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REVIEW

Matricellular protein CCN1/CYR61: a new player in inflammation and leukocyte trafficking

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Abstract Cystein-rich protein 61 (CYR61/CCN1) is a component of the extracellular matrix, which is produced and secreted by several cell types including endothelial cells, fibroblasts and smooth muscle cells. CCN1 has been implicated in leukocyte migration and the inflammatory process, but it is also involved in cardiovascular development and carcinogenesis. It exerts its functions through binding to multiple integrins present in many different cell types. This multiplicity in function is now known to contribute to the diverse array of cellular processes it can regulate. The expression of CCN1 is tightly regulated by cytokines and growth factors. However, CCN1 can directly modulate cell adhesion and migratory processes whilst simultaneously regulating the production of other cytokines and chemokines through paracrine and autocrine feedback loops. This complex functionality of CCN1 has highlighted the pivotal role this molecule can play in regulating the immunosurveillance process. Furthermore, CCN1 has now emerged as an important partner when targeting components of the infectious or chronic inflammatory disease processes such as atherosclerosis or rheumatoid arthritis. This review will focus on CYR61/CCN1 and its ability to control the migration of leukocytes, the production of cytokines and cell proliferation or senescence at the site of inflammation.

Keywords CCN1 · CYR61 · Extracellular matrix · Leukocyte migration · Inflammation

Abbreviations

CCN1/CYR61	Cysteine-rich protein 61
ECM	Extracellular matrix
FLS	Fibroblast-like synoviocytes
LPA	Lysophosphatidic acid
MMP	Metalloproteinase
RA	Rheumatoid arthritis
VSMC	Vascular smooth muscle cells

Introduction

The immune system consists of a heterogeneous cell population that shares the ability to rapidly respond to tissue and organ injury. Research in immunology is mainly focused on the roles of inflammatory cytokines and chemokines, antigenic and co-stimulation signals or receptors for pathogen-associated molecular patterns. However, the extracellular matrix (ECM) is emerging as an essential partner modulating the course of inflammation. Aside from providing a structural support for cell adhesion, the ECM influences various aspects of cellular life not only in physiological conditions but also in pathological conditions [1, 2]. Immune cells infiltrating the site of inflammation secrete not only cytokines but also proteases, such as matrix metalloproteinases (MMP), which modify the synthesis of ECM by tissue resident cells or their cleavage. Consequently, modified ECM can alter cell migration, activation, differentiation and survival. For example, cleavage of type I collagen by MMPs enables a chemotactic activity analogous to CXC-chemokine ligand 8 (CXCL8) on neutrophils during lung inflammation [3]. ECM compounds such as fibronectin, heparin sulphate or hyaluronan, have been

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well studied over many years; however, the CCN family of proteins has not been as well characterised for their role in the inflammatory response [4].

Named after the first three members, cysteine-rich protein 61 (CYR61; CCN1), connective tissue growth factor (CTGF; CCN2) and nephroblastoma overexpressed protein (NOV; CCN3), the CCN family comprises six secreted proteins that share a similar modular structure consisting of four domains exhibiting sequence homologies to insulin-like growth factor binding protein, von Willebrand factor type C, thrombospondin type I and a cysteine knot motif [4, 5]. CCN proteins were initially identified as immediate-early genes whose secretion was induced by mitogenic factors [4–6]. More recently, studies revealed that these matricellular proteins play critical roles in cardiovascular development, inflammation, injury repair and cancer. Their functionality is mediated via specific integrin binding and heparin sulphate proteoglycans, which in turn can activate signalling pathways responsible for the regulation of cell adhesion, migration, proliferation or senescence. In addition, CCN binds growth factors (BMP, TGFβ) or ECM-associated proteins such as laminin, thus bridging cells and extracellular ligands (Fig. 1) [4, 5, 7].

In this review, we will focus on CYR61/CCN1 and how it is implicated in many different pathways of the immune and inflammatory process.

