factor induction, and prevents renal fibrogenesis in 5/6 nephrectomized mice. FASEB J 17:268–270. https://doi.org/10.1096/fj.02-0442fj

Ishihara J, Uemoto T, Yamato M, Shiratsuchi Y, Takaki S, Petrich BG, Nakauchi H, Eto K, Kitamura T, Okano T (2014) Cyclopeptide PRS.000000000004590

Istvanffy R, Viline B, Schreck C, Ruf F, Pagel C, Grziwok S, Henkel L, Przerzes da Costa O, Berndt J, Stumpfien V, Gotze KS, Schiemann M, Pelschel C, Mewes HW, Oostendorp RAJ (2015) Stromata-induced connective tissue growth factor maintains cell cycle progression and repopulation activity of hematopoietic stem cells in vitro stem. Cell Rep 5:702–715. https://doi.org/10.1016/j.celrep.2015.09.018

Ito Y, Aten J, Bende RJ, Oemar BS, Rabelink TJ, Weening JJ, Goldschmeding R (1998) Expression of connective tissue growth factor in human renal fibrosis. Kidney Int 53:835–861. https://doi.org/10.1046/j.1523-1755.1998.00820.x

Ivko SE, Yoon BS, Popoff SN, Safadi FF, Libuda DE, Stephenson RC, Daluiski A, Lyons KM (2003) Connective tissue growth factor coordinates chondrogenesis and angiogenesis during skeletal development. Development 130:2779–2791. https://doi.org/10.1242/dev.00505

Jed sadayn mats A, Chen CC, Kiree vl MA, L au LF, Lam SC (1999) Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin alpha(Hb)beta(3). J Biol Chem 274:24321–24327

Jensen J, Gentzkow G, Berman G, Senne L, Jewell M, Connall TP, Miller SR, Galiano RD, Young L (2018) Anti-CTGF oligonucleotide reduces severity of postural hypotensive scars in a randomized double-blind, within-subject, placebo-controlled study. Plast Reconstr Surg 142:192e–201e. https://doi.org/10.1097/PRS.0000000000005490

Jeon K-I, Phipps RP, Sime PJ, Huxlin KR (2015) Inhibitory effects of PPARalpha ligands on TGF-beta1-induced CTGF expression in cat corneal fibroblasts. Exp Eye Res 138:52–58. https://doi.org/10.1016/j.exer.2015.06.028

Ji X, Song L, Sheng L, Gao A, Zhao Y, Han S, Zhang Y, Zhu C, Zhao S, Wang Z, Xu B, Li L, Li J, Tan N, Zhao B (2018) Cyclopeptide RA-V inhibits organ enlargement and tumorigenesis induced by YAP activation. Cancers (Basel). https://doi.org/10.3390/cancers10110449

Johnson SK, Stewart JP, Bani R, Qu P, Barlogie B, van Rhee F, Shaughnessy JD Jr, Epstein J, Yaccoby S (2014) CYR61/CCN1 overexpression in the myeloma microenvironment is associated with superior survival and reduced bone disease. Blood 124:2051–2060. https://doi.org/10.1182/blood-2014-02-555813

Johnson BG, Ren S, Karaca G, Gomez IG, Fligny C, Smith B, Ergun A, Locke G, Gao B, Hayes S, MacDonnell S, Duffield JS (2017) Connective tissue growth factor domain 4 amplifies fibrotic kidney disease through activation of LDL receptor-related protein 6. J Am Soc Nephrol 28:1769–1782. https://doi.org/10.1681/ASN.2016080826

Jun JI, Lau LF (2011) Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. Nat Rev Drug Discov 10:945–963. https://doi.org/10.1038/nrd3599

Jung Y, Wang J, Schneider A, Sun YX, Koh-Paige AJ, Osman NI, McCauley LK, Taichman RS (2006) Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. Bone 38:497–508. https://doi.org/10.1016/j.bone.2005.10.003

Kaassboll OJ, Gadicherla AK, Wang JH, Mensen VT, Hagelin EMV, Dong MQ, Attramadal H (2018) Connective tissue growth factor (CCN2) is a matricellular preproprotein controlled by proteolytic activation. J Biol Chem 293:17953–17970. https://doi.org/10.1074/jbc.RA118.004559

Kafi R, Fisher GJ, Quan T, Shao Y, Wang R, Voorhees JJ, Kang S (2004) UV-A1 phototherapy improves nephrogenic fibrosing dermopathy. Arch Dermatol 140:1322–1324. https://doi.org/10.1001/archderm.140.11.1322

Kang H, Chen IM, Wilson CS, Bedrick EJ, Harvey RC, Atlas SR, Devi-
das M, Mullighan CG, Wang X, Murphy M, Ar K, Wharton W,
Kubota S, Kawata K, Yanagita T, Doi H, Kitoh T, Takigawa M (2004) Abundant retention and release of connective tissue growth factor (CTGF/CCN2) by platelets. J Biochem 136:279–282. https://doi.org/10.1093/jb/mvh126

Kubota S, Kawaki H, Kondo S, Yosimichi G, Minato M, Nishida T, Hanagata H, Miyauchi A, Takigawa M (2006) Multiple activation of mitogen-activated protein kinases by purified independent CCN2 modules in vascular endothelial cells and chondrocytes in culture. Biochimie 88:1973–1981. https://doi.org/10.1016/j.biochi.2006.07.007

Lachowski D, Cortes E, Robinson B, Rice A, Rombouts K, Del Rio (2013) Conjunction junction, what’s the function? CCN proteins. J Cell Sci 126:1631–1639. https://doi.org/10.1242/jcs.164006

Lau LF, Lam SC-T (1999) The CCN family of angiogenic regulators: The integrin connection. Ex Cell Res 248:44–57

Laug R, Fehrholz M, Schutze N, Kramer BW, Krump-Konvalinkova V, Speer CP, Kunzmann S (2012) IFN-gamma and TNF-alpha synergize to inhibit CTGF expression in human lung endothelial cells. PLoS ONE 7:e45430. https://doi.org/10.1371/journal.pone.0045430

Lee Y-S, Kim J-A, Kim KL, Jang H-S, Kim J-M, Lee J-Y, Shin I-S, Lee J, Hwang J, Kim S, Kang S, Lee S, Yoon J (2019) Aldosterone upregulates connective tissue growth factor expression via p38 MAPK pathway and mineralocorticoid receptor in ventricular myocytes. J Korean Med Sci 19:805–811. https://doi.org/10.3346/jkms.2004.19.6.805

