The matricellular protein CCN1 in tissue injury repair

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Abstract

The expression of *Ccn1* (*Cyr61*) is essential for cardiovascular development during embryogenesis, whereas in adulthood it is associated with inflammation, wound healing, injury repair, and related pathologies including fibrosis and cancer. Recent studies have found that CCN1 plays a critical role in promoting wound healing and tissue repair. Mechanistically, CCN1 functions through direct interaction with specific integrin receptors expressed in various cell types in the wound tissue microenvironment to coordinate diverse cellular functions for repair. Here we briefly summarize the current knowledge on the functions of CCN1 in tissue injury repair and discuss pertinent unanswered questions.

Keywords wound healing · tissue regeneration · injury repair · integrin · inflammation

Introduction

CCN1, previously named Cyr61, was identified as a cysteine-rich protein encoded by a growth factor-inducible immediate-early gene (Lau and Nathans 1987; O'Brien et al. 1990). Subsequent identification of other highly conserved proteins gave rise to the CCN family of six secreted matricellular proteins with a similar modular structure (Lau and Lam 1999; Leask and Abraham 2006; Holbourn et al. 2008; Chen and Lau 2009). *Ccn1* is an essential gene required for cardiovascular development during embryogenesis (Mo et al. 2002; Mo and Lau 2006), whereas in adulthood its expression is tightly associated with inflammation and injury repair (Lau 2011). Numerous studies have found elevated expression of *Ccn1* and other members of the *Ccn* gene family in tissue injury, chronic diseases and related pathologies, including fibrosis and cancer in animal models and human patients (Jun and Lau 2011; Kubota and Takigawa 2013). In some instances, the expression of members of the *Ccn* gene family is shown to promote disease pathology or cancer progression (Kubota and Takigawa 2015; Kleer 2016). For example, *Ccn2* expression has been widely associated with fibrotic conditions and appears to mediate or exacerbate fibrosis (Leask 2013). By

contrast, recent findings have indicated that CCN1 plays important roles in promoting injury repair in several organs and tissues.

Wound healing and injury repair, irrespective of the organ or tissue, generally proceed through three broad phases that are temporally overlapping but functionally distinct (Stramer et al. 2007; Gurtner et al. 2008). Infiltration of neutrophils and macrophages comprise the initial inflammatory phase, whereby immune cells combat microbes and eliminate cell debris. This is followed by the proliferation phase when myofibroblasts produce a large amount of extracellular matrix (ECM) to promote tissue integrity and parenchymal cell proliferation. Finally, the provisional ECM is remodeled in the maturation phase to rebuild the architecture of the original uninjured tissue. Interestingly, CCN1 has been shown to regulate each of these phases in injury repair by interacting with disparate cell types through specific integrin receptors (Lau 2016). For example, CCN1 binds integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in epithelial cells and $\alpha_6\beta_1$ in fibroblastic cells, and its specific binding sites for these receptors have been identified (Fig. 1) (Leu et al. 2003; Chen et al. 2004; Leu et al. 2004). Furthermore, site-specific CCN1 mutations targeting these binding sites that abolish integrin binding have been prepared, and knockin mice with these mutant alleles replacing the *Ccn1* genomic locus have been constructed (Chen et al. 2007; Kim et al. 2013, 2015). Thus, *Ccn1*^{D125A/D125A} mice express CCN1 with a single amino acid change (D125 to A) that abrogates binding to $\alpha_v\beta_3/\alpha_v\beta_5$, and *Ccn1*^{DM/DM} mice carry an allele encoding CCN1 unable to bind $\alpha_6\beta_1$. In contrast to the

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Fig. 1 Schematic diagram of CCN1. Human CCN1 is a 381 a.a. protein with an N-terminal secretory peptide followed by 4 structural domains conserved in the CCN protein family. A binding site for integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ is located in the von Willebrand factor C domain (VWC), and the C-terminal (CT) domain contains two $\alpha_6\beta_1$ binding site that partially overlap with an $\alpha_M\beta_2$ binding site (Leu et al. 2004; Schober et al. 2002). The thrombospondin type 1 repeat (TSR) domain binds phosphatidylserine (Jun et al. 2015), and contains an $\alpha_6\beta_1$ binding site that functions when CCN1 acts as an immobilized substrate (Leu et al. 2003; Leu et al. 2004)

embryonic lethality of *Ccn1* knockout mice (Mo et al. 2002; Mo and Lau 2006), these knockin mice are fertile and viable, allowing the investigation of CCN1 functions through specific integrin receptors in various mouse models of injury (Chen et al. 2007; Kim et al. 2013, 2015).