Expression and regulation of CCN1 under homeostasis

Under homeostatic conditions, matricellular protein CYR61/CCN1 is expressed at low levels in most adult tissues by endothelial cells, fibroblasts and vascular smooth muscle cells (VSMC). In addition, intracellular flow cytometry studies revealed constitutive expression of CCN1 in human leukocytes circulating in the blood [8]. No expression was reported in developing murine thymocytes [9]. During embryonic development, the importance of CCN1 for the cardiovascular system has been demonstrated by the phenotype of *Ccn1*-deficent mice, which are embryonic lethal because of cardiovascular defects [10] and deficiencies in promoting angiogenesis [11, 12].

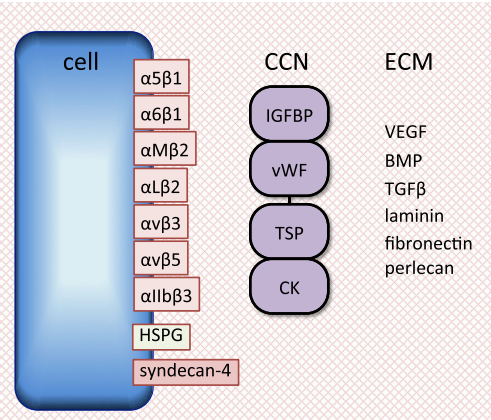

Fig. 1 Schematic representation of CCNs. CCNs share a modular structure consisting of four domains exhibiting sequence homologies to insulin-like growth factor binding protein (*IGFBP*), von Willebrand factor type C (*vWF*), thrombospondin type I (*TSP*) and a cysteine knot motif (*CK*). They bridge cells to ECM compounds by binding cell surface integrins and heparin sulphate proteoglycans (*HSPG*)

Expression and regulation under pathological conditions

Although expressed at low levels during steady-state homeostasis, it is remarkable how CCN1 expression is induced by serum growth factors, cytokines and environmental stress.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease that principally manifests in the synovial joints. Overexpression of CCN1 protein has been reported in fibroblast-like synoviocytes (FLS), with elevated levels observed in synovial fluid samples from RA patients as compared with healthy controls and patients suffering from osteoarthritis [13]. Similarly, CCN1 mRNA was strongly increased in lymphoblastoid B cell lines derived from RA-discordant monozygotic twins [13, 14], being one of the three most overexpressed genes [14]. Interestingly, mRNA and protein expression of CCN1 is inhibited by TNFα in chondrocytes [15–17], whereas it is strongly induced in primary human osteoblasts and U2OS cells by TNFα, oncostatin M and the pro-inflammatory cytokines IL6 and IL1β [16, 17]. However, TNFα was shown to have no effect on CCN1 expression in FLS. The expression of CCN1 in FLS is induced by IL17 in a p38 MAPK and NF-κB-dependent manner and suppressed by simvastatin treatment [16–19]. These findings strongly suggest that CCN1 plays a key role in inflammation and chronic inflammatory disease.

Bacterial and viral infection

Lysophosphatidic acid (LPA) is a bioactive lysophospholipid that can bind to specific G protein-coupled receptors termed LPA receptors. These interactions have been shown promote morphological changes and cell proliferation/survival. Production of LPA is a common feature of many bacteria. In vitro infection of primary murine epithelial cells or a human epithelial cell line with the Gram-negative bacteria *Yersinia enterocolitica* led to increased mRNA expression of CCN1 through the activation of LPA receptors [20, 21]. Similar results have also been obtained using Gram-negative bacteria

Escherichia coli and *Pseudomonas aeruginosa* and Gram-positive bacteria *Enterococcus faecalis* or *Staphylococcus aureus* [21]. Induction of CCN1 protein has also been shown to occur in primary murine hepatocytes stimulated with LPS in vitro through the TLR4/MyD88/AP-1 pathway and hepatocytes of LPS-stimulated normal and obese mice [22]. Consistent with these observations, increased levels of CCN1 have been reported in the blood of patients with sepsis, compared to healthy controls [8].

Comparably, in vitro infection of human epithelial cell lines with poliovirus, hepatitis C virus [23] and coxsackievirus B3 [24] or in vivo with oncolytic HSV-1 in rats [25] also led to upregulation of CCN1 expression in epithelial cells. The induction of CCN1 following coxsackievirus B3 infection occurs through the activation of JNK, which was induced by virus replication and viral protein expression in infected epithelial cells [24]. Interestingly, inhibition of CCN1 expression with shRNA reduces coxsackievirus B3 growth [24] whilst CCN1 treatment reduced HSV-1-derived oncolytic virus replication and cytotoxicity [26].