Lee CH, Shah B, Moioli EK, Mao JJ (2010) CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. J Clin Invest 120:3340–3349. https://doi.org/10.1172/JCI43230

Lee MS, Ghim J, Kim SJ, Yun YS, Yoo SA, Suh PG, Kim WU, Ryu SH (2015) Functional interaction between CTGF and FPR1 regulates VEGF-A-induced angiogenesis. Cell Signal 27:1439–1448. https://doi.org/10.1016/j.cellsig.2015.04.001

Li AJ, Calvi LM (2017) The microenvironment in myelodysplastic syndromes: Niche-mediated disease initiation and progression. Exp Hematol 55:3–18. https://doi.org/10.1016/j.exphem.2017.08.003

Li G, Xie Q, Shi Y, Li D, Zhang M, Jiang S, Zhou H, Lu H, Jin Y (2006) Inhibition of connective tissue growth factor by siRNA prevents liver fibrosis in rats. J Gene Med 8:889–900. https://doi.org/10.1002/jgm.894

Li X, Ling W, Khan S, Yaccoby S (2012) Therapeutic effects of intrabone and systemic mesenchymal stem cell cytotherapy on myeloma bone disease and tumor growth. J Bone Miner Res 27:1635–1648. https://doi.org/10.1002/jbmr.1620

Li H, Li J, Cheng J, Chen X, Zhou L, Li Z (2019) AML-derived mesenchymal stem cells upregulate CTGF expression through the BMP pathway and induce K562ADM fusiform transformation and chemoresistance. Oncol Rep 42:1035–1046. https://doi.org/10.3892/or.2019.7237

Lin J, Liliensiek B, Kanitz M, Schimanski U, Böhrer H, Walldherr R, Martin E, Kauthmann G, Ziegler R, Narwoth PP (1998) Molecular cloning of genes differentially regulated by TNF-alpha in bovine aortic endothelial cells, fibroblasts and smooth muscle cells. Cardiovasc Res 38:802–813. https://doi.org/10.1016/s0008-6363(98)00055-8

Lindner U, Kramer J, Rohwedel J, Schlenke P (2019) Mesenchymal stem or stromal cells: toward a better understanding of their biology? Transfus Med Hemother 37:75–83. https://doi.org/10.1159/000290897

Liu S, Leask A (2013) CCN2 modulates hair follicle cycling in mice. Mol Biol Cell 24:3939–3944. https://doi.org/10.1091/mbc.E13-08-0472

Liu X, Luo F, Li J, Wu W, Li L, Chen H (2008) Homocysteine induces connective tissue growth factor expression in vascular smooth muscle cells. J Thromb Haemost 6:184–192. https://doi.org/10.1111/j.1538-7836.2007.02801.x

Liu SC, Hsu CJ, Chen HT, Tsou HK, Chung SM, Tang CH (2012) CTGF increases IL-6 expression in human synovial fibroblasts through integrin-dependent signaling pathway. PLoS ONE 7:e51097. https://doi.org/10.1371/journal.pone.0051097

Liu S, Herault Y, Pavlovic G, Leask A (2014) Skin progenitor cells contribute to bleomycin-induced skin fibrosis. Arthritis Rheumatol 66:707–713. https://doi.org/10.1002/art.38276

Liu H, Peng F, Liu Z, Jiang F, Li L, Gao S, Wang G, Song J, Ruan E, Shao Z, Fu R (2017) CYR61/CCN1 stimulates proliferation and differentiation of osteoblasts in vitro and contributes to bone remodeling in vivo in myeloma bone disease. Int J Oncol 50:631–639. https://doi.org/10.3892/ijo.2016.3815

Long X, Yu Y, Perlaky L, Man TK, Redell MS (2015) Stromal CYR61 confers resistance to mitoxantrone via spleen tyrosine kinase activation in human acute myeloid leukaemia. Br J Haematol 170:704–718. https://doi.org/10.1111/bjh.13492

Lu H, Kojima K, Battula VL, Korchin B, Shi Y, Chen Y, Spong S, Thomas DA, Kantarjian H, Lock RB, Andreeff M, Konopleva M (2014) Targeting connective tissue growth factor (CTGF) in acute lymphoblastic leukemia preclinical models: anti-CTGF monoclonal antibody attenuates leukemia growth. Ann Hematol 93:485–492. https://doi.org/10.1007/s00277-013-1939-2

Luo Q, Kang Q, Si W, Jiang W, Park JK, Peng Y, Li X, Luu HH, Luo J, Montag AG, Haydon RC, He TC (2004) Connective tissue growth factor (CTGF) is regulated by Wnt and bone morphogenetic proteins signaling in osteoblast differentiation of mesenchymal stem cells. J Biol Chem 279:55958–55968. https://doi.org/10.1074/jbc.M407810200

Maeda A, Nishida T, Aoyama E, Kubota S, Lyons KM, Kuboki T, Takigawa M (2009) CCN family 2/connective tissue growth factor modulates BMP signalling as a signal conductor, which action regulates the proliferation and differentiation of chondrocytes. J Biochem 145:207–216. https://doi.org/10.1093/jb/mvq159

Makino K, Makino T, Stawski L, Lipson KE, Leask A, Trojanowska M (2017) Anti-connective tissue growth factor (CTGF/CCN2) monoclonal antibody attenuates skin fibrosis in mice models of systemic sclerosis. Arthritis Res Ther 19:134. https://doi.org/10.1186/s13075-017-1356-3

Masuko K, Murata M, Yudoh K, Shimizu H, Beppu M, Nakamura H, Kato T (2010) Prostaglandin E2 regulates the expression of connective tissue growth factor (CTGF/CCN2) in human...
osteoarthritic chondrocytes via the EP4 receptor. BMC Res Notes 3:5. https://doi.org/10.1186/1756-0500-3-5

McCallum L, Price S, Plange N, Perbal B, Pierce A, Whetton AD, Irvine AE (2006) A novel mechanism for BCR-ABL action: stimulated secretion of CCN3 is involved in growth and differentiation regulation. Blood 108:1716–1723. https://doi.org/10.1182/blood-2006-04-016113