CCN1 in cutaneous wound healing

Upon skin injury, *Ccn1* is upregulated in a biphasic manner during the inflammatory phase and the maturation phase (Jun et al. 2015). A remarkable activity of CCN1 in the inflammatory phase of cutaneous wound healing is the stimulation of efferocytosis, or phagocytic clearance of apoptotic cells (Jun et al. 2015). Infiltration of neutrophils is one of the first events to occur in response to wounding to defend against microbial infections. However, neutrophils are short-lived and rapidly undergo apoptosis. The apoptotic neutrophils must be removed in order for healing to proceed, since these cells can undergo secondary necrosis and secrete proteases and cytokines that cause further tissue damage (Silva 2010). *Ccn1*^{D125A/D125A} knockin mice, which express a mutant CCN1 unable to bind $\alpha_v\beta_3/\alpha_v\beta_5$, suffer impaired wound healing with the accumulation of apoptotic neutrophils. Mechanistically, CCN1 acts as a bridging molecule and binds phosphatidylserine (PS), the “eat-me” signal on apoptotic cells, and $\alpha_v\beta_3/\alpha_v\beta_5$ in macrophages to trigger phagocytic removal of the apoptotic neutrophils (Fig. 2). Wounds of *Ccn1*^{D125A/D125A} mice are deficient in CCN1-mediated clearance of apoptotic neutrophils and are stalled at the

inflammatory phase, resulting in impaired progression to the proliferation phase (Jun et al. 2015).

During the proliferation phase, resident fibroblasts, pericytes, and mesenchymal stem cell-like cells may transdifferentiate into myofibroblasts and produce ECM to promote tissue integrity and cell proliferation (Jun and Lau 2018). When healing enters the maturation phase, these fibrogenic myofibroblasts cease to produce ECM by undergoing apoptosis, senescence, dedifferentiation, or lineage reprogramming (Jun and Lau 2018). However, if the injury is chronic or wound healing is deregulated, uncontrolled stimulation of myofibroblasts can lead to fibrotic scar formation and loss of organ function (Bochaton-Piallat et al. 2016; Wynn and Vannella 2016). CCN1 level declines after the inflammatory phase in skin wounds but is elevated again in the maturation phase (Jun et al. 2015). CCN1 triggers myofibroblasts to undergo cellular senescence through engagement of integrin $\alpha_6\beta_1$, whereupon senescent myofibroblasts express the senescence-associated secretory phenotype (SASP) that includes upregulation of matrix degradation enzymes and downregulation of collagen (Jun and Lau 2010a). Thus, CCN1 turns the ECM-producing, fibrogenic myofibroblasts into ECM-degrading, anti-fibrotic senescent cells in the maturation phase (Jun and Lau 2010b). As such, CCN1 accelerates matrix remodeling in the maturation phase and limits fibrosis. Consistently, *Ccn1*^{DM/DM} mice that express CCN1 unable to bind $\alpha_6\beta_1$ suffer exacerbated fibrosis as myofibroblasts fail to undergo senescence (Jun and Lau 2010a). Treatment of wounds with CCN1 protein reduces accumulation of collagen with a concomitant increase in senescent myofibroblasts. CCN1 triggers senescence in fibroblastic cells through $\alpha_6\beta_1$ -mediated activation of RAC1 and NOX1 to generate the sustained accumulation of reactive oxygen species (ROS), which trigger senescence by activating the p53 and p16^{INK4a}/pRb pathways (Jun and Lau 2010a).

CCN1 in hepatobiliary injury repair

The liver is a highly regenerative organ and CCN1 plays critical roles in the proliferation and maturation phases of hepatobiliary injury repair. Hepatocytes and cholangiocytes