These studies have strongly highlighted the induction of CCN1 as a common host response to bacterial or viral infection.

Vascular injury

Protein and mRNA levels of CCN1 are increased in the plasma of patients with giant cell myocarditis [8] and cardiac biopsies of patients with dilated cardiomyopathy, respectively [27]. This was also observed in a mouse model of myocardial infarction [28] and diabetic retinopathy [29]. In the context of diabetic retinopathy, CCN1 is processed by MMP2 and MMP14 [30–32], where different domains are observed to exhibit proangiogenic or antiangiogenic activities [32]. The expression of CCN1 was upregulated in arterial smooth muscle walls during restenosis following balloon angioplasty in rat carotid artery, as part of ECM remodelling resulting from vascular injury [33]. Furthermore, CCN1 was shown to be highly expressed, via angiotensin 2, in aortic arteriosclerotic plaques of *ApoE*-deficient mice and in human arteriosclerotic lesions [34–37].

Colon inflammation

A role for angiogenesis and angiogenic factors is established in inflammatory bowel diseases. In this context, increased levels of CCN1 were measured in the colons of patients with Crohn's disease and ulcerative colitis and in colons of mice treated with dextran sodium sulphate to induce inflammatory colitis [38].

Receptors of CCN1

Matricellular CCN1 has the ability to mediate cell adhesion, mainly through binding specific integrins although it does not contain the canonical RGD sequence (Fig. 1 and Table 1). This has been reported not only for human T cells, B cells, natural killer (NK) cells or monocytes in the blood [8, 34, 37, 39] but also for $CD34^+$ circulating progenitors and platelets [40, 41]. Some discrepancies have been observed between human and mouse cells. Hence, in mouse, CCN1 binds to $CD11b^+$ cells and B cells but not T cells or NK cells [39]. Regarding $CD34^+$ progenitors, interactions occur through αMβ2 and αVβ3 [40]. Interactions with monocytes and macrophages are mediated by integrin αMβ2, αDβ2 and syndecan-4 [22, 34, 37, 42, 43], whilst integrin α6β1 and αLβ2 are important for thymocytes [9]. For mesenchymal cell populations, integrins αVβ5, α6β1 and syndecan-4 support CCN1 binding to fibroblasts [44, 45] whilst heparan sulphate proteoglycans and integrin α6β1 are implicated for vascular smooth muscle cells [33, 46, 47] and only integrin α6β1 for thymic epithelial cells [9].

Table 1 Receptors of CCN1

Cell type	Species	Receptors	References
B cells T cells NK cells	Human	Unknown	[8, 34, 37, 39]
T cells NK cells Spleen dendritic cells	Mouse	No binding	[39]
CD34+ circulating progenitors	Human	Integrin αMβ2 Integrin αVβ3	[40]
Monocyte Macrophage	Human Mouse	Integrin αMβ2 Integrin αDβ2 Syndecan-4	[22, 34, 37, 42, 43]
Osteoblast Osteoclast	Human Mouse	Integrin αVβ3 Integrin αVβ5	[61–63]
Spleen CD11b+ cells	Mouse	Unknown	[39]
Thymocytes	Mouse	Integrin α6β1 Integrin αLβ2	[9]
Thymic epithelial cells	Mouse	Integrin α6β1	[9]
Platelets	Human	Integrin αIIbβ3	[41]
Fibroblasts (including FLS)	Human Mouse	Integrin αVβ5 Integrin α6β1 Syndecan-4	[44, 45]
Vascular smooth muscle cells	Human Mouse Rat	Heparan sulphate proteoglycan Integrin α6β1	[33, 46, 47]