McCallum L, Lu W, Price S, Lazar N, Perbal B, Irvine AE (2009) CCN3: a key growth regulator in chronic myeloid leukaemia. J Cell Commun Signal 3:115–124. https://doi.org/10.1007/s12079-009-0058-8

McCallum L, Lu W, Price S, Lazar N, Perbal B, Irvine AE (2012) CCN3 suppresses mitogenic signalling and reinstates growth control mechanisms in chronic myeloid leukaemia. J Cell Commun Signal 6:27–35. https://doi.org/10.1007/s12079-011-0142-2

Mercurio S, Latinkic B, Itasaki N, Krumlauf R, Smith JC (2004) Connective-tissue growth factor modulates WNT signalling and interacts with the WNT receptor complex. Development 131:2137–2147. https://doi.org/10.1242/dev.01045

Midwood KS, Williams LV, Schwarzbauer JE (2004) Tissue repair and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 36:1031–1037. https://doi.org/10.1016/j.biocel.2003.12.003

Miyazaki O, Kurashita S, Fukamachi I, Endo K, Ng PS, Takehara K (2010) Subtraction method for determination of N-terminal connective tissue growth factor. Ann Clin Biochem 47:205–211. https://doi.org/10.1258/acb.2010.009182

Möhle R, Marcus Schittenhelm M, Failenschmid C, Bautz F, Kratz-Miyazaki O, Kurashita S, Fukamachi I, Endo K, Ng PS, Takehara K (2010) Functional response of leukemic blasts to stromal cell-derived factor-1 correlates with preferential expression of the chemokine receptor CXCR4 in acute myelomonocytic and lymphoblastic leukaemia. Br J Haematol 110:563–572

Mokalled MH, Patra C, Dickson AL, Tainier DY, Poss KD (2016) Injury-induced ctgfa directs glial bridging and spinal cord regeneration in zebrafish. Science 354:630–634. https://doi.org/10.1126/science.aaf2679

Moran-Jones K, Gloss BS, Varga Z, Xu L, Oliver N, Aten J, Joles JA, Vial C, Brandan E, Lyons KM, Goldschmeding R (2008) CTGF inhibits BMP-7 signaling in diabetic nephropathy. J Am Soc Nephrol 19:2098–2107. https://doi.org/10.1681/ASN.2007111261

Mundy C, Gannon M, Popoff SN (2014) Connective tissue growth factor (CTGF/CCN2) negatively regulates BMP-2 induced osteoblast differentiation and signaling. J Cell Physiol 229:672–681. https://doi.org/10.1002/jcp.24491

Nagasawa-Masuda A, Terai K (2017) Yap/Taz transcriptional activity is essential for vascular regression via Ctgf expression and actin polymerization. PLoS ONE 12:e0174633. https://doi.org/10.1371/journal.pone.0174633

Nakanishi T, Kimura Y, Tamura T, Ichikawa H, Yamaai YI, Sugi-Adler K, Serve H, Brugger W, Lothar Kanz L (2000) Functional response of leukemic blasts to stromal cell-derived factor-1 correlates with preferential expression of the chemokine receptor CXCR4 in acute myelomonocytic and lymphoblastic leukaemia. Br J Haematol 110:563–572

Nguyen XX, Muhammad L, Nietert PJ, Feghali-Bostwick C (2018) CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreatic cancer. Proc Natl Acad Sci U S A 110:12325–12330. https://doi.org/10.1073/pnas.1300415110

Neve A, Corrado A, Cantatore FP (2011) Osteoblast physiology in normal and pathological conditions. Cell Tissue Res 343:289–302. https://doi.org/10.1007/s00441-010-1086-1

Nguyen TQ, Roestenberg P, van Nieuwenhoven FA, Bovenschen N, Li Z, Xu L, Oliver N, Aten J, Joles JA, Vial C, Brandan E, Lyons KM, Goldschmeding R (2008) CTGF inhibits BMP-7 signaling in diabetic nephropathy. J Am Soc Nephrol 19:2098–2107. https://doi.org/10.1681/ASN.2007111261

Ong J, van Den Berg-Rabin S, Dikic I, Zon L (2015) Connective tissue growth factor modulates WNT signalling and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 36:1031–1037. https://doi.org/10.1016/j.biocel.2003.12.003

Omo-Asagbunla S, Murphy M, Godson C, Cannon S, Kato S, Mackenzie HS, Martin F, Murphy-Ullrich JE, Sage EH (2014) Revisiting the matricellular concept. Matrix Biol 37:1–14. https://doi.org/10.1016/j.matbio.2014.07.005

Neve A, Frese KK, Bapteiro TE, Nakagawa T, Sterllicht MD, Seeley TW, Pilsarski C, Jodrell DI, Spong SM, Tuveson DA (2013) CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreatic cancer. Proc Natl Acad Sci U S A 110:12325–12330. https://doi.org/10.1073/pnas.1300415110

Ong J, van Den Berg-Rabin S, Dikic I, Zon L (2015) Connective tissue growth factor modulates WNT signalling and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 36:1031–1037. https://doi.org/10.1016/j.biocel.2003.12.003

Omo-Asagbunla S, Murphy M, Godson C, Cannon S, Kato S, Mackenzie HS, Martin F, Murphy-Ullrich JE, Sage EH (2014) Revisiting the matricellular concept. Matrix Biol 37:1–14. https://doi.org/10.1016/j.matbio.2014.07.005

Neve A, Frese KK, Bapteiro TE, Nakagawa T, Sterllicht MD, Seeley TW, Pilsarski C, Jodrell DI, Spong SM, Tuveson DA (2013) CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreatic cancer. Proc Natl Acad Sci U S A 110:12325–12330. https://doi.org/10.1073/pnas.1300415110

Ong J, van Den Berg-Rabin S, Dikic I, Zon L (2015) Connective tissue growth factor modulates WNT signalling and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 36:1031–1037. https://doi.org/10.1016/j.biocel.2003.12.003

Omo-Asagbunla S, Murphy M, Godson C, Cannon S, Kato S, Mackenzie HS, Martin F, Murphy-Ullrich JE, Sage EH (2014) Revisiting the matricellular concept. Matrix Biol 37:1–14. https://doi.org/10.1016/j.matbio.2014.07.005