Fig. 2 CCN1 functions in wound healing. In the inflammatory phase, CCN1 stimulates the clearance of apoptotic neutrophils (Jun et al. 2015), allowing wound healing to progress to the proliferation phase in which

myofibroblasts produce ECM to promote tissue integrity. During the maturation phase, CCN1 induces cellular senescence in myofibroblasts to stimulate matrix remodeling and limit fibrosis (Jun and Lau 2010a)

are the two distinct epithelial cell types of the liver, and they respond to damage differently (Fig. 3a). In cholestatic injury, the major repair response during the proliferation phase is ductular reaction, which includes cholangiocyte proliferation, expansion of bile ducts, and differentiation of hepatic progenitor cells (HPCs) into cholangiocytes. CCN1 is essential for the regeneration of bile ducts after injury from either bile duct ligation (BDL) or diethoxycarbonyl dihydrocollidine (DDC) diet, experimental models that mimic human cholestatic liver disease (Kim et al. 2015). Indeed, *Ccn1*^{D125A/D125A} mice perish within days after BDL, whereas wild-type mice survive during the same experimental period. The rapid mortality is because ductular reaction fails to occur, and the bile acids that cannot drain into the gallbladder and intestine leak out to the liver parenchyma and cause massive hepatic necrosis. CCN1 acts by engagement of integrins $\alpha_v\beta_5$ in cholangiocytes, thus activating NF κ B-mediated *Jag1* expression and Jag1/Notch signaling required for cholangiocyte proliferation (Kim et al. 2015). Consistent with the essential role of CCN1 for cholangiocyte proliferation and bile duct expansion through the α_v -NF κ B axis, wild-type mice phenocopy *Ccn1*^{D125A/D125A} mice in response to BDL when subjected to blockade of integrins $\alpha_v\beta_3/\alpha_v\beta_5$ or inhibition of NF κ B activation (Kim et al. 2015). Moreover, injection of Jag1 rescues the bile duct regeneration defects in *Ccn1*^{D125A/D125A} mice. CCN1 also activates *Jag1* expression in hepatic stellate cells, which interact with HPCs through Jag1/Notch signaling and induced the differentiation of HPCs into cholangiocytes to promote bile duct regeneration (Kim et al. 2015; Boulter et al. 2012). These observations show that CCN1 is essential for ductular reaction in bile duct regeneration, acting through $\alpha_v\beta_5$ -mediated activation of NF κ B and Jag1/Notch signaling.

CCN1 also accelerates matrix remodeling in the maturation phase and limits fibrosis. When the liver suffers chronic

injuries due to hepatotoxin intoxication (e.g., carbon tetrachloride) or cholestasis (e.g., BDL), myofibroblasts are activated to produce ECM in the injury response. In both injury models, CCN1 dampens and limits fibrosis by inducing senescence in the myofibroblasts, leading to their expression of the anti-fibrotic SASP (Kim et al. 2013). *Ccn1*^{DM/DM} mice and mice with hepatocyte-specific deletion of *Ccn1* (*Ccn1* ^{Δ Hep}) suffer exacerbated fibrosis concomitant with deficiencies in myofibroblast senescence, whereas overexpression of *Ccn1* in hepatocytes or adenoviral expression of *Ccn1* in the liver reduces liver fibrosis with enhanced senescence (Kim et al. 2013; Borkham-Kamphorst et al. 2014). Moreover, administration of purified CCN1 protein results in accelerated resolution of pre-established fibrosis (Kim et al. 2013). Together, these results show that CCN1 is required for bile duct regeneration after cholestasis and for limiting fibrosis following hepatobiliary injuries.

CCN1 in intestinal mucosal healing

As the intestinal epithelium is constantly exposed to microbes and ingested toxins, the intestinal epithelial cells are prone to damage. Therefore, differentiated intestinal epithelial cells are removed continuously and are replaced by new cells generated from stem cells located at the base of the intestinal crypt. Typically, epithelial cells of the small intestine are replaced every 4–5 days (Blander 2016). Inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, are associated with inflammatory injury of the bowel and impaired mucosal healing (Abraham and Cho 2009). A common murine model for inflammatory injury to the bowel is exposure to dextran sodium sulfate (DSS), which causes intestinal epithelial cell death and inflammation, followed by mucosal healing and

Fig. 3 Parenchymal-specific CCN1 functions during the proliferation phase in repair. **a** In the liver, CCN1 inhibits compensatory proliferation of hepatocytes when damage is induced by the hepatocarcinogen DEN, thus suppressing tumor initiation (Chen et al. 2016). However, when

cholangiocytes are damaged by cholestasis, CCN1 induces the proliferation of cholangiocytes and bile duct regeneration (Kim et al. 2015). **b** In the bowel, CCN1 promotes intestinal epithelial restitution in response to inflammatory injury (Choi et al. 2015)