Role of CCN1 in cytokine production

In diabetic retinopathy, many defects are associated with localised inflammation. The upregulation of CCN1 leads to increased expression of MCP-1 in chorioretinal vascular endothelial cells through the αVβ3/PI3K/Akt signalling pathway [48]. In the same way, CCN1 treatment stimulates the production of IL6 in FLS of RA patients via the αvβ5 integrin/Akt/NF-κB pathway [18], promoting Th17 cell differentiation in vitro. Interestingly, targeting CCN1 with blocking antibodies has been shown to reduce IL6 levels and a Th17 response in a collagen-induced arthritis model of RA [18]. In addition, CCN1 has been observed to be increased in the synovial fluid of RA patients, which is known to promote the proliferation and survival of FLS [13]. CCN1 also promotes CCL2 expression in osteoblasts [16], skin fibroblasts [49] and renal tubular epithelial cell [50]. In murine macrophages, treatment with CCN1 induced transcriptional changes characteristic of M1 macrophages, including the upregulation of TNFα, IL1α, IL1β, IL6, IL12b, MIP1α, MCP3 and IP10 in an NF-κB-dependent manner [42]. In summary, these studies have shown that CCN1 plays a diverse role in promoting cytokine production and may be a key player in the inflammatory response and chronic disease.

Role of CCN1 in adhesion and migration

CCN1 supports the adhesion of a variety of cell types including monocytes [34, 37], macrophages [42], developing thymocytes and thymic epithelial cells [9], human blood leukocytes [8], fibroblasts [45, 51], vascular smooth muscle cells [52] or endothelial cells [53].

As well as mediating cell adhesion to multiple cell types, CCN1 has the ability to promote cell migration events of endothelial cells [32, 38, 53, 54], cancer cells [5, 55, 56] and vascular smooth muscle cells [33]. CCN1 acts as a chemoattractant for lymphocytes, monocytes and murine macrophages in multiple in vitro studies [8, 22, 39]. Consistent with these observations, CCN1 had no effect on integrin density or chemokine receptor expression in human monocyte and lymphocyte populations [8]. It activates the PI3K/Akt and p38 signalling pathways and actin polymerisation [8] while murine macrophage chemotaxis occurs via MEK/ERK signalling [22]. A role for CCN1 in disease has been confirmed in numerous animal studies, CCN1 protein treatment or overexpression in the liver exacerbates hepatic inflammation and macrophage infiltration in high-fat diet mice compared to normal fed mice [22]. Similarly, the importance of CCN1 in kidney fibrosis was demonstrated in a model of unilateral ureteral obstruction surgery in mice [50]. In this model, treatment with anti-CCN1 antibodies reduced kidney fibrosis by decreasing macrophage infiltration [50]. Finally, simvastatin treatment inhibits CCN1 expression and CCN1-dependent infiltration of macrophages and $CCN1^+$ osteoblasts and slows down the progression of disease in the mouse model of collagen-induced arthritis [16, 17, 19].

The chemotactic activity of CCN1, secreted by vascular endothelial growth factor (VEGF)-stimulated osteoblasts, regulates the formation of capillary-like sprouts by endothelial cells in vitro and promotes angiogenic processes in vivo [54]. CCN1 attracts endothelial cells and promotes angiogenesis in the context of bone fracture in mouse models, thereby contributing to the fracture healing process [54]. In addition, CCN1 promotes the recruitment and the differentiation of circulating $CD34^+$ progenitors to endothelial cells, hinting at a role in cardiovascular regeneration [40].

Consistent with observations using chemokines, prolonged exposure to CCN1 inhibits the migration of spleen macrophages, T cells and monocytes [8, 39]. Actually CCN1 abolishes their chemotactic response to CCL2 or SDF1 [8, 39] by downregulating PI3K, p38 and Akt signalling [8]. A regulatory role for CCN1 in immune cell migration was also demonstrated in inflammatory cardiomyopathy. In experimental autoimmune myocarditis, systemic CCN1 overexpression inhibits the migration of circulating immune cells, without affecting cardiac chemokine or chemokine receptor expression thereby ameliorating the disease process and reducing disease scores [39].

Role of CCN1 in cell proliferation and survival

Vascular endothelium

CCN1 promotes cell survival and tubule formation in human umbilical vein endothelial cells. Interestingly, proliferation can be mediated through the integrin α6β1 in the unactivated state, whereas VEGF stimulation is required to activate the integrin αVβ3 for proliferation and adhesion/migration mechanisms of endothelial cells [53]. A truncated form of CCN1 consisting of the insulin-like growth factor binding protein and von Willebrand factor type C domains has been shown to exhibit proangiogenic properties on retinal endothelial cells, whilst the presence of the thrombospondin type I domain to the previous variant suppressed cell growth [32].

