

Deadly liaisons: fatal attraction between CCN matricellular proteins and the tumor necrosis factor family of cytokines

Chih-Chiun Chen · Lester F. Lau

Received: 15 September 2009 / Accepted: 6 October 2009 / Published online: 7 November 2009
© The Author(s) 2009. This article is published with open access at Springerlink.com

Abstract Recent studies have revealed an unexpected synergism between two seemingly unrelated protein families: CCN matricellular proteins and the tumor necrosis factor (TNF) family of cytokines. CCN proteins are dynamically expressed at sites of injury repair and inflammation, where TNF cytokines are also expressed. Although TNF α is an apoptotic inducer in some cancer cells, it activates NF κ B to promote survival and proliferation in normal cells, and its cytotoxicity requires inhibition of de novo protein synthesis or NF κ B signaling. The presence of CCN1, CCN2, or CCN3 overrides this requirement and unmasks the apoptotic potential of TNF α , thus converting TNF α from a proliferation-promoting protein into an apoptotic inducer. These CCN proteins also enhance the cytotoxicity of other TNF cytokines, including LT α , FasL, and TRAIL. Mechanistically, CCNs function through integrin $\alpha_6\beta_1$ and the heparan sulfate proteoglycan (HSPG) syndecan-4 to induce reactive oxygen species (ROS) accumulation, which is essential for apoptotic synergism. Mutant CCN1 proteins defective for binding $\alpha_6\beta_1$ -HSPGs are unable to induce ROS or apoptotic synergism with TNF cytokines. Further, knockin mice that express an $\alpha_6\beta_1$ -HSPG-binding defective CCN1 are blunted in TNF α - and Fas-mediated apoptosis, indicating that CCN1 is a physiologic regulator of these processes. These findings implicate CCN proteins as contextual regulators of the inflammatory response by dictating or enhancing the cytotoxicity of TNF α and related cytokines.

Keywords Inflammation · Apoptosis · TNF · FasL · TRAIL · CYR61 · CTGF · NOV

Introduction

Apoptosis is an evolutionarily conserved process in multicellular organisms for eliminating unwanted or damaged cells, and is critical for normal development and tissue homeostasis. Environmental factors, such as UV irradiation or oxidative stress, can cause or increase susceptibility to apoptosis. A number of endogenously produced proteins, notably members of the tumor necrosis factor (TNF) family of cytokines including TNF α , lymphotoxin- α (LT α), Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL), can induce apoptosis by binding to specific receptors on the surface of target cells. The ensuing signaling events, characterized by the activation of the caspase family of intracellular proteinases, lead to cell shrinkage and DNA fragmentation. The resulting cellular debris is engulfed and removed by macrophages and other surrounding cells.

The extracellular matrix (ECM) has long been recognized as a critical survival factor. Cell adhesion to ECM proteins such as fibronectin through integrin receptors activates cytoprotective signaling pathways involving PI3K, JNK, and ERK to suppress apoptosis (Almeida et al. 2000). When deprived of proper interaction with the ECM or if integrins are unligated or improperly ligated, normal cells undergo a form of apoptosis called anoikis (Cheresh and Stupack 2008; Chiarugi and Giannoni 2008). Contrary to the prevailing view that the ECM serves a pro-survival role, recent studies have revealed that certain ECM molecules can promote or induce apoptosis (Marastoni et al. 2008). For example, the CCN family of proteins can

C.-C. Chen · L. F. Lau (✉)
Department of Biochemistry and Molecular Genetics,
College of Medicine, University of Illinois at Chicago,
Chicago, IL 60607, USA
e-mail: LFLau@uic.edu

promote apoptosis through integrin-dependent activation of p53 even as they support cell adhesion and induce adhesive signaling (Todorovic et al. 2005), whereas EMILIN2 can induce apoptosis by binding to the death receptors DR-4 and DR-5 (Mongiati et al. 2007). Surprisingly, CCN proteins can also enable or promote the cytotoxicity of the TNF family cytokines both in vitro and in vivo, thus altering the matrix microenvironment to dictate or support TNF cytokine-dependent cell death (Fig. 1). This review focuses on the novel signaling crosstalk that underpins the unexpected synergism between these two seemingly unrelated protein families.