repair. Remarkably, administration of CCN1 protein in wild-type mice greatly accelerates mucosal healing following exposure to DSS, and enhances intestinal epithelial restitution (Choi et al. 2015). Furthermore, *Ccn1*^{DM/DM} mice suffer high mortality, impaired mucosal healing, and diminished interleukin-6 (IL-6) expression during the repair phase of DSS-induced colitis. Moreover, CCN1 induces IL-6 expression in macrophages through integrin $\alpha_M\beta_2$ and in fibroblasts through $\alpha_6\beta_1$, and IL-6 promotes intestinal epithelial cell proliferation (Choi et al. 2015). Administration of CCN1 protein or IL-6 resulted in full or partial rescue of the mucosal healing defects and mortality of *Ccn1*^{DM/DM} mice, respectively (Choi et al. 2015). These findings show that CCN1 plays a critical role in intestinal mucosal healing after colitis in part through integrin-mediated activation of IL-6 expression, and suggest a therapeutic potential for CCN1 for treating inflammatory bowel disease (Fig. 3b).

CCN1 in injury repair of other tissues

Tissue repair often requires angiogenesis to support new cell growth and for vascularization of the new tissue. CCN1 can induce angiogenesis through direct binding to integrin $\alpha_V\beta_3$ in endothelial cells (Babic et al. 1998; Leu et al. 2002). Endothelial cells of the angiogenic sprouts are organized into the tip cell at the leading edge that migrates directionally toward the angiogenic signal gradient, followed by stalk cells that form the endothelial lumen of the vessel (Blanco and Gerhardt 2013). The tip cell expresses the Notch ligand Dll4 and interacts with stalk cells to activate Notch signaling, thereby establishing a hierarchy that maintains organization of the angiogenic sprout (Blanco and Gerhardt 2013). Interestingly, CCN1 regulates *Dll4* expression and Dll4-Notch signaling between tip and stalk cells as well as VEGFR2 signaling in the angiogenic sprout, thus regulating the interaction between tip and stalk cells (Hasan et al. 2011; Chintala et al. 2015). Consequently, endothelial cell-specific deletion of *Ccn1* leads to endothelial hyperplasia and loss of the distinctive hierarchical organization, resulting in blood vessels that form a flat hyperplastic sinus (Chintala et al. 2015). Thus, CCN1 may contribute to wound repair through its angiogenic function. For example, *Ccn1* is highly expressed during bone fracture healing in rat and sheep (Hadjiargyrou et al. 2000; Lienau et al. 2006), potentially enhancing fracture healing through its angiogenic activity (Athanasopoulos et al. 2007). Indeed, application of CCN1 protein in a collagen matrix to the site of osteotomy improves fracture repair by increasing callus diameter, bone volume, and torsional strength in a rabbit trauma model (Frey et al. 2012).

In the lung, both positive and negative effects of CCN1 have been reported in injury repair, although differences in the injury models and experimental protocols may underlie

the disparate observations. Treatment with recombinant CCN1 protein curtails hyperoxia-induced injury in neonatal rats, in part by reducing macrophage and neutrophil infiltration and inflammasome activation (Vaidya et al. 2017). CCN1 also protects against hyperoxia-induced cell death in lung epithelial cells via activation of the Akt (Jin et al. 2005). Furthermore, recombinant CCN1 accelerated repair whereas anti-CCN1 antibodies impaired repair from inflammatory injury to the lung epithelium in an in vitro assay (Zemans et al. 2013). In contrast to these repair-promoting functions, it has also been reported that adenoviral overexpression of CCN1 in the lung induces injury with weight loss, increased neutrophil counts in bronchoalveolar lavage, and increased mortality (Grazioli et al. 2015). However, it is not clear which cell types are overexpressing CCN1, and the mechanistic basis for this injury is unknown. It has also been reported that siRNA knockdown of *Ccn1* downregulates profibrotic gene expression and TGF- β 1-induced SMAD3 signaling in lung fibroblasts, thus attenuating bleomycin-induced lung injury (Kurundkar et al. 2016). These findings suggest that CCN1 may promote TGF- β 1 actions in specific contexts.