Neve A, Frese KK, Bapteiro TE, Nakagawa T, Sterllicht MD, Seeley TW, Pilsarski C, Jodrell DI, Spong SM, Tuveson DA (2013) CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreatic cancer. Proc Natl Acad Sci U S A 110:12325–12330. https://doi.org/10.1073/pnas.1300415110

Neve A, Corrado A, Cantatore FP (2011) Osteoblast physiology in normal and pathological conditions. Cell Tissue Res 343:289–302. https://doi.org/10.1007/s00441-010-1086-1

Nguyen TQ, Roestenberg P, van Nieuwenhoven FA, Bovenschen N, Li Z, Xu L, Oliver N, Aten J, Joles JA, Vial C, Brandan E, Lyons KM, Goldschmeding R (2008) CTGF inhibits BMP-7 signaling in diabetic nephropathy. J Am Soc Nephrol 19:2098–2107. https://doi.org/10.1681/ASN.2007111261

Nguyen XX, Muhammad L, Nietert PJ, Feghali-Bostwick C (2018) IGFBP-5 promotes fibrosis via increasing its own expression and that of other pro-fibrotic mediators. Front Endocrinol (Lausanne) 9:601. https://doi.org/10.3389/fendo.2018.00601

Nishida T, Nakanshi T, Shimo T, Takigawa M (2000) Effects of CTGF/Hcs24, a hypertrophic chondrocyte-specific gene product, on the proliferation and differentiation of osteoblastic cells in vitro. J Cell Physiol 184:197–206
Paradis V, Perlemuter G, Bonvoult F, Dargere D, Parfait B, Vidaud M, Conti M, Huet S, Ba N, Buffet C, Bedossa P (2001) High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 34:738–744. https://doi.org/10.1053/jhep.2001.28055

Peidí A, Perbal B, Leask A (2019) Yin/Yang expression of CCN family members: Transforming growth factor beta 1, via ALK5/FAK/MEK, induces CCN1 and CCN2, yet suppresses CCN3, expression in human dermal fibroblasts. PLoS ONE 14:e0218178. https://doi.org/10.1371/journal.pone.0218178

Perbal B (2018) The concept of the CCN protein family revisited: a centralized coordination network. J Cell Commun Signal 12:3–12. https://doi.org/10.1007/s12079-018-0455-5

Perbal B, Tweedie S,布鲁福德 E (2018) The official unified nomenclature adopted by the HGNC calls for the use of the acronyms, CCN1–6, and discontinuation in the use of the acronyms, CYR61, CTGF, NOV and WISP 1–3 respectively. J Cell Commun Signal 12:625–629. https://doi.org/10.1007/s12079-018-0491-1

Pereira RC, Durant D, Canalis E (2000) Transcriptional regulation of connective tissue growth factor by cortisol in osteoblasts. Am J Physiol Endor Metab 279:E570–576. https://doi.org/10.1152/ajpendo.2000.279.3.E570

Pi L, Ding X, Jorgensen M, Pan J, Oh SH, Pintilie D, Brown A, Song WY, Petersen BE (2008) Connective tissue growth factor with a novel fibroconnectin binding site promotes cell adhesion and migration during rat oval cell activation. Hepatology 47:996–1004. https://doi.org/10.1002/hep.22079

Pi L, Shenoy AK, Liu J, Kim S, Nelson N, Xia H, Hauswirth WW, Petersen BE, Schultz GS, Scott EW (2012) CCN2/CTGF regulates neovessel formation via targeting structurally conserved cystine knot motifs in multiple angiogenic regulators. FASEB J 26:3365–3379. https://doi.org/10.1096/fj.11-200154

Pinho S, Frenette PS (2019) Haematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol 20:303–320. https://doi.org/10.1038/s41580-019-0103-9

Piszczałowski RT, Rafferty BJ, Rozado A, Parziale JV, Lents NH (2015) Myeloid Zinc Finger 1 (MZF-1) regulates expression of the CCN2/CTGF and CCN3/NOV genes in the hematopoietic compartment. J Cell Physiol 230:2634–2639. https://doi.org/10.1002/jcp.25021

Pobbati AV, Hong W (2020) A combat with the YAP/TAZ-TEAD oncoproteins for cancer therapy. Theranostics 10:3622–3635. https://doi.org/10.7150/thno.40889

Preisser F, Giehl K, Rehm M, Goppelt-Struebe M (2016) Inhibitors of oxygen sensing prolyl hydroxylases regulate nuclear localization of the transcription factors Smad2 and YAP/TAZ involved in CTGF synthesis. Biochim Biophys Acta 1863:2027–2036. https://doi.org/10.1016/j.bbamcr.2016.05.001

Qiao G, Xia D, Cheng Z, Zhang G (2017) miR132 in atrial fibrillation directly targets connective tissue growth factor. Mol Med Rep 16:4143–4150. https://doi.org/10.3892/mmr.2017.7045

Quan T, Shin S, Qin Z, Fisher GJ (2009) Expression of CCN family of genes in human skin in vivo and alterations by solar-simulated ultraviolet irradiation. J Cell Commun Signal 3:19–23. https://doi.org/10.1007/s12079-009-0044-8

Raghu G, Scholand MB, de Andrade J, Lancaster L, Mago D, Goldin J, Brown KK, Flaherty KR, Wencel M, Wanger J, Neff T, Valone F, Stauffer J, Porter S (2016) FG-3019 anti-connective tissue growth factor monoclonal antibody: results of an open-label clinical trial in idiopathic pulmonary fibrosis. Eur Respir J 47:1481–1491. https://doi.org/10.1183/13993003.01030-2015

Raghunathan VK, Dreier B, Morgan JT, Tuyen BC, Rose BW, Reilly CM, Russell P, Murphy CJ (2014) Involvement of YAP, TAZ and HSP90 in contact guidance and intercellular junction formation
in corneal epithelial cells. PLoS ONE 9:e109811. https://doi.org/10.1371/journal.pone.0109811

Ramazani Y, Knops N, Elmonem MA, Nguyen TQ, Arcolino FO, van den Heuvel L, Levchenko E, Kuypers D, Goldsmeding R (2018) Connective tissue growth factor (CTGF) from basics to clinics. Matrix Biol 68–69:44–66. https://doi.org/10.1016/j.matbio.2018.03.007