Apoptotic synergism between members of the CCN and TNF families

The CCN family is comprised of six structurally conserved, ECM-associated signaling proteins in vertebrates (Leask and Abraham 2006; Holbourn et al. 2008; Chen and Lau 2009). The first three members described, Cyr61 (cysteine rich 61, CCN1), Connective tissue growth factor (CTGF, CCN2), and Nephroblastoma overexpressed (Nov, CCN3), provided the acronym for the CCN family. CCNs regulate diverse aspects of cell behavior including cell adhesion, migration, proliferation, survival, and differentiation without being integral structural components of the matrix, and as such they fit the characteristics of “matricellular” proteins (Bornstein 1995; Lau and Lam 1999). Like many

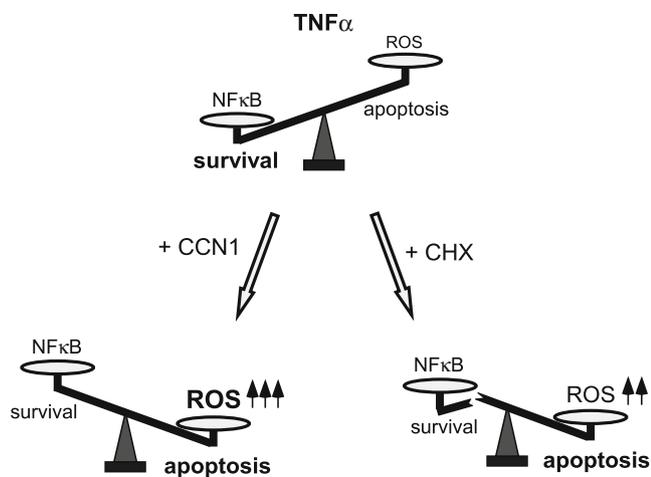


Fig. 1 CCN1 tips the balance in TNF α -regulated life and death decisions. TNF α is a potent activator of NF κ B, a master transcription factor that induces the expression of genes that promote cell proliferation and survival, and suppress apoptosis. Blockade of de novo protein synthesis or NF κ B signaling unleashes the apoptotic potential of TNF α , allowing TNF α -induced apoptosis to proceed. The presence of CCN1 overrides the effects of NF κ B by increasing the cellular accumulation of ROS, leading to apoptosis without inhibiting protein synthesis or NF κ B activity

ECM proteins, they function through direct interaction with integrin receptors and cell surface heparan sulfate proteoglycans (HSPGs) to mediate many of their activities (Kireeva et al. 1998; Chen et al. 2000).

The TNF superfamily includes at least 19 cytokines that play critical roles in regulating the development and function of the immune system (Locksley et al. 2001; Aggarwal 2003). A subset of this family, such as TNF α , LT α , FasL, and TRAIL, can also induce cell death. TNF cytokine-dependent cell death may play important roles in the removal of damaged or potentially cancerous cells as part of immune surveillance, but can also contribute to the etiology of a variety of diseases if it occurs excessively. TNF α was initially found to induce necrotic and apoptotic death in certain cancer cells; however, subsequent studies showed that TNF α alone promotes survival of normal cells in culture and its cytotoxicity is only revealed when de novo protein synthesis or NF κ B signaling is blocked (Varfolomeev and Ashkenazi 2004; Muppidi et al. 2004). TNF α activates the transcription factor NF κ B, which induces the expression of a large number of proteins critical for cell survival and inflammation. Since TNF α can activate both survival and apoptotic signaling, its cytotoxicity is highly contextual and dependent on the presence of sensitizing contributors such as viral infection or IFN- γ that perturbs NF κ B signaling or protein synthesis (Varfolomeev and Ashkenazi 2004; Muppidi et al. 2004). LT α binds the same receptors as TNF α and is thought to act similarly. By contrast, FasL and TRAIL are weak inducers of NF κ B and can induce cell death on their own; however, their cytotoxicity is also regulated by other environmental factors.

In adults, CCNs are highly expressed at sites of tissue damage and inflammation where TNF family cytokines are also expressed in many instances, providing the opportunity for CCNs and TNF cytokines to interact. Recently we showed that CCN1 and CCN2, either in a soluble form or as adhesion substrates, enable TNF α to induce apoptosis without perturbation of protein synthesis or NF κ B signaling, and enhance the cytotoxic effects of FasL and TRAIL (Chen et al. 2007; Juric et al. 2009; Franzen et al. 2009). Indeed, the interactions between the CCN and TNF families extend even further: CCN1, CCN2, and CCN3, can each unmask the cytotoxicity of TNF α and LT α , and promote the apoptotic activity of FasL and TRAIL in normal human fibroblasts, resulting in rapid apoptosis within 4–6 h (Fig. 2). Although CCNs do not trigger cell death on their own under these conditions, they induced apoptosis in ~25% of cells in combination with TNF α or LT α , and enhanced the apoptotic efficacy of FasL and TRAIL by at least 2-fold (Fig. 2). These observations are remarkable, since TNF α normally promotes the proliferation of fibroblasts by inducing the expression of PDGF and is not