CCN1 inhibits injury-induced hepatocellular carcinogenesis

Elevated expression of *CCN1* has been observed in many cancers (Lau 2011), and overexpression of *Ccn1* in xenografts of established cancer cell lines generally promotes tumor growth, potentially through stimulation of angiogenesis, cell migration, epithelial-mesenchymal transition, and cancer stemness (Babic et al. 1998; Haque et al. 2011; Huang et al. 2017). However, CCN1 plays an inhibitory role in cancer initiation induced by injury. Hepatocellular carcinoma (HCC) is often driven by chronic inflammatory damage occurring over an extended period of time. A commonly used mouse model of HCC is the exposure to diethylnitrosamine (DEN), which causes DNA damage and apoptosis in hepatocytes. This leads to the phagocytic elimination of cell debris by Kupffer cells, which produce hepatomitogens that trigger hepatocyte proliferation to compensate for the cell loss. However, this compensatory proliferation may stimulate the expansion of damaged cells that are prone to oncogenic transformation and is a critical driver of hepatocarcinogenesis resulting from hepatocyte damage (Kuraishy et al. 2011). In this context, the epidermal growth factor receptor (EGFR) plays a critical role, and inhibition of EGFR signaling by erlotinib abolishes compensatory hepatocyte proliferation and abrogates development of HCC (Chen et al. 2016). CCN1 suppresses tumorigenesis in DEN-induced HCC by inhibiting EGFR-dependent hepatocyte compensatory proliferation through $\alpha_6\beta_1$ -mediated generation of ROS and p53 activation, leading to a cell cycle block (Chen et al. 2016).

Table 1 CCN1-induced functions mediated through specific integrins in tissue injury repair

Cell type	$\alpha_V\beta_3/\alpha_V\beta_5$	$\alpha_6\beta_1$	$\alpha_M\beta_2$	References
Endothelial cells	Angiogenesis			(Babic et al. 1998; Leu et al. 2002)
Hepatocytes		Inhibition of EGFR-dependent proliferation		(Chen et al. 2016)
Cholangiocytes	Jag1/Notch signaling and cell proliferation			(Kim et al. 2015; Kim and Lau 2015)
Macrophages	Phagocytic clearance of apoptotic cells		Expression of cytokines	(Jun et al. 2015; Choi et al. 2015; Bai et al. 2010)
Myofibroblasts		Senescence		(Jun and Lau 2010a)

As a result, *Ccn1* ^{Δ Hep} and *Ccn1*^{DM/DM} mice suffer increased compensatory proliferation and tumorigenicity after exposure to DEN (Chen et al. 2016).

Summary and perspectives

As summarized above, CCN1 interacts with distinct cell types through specific integrin receptors to coordinate diverse and critical functions in wound healing and injury repair (Table 1). In the inflammatory phase, CCN1 stimulates macrophage efferocytosis of apoptotic neutrophils, thereby facilitating the progression to the proliferation phase (Jun et al. 2015). In cholestatic injury to the liver, CCN1 is essential for inducing Jag1/Notch signaling and cholangiocyte proliferation (Kim et al. 2015). However, when hepatocytes are damaged due to hepatocarcinogens such as DEN, CCN1 restricts compensatory proliferation and therefore suppresses cancer initiation (Chen et al. 2016). In the intestinal epithelium, CCN1 greatly promotes mucosal restitution following inflammatory injury, in part through the induction of IL-6 (Choi et al. 2015). Finally, in the maturation phase, CCN1 accelerates matrix remodeling and dampens fibrosis by inducing myofibroblast senescence (Kim et al. 2013; Jun and Lau 2010a). Moreover, CCN1 may contribute to tissue repair in wound healing through its induction of angiogenesis (Babic et al. 1998; Chintala et al. 2015). These findings illustrate the broad range of biological functions that CCN1 can coordinate through various cell types in tissue repair and portend additional functions yet to be uncovered. Explorations using disparate injury models will provide additional insights. Since CCN1 induces diverse functions in distinct cell types at various stages of wound healing and tissue repair, inhibition or overexpression of CCN1 may have different consequences depending on the tissue, nature of the damage, and timing of perturbation. These diverse effects may be important considerations in contemplating CCN1 as a therapeutic target.

Currently much is unknown about how *Ccn1* expression is regulated in diverse cell types in the context of injury repair.

Furthermore, since the specific functions of CCN1 are mediated through its binding to specific integrins and their associated co-receptors, it will be important to understand how the molecular interaction between CCN1-integrin complexes with specific co-receptors affects signaling mechanisms (Lau 2016; Juric et al. 2012). The functional significance and regulation of post-translational modification of CCN1, including fucosylation and proteolysis, are also not well understood (Niwa et al. 2015; Pendurthi et al. 2005; Choi et al. 2013). The large number of conserved cysteine residues in CCN proteins suggests that specific tertiary structure is important for their function, although little structural information is currently available. Future determination of the 3-dimensional structure of CCN1 will greatly enhance our understanding of its mechanism of action.

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