Rayego-Mateos S, Rodrigues-Diez R, Morgado-Pascual JL, Rodrigues Diez RR, Mas S, Lavoiz C, Alique M, Pato J, Keri G, Ortiz A, Egido J, Ruíz-Ortega M (2013) Connective tissue growth factor is a new ligand of epidermal growth factor receptor. J Mol Cell Biol 5:323–335. https://doi.org/10.1093/jmcb/mjt030

Ren J, Jin P, Sabatino M, Balakumaran A, Feng J, Kuznetsov SA, Klein HG, Robey PG, Strongeck DF (2011) Global transcriptome analysis of human bone marrow stromal cells (BMSC) reveals proliferative, mobile and interactive cells that produce abundant extracellular matrix proteins, some of which may affect BMSC potency. Cytotherapy 13:661–674. https://doi.org/10.3109/1465249.2010.548379

Ren S, Johnson BG, Kida Y, Ip C, Davidson KC, Lin S-L, Kobayashi A, Lang RA, Hadjantonakis A-K, Moon RT, Duffield JS (2013) LRP-6 is a coreceptor for multiple fibrogenic signaling pathways in pericytes and myofibroblasts that are inhibited by DKK-1. Proc Natl Acad Sci U S A 110:1440–1445. https://doi.org/10.1073/pnas.1111791110

Ren Y, Du C, Shi Y, Wei J, Wu H, Cui H (2017) The Sirt1 activator by prostaglandin E(2). Am J Physiol 277:L1165–1171. https://doi.org/10.1152/ajplung.1999.277.6.L1165

Richeldi L, Fernández Pérez ER, Costabel U, Albera C, Lederer DJ, Flaherty KR, Ettinger N, Perez R, Scholand MB, Golchin J, Poeny Yu K-H, Neff T, Porter S, Zhong M, Gorina E, Kouchaki E, Raghu G (2020) Pamrevelumab, an anti- connective tissue growth factor therapy, for idiopathic pulmonary fibrosis (PRAISE): a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Respir Med 8:25–33. https://doi.org/10.1016/S2213-2600(19)30262-0

Ricupero DA, Rishikof DC, Kuang PP, Poliks CF, Goldstein RH (2017) The Sirt1 activator by prostaglandin E(2). Am J Physiol 277:L1165–1171. https://doi.org/10.1152/ajplung.1999.277.6.L1165

Riser BL, Denichilo M, Cortes P, Baker C, Grondin JM, Yee J, Narins DH, Radich JP (2007) Connective tissue growth factor (CTGF) and the fibrosis of diabetic renal disease. Am J Pathol 174:1725–1734. https://doi.org/10.1016/j.ajpath.2007.08.025

Rooney B, O’Donovan H, Gaffney A, Browne M, Faherty N, Curran SP, Sadlier D, Godson C, Brazil DP, Crean J (2011) CTGF/ CCN2 activates canonical Wnt signalling in mesangial cells through LRP6: implications for the pathogenesis of diabetic nephropathy. FEBS Lett 585:531–538. https://doi.org/10.1016/j.febslet.2011.01.004

Rozado A, Piszczatowski RT, Rafferty BJ, Lents NH (2014) Regulation of CCN2 and CCN3 in bone marrow through myloid zinc finger-1 and its medical implication in hematopoesis (1005.4). FASEB J 28:1005.4

Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131:324–336. https://doi.org/10.1016/j.cell.2007.08.025

Safadi FF, Xu J, Smock SL, Kanaan RA, Selim AH, Odgren PR, Marks SC Jr, Owen TA, Popoff SN (2003) Expression of connective tissue growth factor in bone: its role in osteoblast proliferation and differentiation in vitro and bone formation in vivo. J Cell Physiol 196:51–62. https://doi.org/10.1002/jcp.10319

Sacai K, Nakamura M, Lipson KE, Miyake T, Kamikawa Y, Sagara A, Shinozaki Y, Kitajima S, Toyama T, Hara A, Iwata Y, Shimizu M, Furuiuchi K, Kaneko S, Tager AM, Wada T (2017) Inhibition of CTGF ameliorates peritoneal fibrosis through suppression of fibroblast and myofibroblast accumulation and angiogenesis. Sci Rep 7:5392. https://doi.org/10.1038/s41598-017-05624-2

Sala-Torra O, Gundacker HM, Stirewalt DL, Ladne PA, Pogosova-Agadjanyan EL, Slovak ML, Willman CL, Heimfeld S, Boldt DH, Radich JP (2007) Connective tissue growth factor (CTGF) expression and outcome in adult patients with acute lymphoblastic leukemia. Blood 109:3080–3083. https://doi.org/10.1182/blood-2006-06-031096

Sangaletti S, Chiodoni C, Tripodo C, Colombo MP (2017) Common extracellular matrix regulation of myeloid cell activity in the bone marrow and tumor microenvironments. Cancer Immunol Immunother 66:1059–1067. https://doi.org/10.1007/s00262-017-1942-5

Scheper K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T, Wagers AJ, Hsiao EC, Passegue E (2013) Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell 13:285–299. https://doi.org/10.1016/j.stem.2013.06.009

Schilld C, Trueb B (2002) Mechanical stress is required for high-level expression of connective tissue growth factor. Exp Cell Res 274:83–91. https://doi.org/10.1006/excr.2001.5458

Schilld C, Trueb B (2004) Three members of the connective tissue growth factor family CCN are differentially regulated by mechanical stress. Biochim Biophys Acta 1691:33–40. https://doi.org/10.1016/j.bbamcr.2003.12.001

Schober JM, Chen N, Grzeszkiewicz TM, Jovanovic I, Emsen EE, Ugarova TP, Ye RD, Lau LF, Lam SC-T (2002) Identification of integrin alpha(M)beta(2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): immediate-early gene products expressed in atherosclerotic lesions. Blood 99:4457–4465. https://doi.org/10.1182/blood-vol.99.12.4457

Schotte D, Chau JC, Sylvester G, Liu G, Chen C, van der Velden VH, Broekhuis MJ, Peters TC, Pieters R, den Boer ML (2009) Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia 23:313–322. https://doi.org/10.1038/leu.2008.286