As mentioned above, CCN1 can regulate the recruitment and differentiation of $CD34^+$ progenitor cells, which are important in cardiovascular tissue regeneration [40]. Furthermore, treatment with CCN1 or supernatants from CCN1-stimulated human $CD34^+$ cells has been shown to promote the proliferation of endothelial cells and enhance endothelial proliferation and neovascularization [40].

VSMC proliferation is characteristic of many vascular diseases, such as atherosclerosis. Forced expression of FOXO3a in VSMC decreased their viability through

inhibition of CCN1 in a rat balloon carotid arterial injury model [52].

Rheumatoid arthritis

In addition to sustaining a Th17 response in RA [18], the high levels of CCN1 detected in synovial fluid and tissue are responsible for the proliferation of FLS in RA patients. Moreover, CCN1 also protects FLS from apoptosis by maintaining Bcl-2 expression in FLS [13].

Thymic epithelial cells and thymus function

In the thymus, not only thymocytes are undergoing massive proliferation but also thymic epithelial cells (TEC) too. TEC are the main stromal cell populations of the thymic microenvironment that provide key signals to developing thymocytes. Alteration in TEC architecture is characteristic of age-associated thymic involution or cytoablative treatments [57, 58]. In vitro, CCN1 treatment of foetal thymic lobes favours the expansion of thymic stroma by promoting the proliferation of TEC through integrin α6β1/Akt axis. In vivo, the overexpression of CCN1 in thymic stroma increases the production of T cells via expansion of the TEC compartment. Thereby additional space is available for the recruitment and hosting of circulating hematopoietic progenitors and their development into T cells [9]. It is important to mention that CCN1 does not affect the proliferation and development of thymocytes per se.

Role of CCN1 in cell death and senescence

In total opposition to its proliferative activities, CCN1 can also contribute to senescence or cell death induction, which has been evidenced in several models.

In TNFα-resistant primary human fibroblasts, CCN1 unmasks the cytotoxic activity of TNFα. In this way, mice expressing a mutant *Ccn1* are resistant to TNFα-induced apoptosis in vivo [44]. During the healing process of cutaneous wounds, senescent fibroblasts accumulated in granulation tissues, where CCN1 was strongly expressed [49]. CCN1 induced the expression of antifibrotic genes in fibroblasts, thus limiting fibrosis during tissue repair. Induction of senescence by CCN1 occurred through binding to integrin α6β1 and heparan sulphate proteoglycans, activating p53 and Rac1/Nox1 signalling. As a consequence, mice bearing a mutant *Ccn1* gene exhibited an exacerbated fibrosis [45]. Comparably, treatment of muscle progenitor cells with CCN1 hampered their proliferative potential through the increase of p53 and p16Ink4A levels but without affecting the myogenic marker myoD [59].

Fibrosis is also observed in liver injuries. Upon carbon tetrachloride intoxication or bile duct ligation, CCN1 was induced, promoting senescence of hepatic stellate cells and portal fibroblasts [60]. In this model, integrin α6β1 and the Rac1/Nox1 pathway also played a regulatory role [60], as described in cutaneous wound healing [45]. Consequently, mice with hepatocyte-specific Ccn1-deletion displayed aggravated fibrosis due to a lack of cellular senescence [60].

Conclusion

The molecule CCN1 can exhibit diverse and different functions based on its modular structure and its ability to bind different integrins, thereby implicating it in distinct and complex processes. It is therefore remarkable that CCN1 expression is induced by proinflammatory cytokines while promoting itself the production of cytokines and chemokines. Additionally, locally produced CCN1 supports immune cell trafficking not only by both attracting and immobilising immune cells but also by driving differentiation by turning macrophages into M1-type cells. However, despite its upregulation upon viral or bacterial infections, the multiple roles of CCN1 have not been investigated in detail for these conditions. Finally, the expression of CCN1 in thymic stromal cells and its ability to improve thymus size arise interesting perspectives for studies in lymph nodes and bone marrow, two immune organs, which share structural similarities with the thymus and the setup of an appropriate immune response.

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Conflict of interest The authors declare that they have no competing financial interests.

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