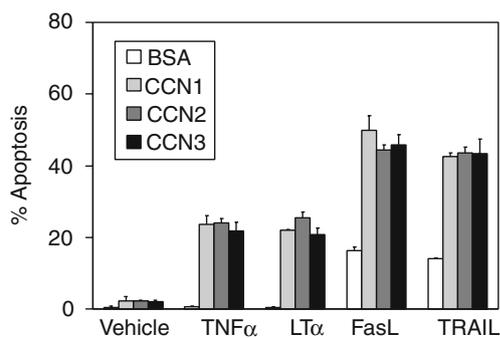


Fig. 2 Apoptotic synergism between the CCN and TNF protein families. Normal human skin fibroblasts were serum-starved overnight before being treated for 6 h at 37°C with serum-free media containing purified recombinant CCN1, CCN2, or CCN3 proteins (5 μ g/ml each), with or without TNF α (10 ng/ml), LT α (10 ng/ml), FasL (50 ng/ml), or TRAIL (20 ng/ml) as indicated. Apoptotic cells were scored as described (Chen et al. 2007)

cytotoxic (Battegay et al. 1995). Thus, the presence of CCNs turns TNF α from being a proliferation-enhancing agent into a cytotoxic factor. These activities appear unique to members of the CCN family and were not found in other ECM proteins tested, such as collagen, fibronectin, laminin, and vitronectin.

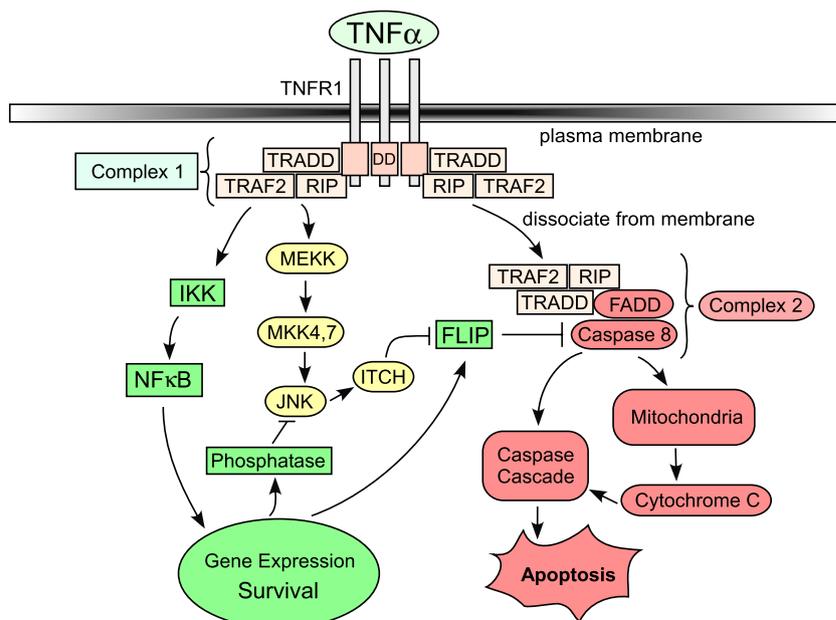
NF κ B suppresses the cytotoxicity of TNF α

When TNF α engages its cell surface receptor TNFR1, the death domain in the receptor cytoplasmic tail recruits the adaptor protein TRADD, which further recruits RIP and TRAF2 that participate in the activation of NF κ B and JNK

(Fig. 3). The RIP, TRAF2, and TRADD complex (complex I) subsequently dissociates from the receptor and recruits FADD and procaspase-8/-10 into a secondary complex (complex II) in which activation of procaspase-8/-10 occurs (Muppidi et al. 2004; Bertazza and Mocellin 2008). Whereas caspases-8/-10 can directly activate caspase-3 by proteolysis to trigger apoptosis, in some cell types caspase-8 cleaves and activates the BH3-only protein Bid, leading to cytochrome c release from the mitochondria and amplification of the apoptotic signal. Cytoplasmic cytochrome c complexes with Apaf-1 and activates caspase-9, which further activates caspase-3 to trigger apoptosis.