Roestenberg P, van Nieuwenhoven FA, Wieten L, Boer P, Diekman T, Tiller AM, Wiersinga WM, Oliver N, Usinger W, Weitz S, Schlingemann RO, Goldsmeding R (2004) Connective tissue growth factor is increased in plasma of type 1 diabetic patients with nephropathy. Diabetes Care 27:1164–1170
Shimotani T, Nakanishi T, Nishida T, Asano M, Sasaki A, Kanyama M, Segarini PR, Nesbitt JE, Li D, Hays LG, Yates JR 3rd, Carmichael DF (2001) Inhibition of endogenous expression of connective tissue growth factor by its antisense oligonucleotide and antisense RNA suppresses proliferation and migration of vascular endothelial cells. J Biochem 124:130–140

Shimotani T, Nakanishi T, Nishida T, Asano M, Kanyama M, Kuboki T, Tamatani T, Tzeku K, Takemura M, Matsumura T, Takigawa M (1999) Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells in vitro, and angiogenesis in vivo. J Biochem 126:137–145. https://doi.org/10.1093/jbc/M105180200

Shimo T, Nakanishi T, Shi-Wen X, Renzoni EA, Kennedy L, Howat S, Chen Y, Pearson JD, Shi-wen X, Pennington D, Holmes A, Leask A, Bradham D, Beau

Shimo T, Nakanishi T, Nishida T, Asano M, Sasaki A, Kanyama M, Kuboki T, Matsumura T, Takigawa M (2001a) Involvement of CTGF, a hypertrophic chondrocyte-specific gene product, in tumor angiogenesis. Oncology 61:315–322

Shimo T, Kubota S, Kondo S, Nakanishi T, Sasaki A, Mese H, Matsumura T, Takigawa M (2001b) Connective tissue growth factor as a major angiogenic agent that is induced by hypoxia in a human breast cancer cell line. Cancer Lett 174:57–64. https://doi.org/10.1016/S0304-3835(01)00683-8

Shinde A, Epperly MW, Cao S, Holt D, Goff J, Shields D, Franicola D, Wipf P, Wang H, Greenberger JS (2014) Improved hematopoiesis in GS-Nitroxide (JP4–039)-treated mouse long-term bone marrow cultures and radioreistance of derived bone marrow stromal cell lines. In Vivo 28:699–708

Shi-wen X, Pennington D, Holmes A, Pedro W, Nishida T, Hoffman S, Nguyen XX, Pilewski JM, Feghali-Bostwick C (2019) Insulin-like growth factor binding protein-4 exerts antifibrotic activity by reducing levels of connective tissue growth factor and the C-X-C chemokine receptor 4. FASEB J 33:2176–2189. https://doi.org/10.1096/fj.2018-021483

Su Y, Nishimoto T, Hoffman S, Nguyen XX, Pilewski JM, Feghali-Bostwick C (2019) Insulin-like growth factor binding protein-4 exerts antifibrotic activity by reducing levels of connective tissue growth factor and the C-X-C chemokine receptor 4. FASEB J 33:2176–2189. https://doi.org/10.1096/fj.2018-021483

Sugiyama T, Hirose T, Shibata H, Noda M, Nagasawa T (2006) Maintenance of the hematopoietic stem cell pool by CXCL12–CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 25:977–988. https://doi.org/10.1016/j.immuni.2006.10.016

Sumiyoshi K, Kubota S, Furuta RA, Yasui K, Aoyama E, Kawaki H, Kawata K, Oshgawa T, Yamashiro T, Takigawa M (2010) Thrombopoietic-mesenchymal interaction that may facilitate both endothelial ossification and platelet maturation via CCN2. J Cell Commun Signal 4:5–14. https://doi.org/10.1007/s12079-009-0067-1

Sun K, Wang Q, Huang XH (2006) PPAR gamma inhibits growth of rat hepatic stellate cells and TGF beta-induced connective tissue growth factor expression. Acta Pharmacol Sin 27:715–723. https://doi.org/10.1111/j.1745-7256.2006.00299.x

Sun D, Han S, Liu C, Zhou R, Sun W, Zhang Z, Qu J (2016) MicroRNA-199a-5p functions as a tumor suppressor via suppressing connective tissue growth factor (CTGF) in follicular thyroid carcinoma. Med Sci Monit 22:1210–1217. https://doi.org/10.12659/msm.895788

Sung DK, Kong WH, Park K, Kim JH, Kim MY, Kim H, Hahn SK (2013) Noncovalent PE-Glated CTGF siRNA/PDMAEMA complex for pulmonary treatment of bleomycin-induced lung fibrosis. Biomaterials 34:1261–1269. https://doi.org/10.1016/j.biomaterials.2012.09.061

Suresh S, McCallum L, Wu H, Lazar N, Perbal B, Irvine AE (2011) MicroRNAs 130a/9 are regulated by BCR-ABL and downregulate expression of CCN3 in CML. J Cell Commun Signal 5:183–191. https://doi.org/10.1007/s12079-011-0139-x

Suresh S, McCallum L, Crawford LJ, Lu WH, Sharpe DJ, Irvine AE (2013) The matricellular protein CCN3 regulates NOTCH1 signalling in chronic myeloid leukaemia. J Pathol 231:378–387. https://doi.org/10.1002/path.4246

Surman-Schmitt C, Sasaki T, Hattori T, Eitinger N, Schett G, von der Mark K, Stock M (2012) The Wnt antagonist Wif-1 interacts with CTGF and inhibits CTGF activity. J Cell Physiol 227:2207–2216. https://doi.org/10.1002/jcp.22957

Suzuma K, Naruse K, Suzuma I, Takahara N, Ueki K, Aiello LP, King GL (2000) Vascular endothelial growth factor induces expression of connective tissue growth factor via KDR, Flt1, and phosphatidylinositol 3-kinase-akt-dependent pathways in retinal vascular cells. J Biol Chem 275:40725–40731. https://doi.org/10.1074/jbc.M00509200

Tabe Y, Konopleva M, Munsell MF, Marini FC, Zompetta C, McQueen T, Tao S, Zhao S, Pilewski JM, Feghali-Bostwick C, Roger WS, Igi J, Estey EH, Andreff M (2004) PML–RARalpha is associated with leptin-receptor induction: the role of mesenchymal stem cell-derived adipocytes in APL cell survival. Blood 103:1815–1822. https://doi.org/10.1182/blood-2003-03-0802

Taichman RS, Emerson SG (1994) Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J Exp Med 179:1677–1682. https://doi.org/10.1084/jem.179.5.1677

Takigawa M (2013) CCN2: a master regulator of the genesis of bone and cartilage. J Cell Commun Signal 7:191–201. https://doi.org/10.1007/s12079-013-0204-8

Takigawa M (2018) An early history of CCN2/CTGF research: the road to CCN2 via hcs24, ctgf, ecmogen, and regenerin. J Cell Commun Signal 12:253–264. https://doi.org/10.1007/s12079-017-0414-6

Tam EM, Morrison CJ, Wu YI, Stack MS, Overall CM (2004) Membrane protease proteomics: Isotope-coded affinity tag MS
identification of undescribed MT1-matrix metalloproteinase substrates. Proc Natl Acad Sci U S A 101:6917–6922. https:// doi.org/10.1073/pnas.0305862101

Tesfai Y, Ford J, Carter KW, Firth MJ, O’Leary RA, Gottardo NG, Cole C, Kees UR (2012) Interactions between acute lymphoblastic leukemia and bone marrow stromal cells influence response to therapy. Leuk Res 36:299–306. https://doi.org/10.1016/j.leukres.2011.08.001

Tikellis C, Cooper ME, Twigg SM, Burns WC, Tolcos M (2004) Connective tissue growth factor is up-regulated in the diabetic retina: amelioration by angiotensin-converting enzyme inhibition. Endocrinology 145:860–866. https://doi.org/10.1210/enu.2003-0967

Tong ZY, Brigstock DR (2006) Intrinsic biological activity of the thrombospondin structural homology repeat in connective tissue growth factor. J Endocrinol 188:R1-8. https://doi.org/10.1677/joe.1.06719

Tsai KD, Chen W, Wang SH, Hsiao YW, Chi JY, Wu HY, Lee YJ, Tong ZY, Brigstock DR (2006) Intrinsic biological activity of the thrombospondin structural homology repeat in connective tissue growth factor. Biochem J 359:89–97

Wahab NA, Weston BS, Mason RM (2005) Connective tissue growth factor CCN2 interacts with and activates the tyrosine kinase receptor TrkA. J Am Soc Nephrol 16:340–351. https://doi.org/10.1681 ASN.2003100905

Wang JJ, Ye F, Cheng LJ, Shi YJ, Bao J, Sun HQ, Wang W, Zhang P, Bu H (2009) Osteogenic differentiation of mesenchymal stem cells promoted by overexpression of connective tissue growth factor. J Zhejiang Univ Sci B 10:355–367. https://doi.org/10.1631/jzus.B0820252

Wang X, McLennan SV, Allen TJ, Twigg SM (2010) Regulation of pro-inflammatory and pro-fibrotic factors by CCN2/CTGF in H9c2 cardiomyocytes. J Cell Commun Signal 4:15–23. https://doi.org/10.1007/s12079-009-0083-1

Wei Q, Frenette PS (2018) Niches for hematopoietic stem cells and their progeny. Immunity 48:632–648. https://doi.org/10.1016/j.immuni.2018.03.024

Welch MD, Greene WK, Kees UR (2013) Hypomethylation of the CTGF gene locus is a common feature of paediatric pre-B acute lymphoblastic leukaemia. Br J Haematol 162:537–541. https://doi.org/10.1111/bjh.12417

Welch MD, Howlett M, Halse HM, Greene WK, Kees UR (2015) Novel CT domain-encoding splice forms of CTGF/CCN2 are expressed in B-lineage acute lymphoblastic leukaemia. Leuk Res 39:913–920. https://doi.org/10.1016/j.leukres.2015.05.008

Wells JE, Howlett M, Cole CH, Kees UR (2015) Deregulated expression of connective tissue growth factor (CTGF/CCN2) is linked to poor outcome in human cancer. Int J Cancer 137:504–511. https://doi.org/10.1002/ijc.28972

Wells JE, Howlett M, Halse HM, Heng J, Ford J, Cheung LC, Samuels AL, Crook M, Charles AK, Cole CH, Kees UR (2016) High expression of connective tissue growth factor accelerates dissemination of leukaemia. Oncogene 35:4591–4600. https://doi.org/10.1038/onc.2015.525

Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM (2003) Adult bone marrow is a rich source of human mesenchymal ‘stem’ cells but umbilical cord and mobilized adult bone are not. Br J Haematol 121:368–374

Winkler IG, Barbier V, Nowlan B, Jacobsen RN, Patton JT, Magnani JL, Levesque JP (2012) Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med 18:1651–1657. https://doi.org/10.1038/nm.2905

Wong M, Siegrist M, Goodwin K (2003) Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes. Bone 33:685–693. https://doi.org/10.1016/s8756-3282(03)00224-4

Wunderlich K, Senn BC, Todesco L, Flammer J, Meyer P (2000) Regulation of connective tissue growth factor gene expression in retinal vascular endothelial cells by angiogenic growth factors. Graefe’s Arch Clin Exp Ophthalmol 238(910):915

Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kutok JL, Rajewsky K (2008) Lymphoproliferative disease and autoimmunity in mice with increased miR-17–92 expression in lymphocytes. Nat Immunol 9:405–414. https://doi.org/10.1038/ni.1575

Xu J, Smock SL, Safadi FF, Rosenzweig AB, Ogden PR, Marks SC Jr, Owen TA, Popoff SN (2000) Cloning the full-length cDNA for rat connective tissue growth factor: implications for skeletal development. J Cell Biochem 77(103):115

Yamaai T, Nakanishi T, Asano M, Nawachi K, Yoshimichi G, Ohyama JT, Magnani JL, Levesque JP (2012) Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med 18:1651–1657. https://doi.org/10.1038/nm.2905

Yao Y, Stachura T, Brauchle K, Karakas E, Shaltiel E, Grunfelder S, Harsch I, Liu Y, Leary DJ, Shapira I (2012) Analysis of miR-17-92 family expression in human benign and malignant breast tumors reveals miR-17-92 overexpression in breast cancer. Breast Cancer Res Treat 131:319–330. https://doi.org/10.1007/s10544-011-1613-7

Zhong Z, Zhang H, Zhang X, Zhang J, Wang H, Li C, Zhang Y (2015) Atypical neurons may promote glioma invasion via connective tissue growth factor. Brain Res 1625:1–13. https://doi.org/10.1016/j.brainres.2015.01.019

Zhou Q, Zheng L, Zhong W, Song Y, Zhang Y (2012) Nuclear factor kappa B regulates the expression of connective tissue growth factor in HepG2 cells. J Zhejiang Univ Sci B 10:710–716. https://doi.org/10.1681/jzus.B1200052
Yamashiro T, Fukunaga T, Kobashi N, Kamioka H, Nakanishi T, Takigawa M, Takano-Yamamoto T (2001) Mechanical stimulation induces CTGF expression in rat osteocytes. J Dent Res 80:461–465. https://doi.org/10.1177/00220345010800021201

Yan LF, Wei YN, Nan HY, Yin Q, Qin Y, Zhao X, Chen BY, Zhao G, Wei JG, Cui GB (2014) Proliferative phenotype of pulmonary microvascular endothelial cells plays a critical role in the over-expression of CTGF in the bleomycin-injured rat. Exp Toxicol Pathol 66:61–71. https://doi.org/10.1016/j.etp.2013.08.004

Yang DH, Kim HS, Wilson EM, Rosenfield RG, Oh Y (1998) Identification of glycosylated 38-kDa connective tissue growth factor (IGFBP-related protein 2) and proteolytic fragments in human biological fluids, and up-regulation of IGFBP-rP2 expression by TGF-beta in Hs578T human breast cancer cells. J Clin Endocrinol Metab 83:2593–2596. https://doi.org/10.1210/jcem.83.7.5097

Yang M, Huang H, Li J, Li D, Wang H (2004) Tyrosine phosphorylation of the LDL receptor-related protein (LRP) and activation of the ERK pathway are required for connective tissue growth factor to potentiate myofibroblast differentiation. FASEB J 18:1920–1921. https://doi.org/10.1096/fj.04-2357fje

Yeger H, Perbal B (2016) CCN family of proteins: critical modulators of the tumor cell microenvironment. J Cell Commun Signal 10:229–240. https://doi.org/10.1007/s12079-016-0346-6

Yeh CH, Moles R, Nicot C (2016) Clinical significance of microRNAs in chronic and acute human leukemia. Mol Cancer 15:37. https://doi.org/10.1186/s12943-016-0518-2

Yendamuri S, Calin GA (2009) The role of microRNA in human leukemia: a review. Leukemia 23:1257–1263. https://doi.org/10.1038/leu.2008.382

Yokoi H, Mukoyama M, Nagae T, Mori K, Suganami T, Sawai K, Yoshioita T, Koshikawa M, Nishida T, Takigawa M, Sugawara A, Nakao K (2004) Reduction in connective tissue growth factor by antisense treatment ameliorates renal tubulointerstitial fibrosis. J Am Soc Nephrol 15:1430–1440. https://doi.org/10.1097/01asn.0000130565.69170.85

Yoon PO, Park JW, Lee CM, Kim SH, Kim HN, Ko Y, Bae SJ, Yun S, Park JH, Kwon T, Kim WS, Lee J, Lu Q, Kang HR, Cho WK, Elias JA, Yang JS, Park HO, Lee K, Lee CG (2016) Self-assembled micelle interfering RNA for effective and safe targetting of dysregulated genes in pulmonary fibrosis. J Biol Chem 291:6433–6446. https://doi.org/10.1074/jbc.M115.693671

Yoshihara H, Arai F, Hosokawa K, Hagiwara T, Takubo K, Nakamura Y, Gomei Y, Iwasaki H, Matsukawa S, Miyamoto K, Miyazaki H, Takahashi T, Suda T (2007) Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell 1:685–697. https://doi.org/10.1016/j.stem.2007.10.020

Yosimichi G, Kubota S, Nishida T, Kondo S, Yanagita T, Naka K, Takano-Yamamoto T, Takigawa M (2006) Roles of PKC, PI3K and JNK in multiple transduction of CCN2/CTGF signals in chondrocytes. Bone 38:853–863. https://doi.org/10.1016/j.bone.2005.11.016

Zhang X, Chen X, Liu J, Dong X, Jin Y, Tian Y, Xue Y, Chen L, Chang Y, Liu Y, Wang J (2015) Knockdown of WISP1 inhibit proliferation and induce apoptosis in ALL Jurkat cells. Int J Clin Exp Pathol 8:15489–15496

Zhang X, Wang Y, Guo Q, Diao Y, Liu H, Song G, Wang W, Zhang Z, Yin H, Li L (2018) Prognostic role of microRNA-155 in patients with leukemia: a meta-analysis. Clin Chim Acta 483:6–13. https://doi.org/10.1016/j.cca.2018.04.015

Zhao C, Chen W, Yang L, Stimpson SA, Diehl AM (2006) PPARgamma agonists prevent TGFbeta1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 350:385–391. https://doi.org/10.1016/j.bbrc.2006.09.069

Zhao M, Tao F, Venkatraman A, Li Z, Smith SE, Unruh J, Chen S, Ward C, Qian P, Perry JM, Marshall H, Wang J, He XC, Li L (2019) N-cadherin-expressing bone and marrow stromal progenitor cells maintain reserve hematopoietic stem cells. Cell Rep 26(652–669):e656. https://doi.org/10.1016/j.celrep.2018.12.093

Zhou BO, Yu H, Yue R, Zhao Z, Rios JJ, Naveiras O, Morrison SJ (2017) Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF. Nat Cell Biol 19:891–903. https://doi.org/10.1038/ncll3570

Zhu RJ, Wu MQ, Li ZJ, Zhang Y, Liu KY (2013) Hematopoietic recovery following chemotherapy is improved by BADGE-induced inhibition of adipogenesis. Int J Hematol 97:58–72. https://doi.org/10.1007/s12185-012-1233-4

Zhu X, Song Y, Wu C, Pan C, Lu P, Wang M, Zheng P, Huo R, Zhang C, Li W, Lin Y, Cao Y, Li N (2016) Cyr61 participates in the pathogenesis of acute lymphoblastic leukemia by enhancing cellular survival via the AKT/NF-kappaB signaling pathway. Sci Rep 6:34018. https://doi.org/10.1038/srep34018

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