In normal cells, the pro-survival protein c-FLIP competes with procaspases-8/-10 for binding to the RIP/TRADD/TRAF2/FADD complex, thus preventing caspase activation. TNF α -activated NF κ B induces the transcription and synthesis of c-FLIP, adding to the existing cellular pool of c-FLIP and creating a further obstacle to caspase activation. The function of c-FLIP is opposed by the stress-induced kinase JNK, which promotes apoptosis by phosphorylating the ubiquitin ligase ITCH and thereby targeting c-FLIP for degradation (Chang et al. 2006). If JNK activation is robust and prolonged, c-FLIP is obliterated and TNF α -induced apoptosis can occur. However, under normal conditions TNF α -activated JNK is rapidly inactivated by phosphatases. Reactive oxygen species (ROS), which are generated as microbicides in phagocytic cells, or as second messengers mediating diverse cellular functions in non-phagocytic cells, are important regulators of JNK because they can inactivate phosphatases by oxidation of the critical cysteine residue in the enzyme active centers (Meng et al. 2002). Thus, a high level of

Fig. 3 TNF α induced signaling. TNF α is capable of inducing both pro-survival and pro-apoptotic signaling through its receptor TNFR1. Formation of complex 1 leads to activation of NF κ B and its downstream effectors, while complex II can trigger apoptosis through activation of the caspase cascade. The apoptotic pathway is blocked by c-FLIP, which is targeted for degradation by the ubiquitin ligase ITCH and apoptosis can proceed if JNK activation is sustained (Chang et al. 2006). Signaling molecules in green provide survival functions, while those in red contribute to apoptosis. Those in yellow promote apoptosis only when superactivated under certain contexts



cellular ROS can inactivate phosphatases and allow TNF α -induced JNK activation to remain sustained, thereby leading to c-FLIP degradation and apoptosis (Kamata et al. 2005). Although TNF α itself induces ROS production, TNF α -induced ROS are quickly downregulated by NF κ B, which activates the synthesis of anti-oxidant proteins such as Mn-superoxide dismutase and ferritin heavy chain (Pham et al. 2004). In addition, NF κ B can induce the expression of phosphatases to restrain JNK activation. Therefore, NF κ B counteracts the TNF α -induced apoptotic pathway by inducing the synthesis of c-FLIP, phosphatases, and antioxidant proteins, all of which conspire to block TNF α -induced apoptosis.

CCNs enables TNF α cytotoxicity and promotes FasL activity through ROS

Although inhibition of de novo protein synthesis or NF κ B signaling is the most common way to enable TNF α induction of apoptosis in normal cells, CCNs do not perturb either process. In addition, CCN1 and TNF α induces ~2-fold more apoptosis and with a much faster kinetics (4–6 h vs. 24 h) compared to apoptosis induction by cycloheximide and TNF α , further suggesting that CCN1 works through a distinct mechanism (Chen et al. 2007). Indeed, CCNs act through inducing the accumulation of a high level of ROS to override the anti-apoptotic effects of NF κ B, thus unmasking the cytotoxicity of TNF α (Fig. 4). Both

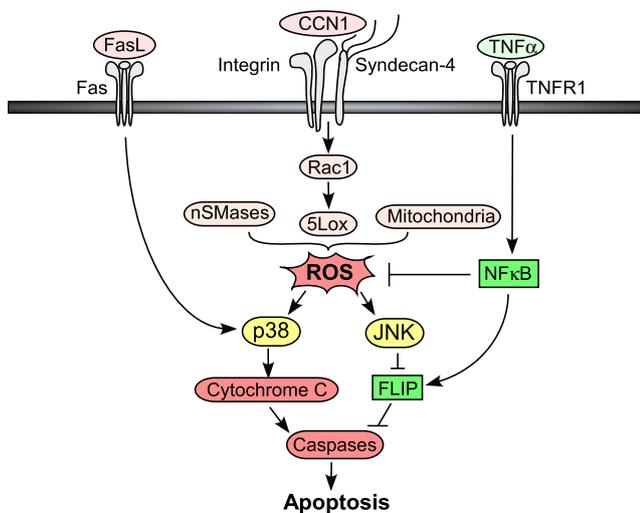


Fig. 4 Signaling crosstalk between CCN1 and TNF α or FasL. In fibroblasts, CCN1 acts through binding integrins $\alpha_6\beta_1$, $\alpha_v\beta_5$, and the cell surface HSPG syndecan-4, leading to generation of ROS by several mechanisms that include the activities of 5-LOX, nSMases, and the mitochondria. A high level of ROS enhances and maintains the activation of the cellular kinases JNK, which targets c-FLIP for degradation and allows TNF α to activate caspases-8 and -10 and induce apoptosis, and p38 MAPK, which promotes FasL-induced Bax activation and cytochrome c release

CCN1 and CCN2 induce ROS production in fibroblasts, and neutralization of ROS with chemical scavengers or inhibiting cellular mechanism of ROS production annihilates their apoptotic synergism with TNF α and FasL (Chen et al. 2007; Juric et al. 2009). TNF α alone induces a transient JNK activation and a low level of ROS that is quickly dampened by NF κ B-induced anti-oxidant proteins. Despite NF κ B actions, the combination of CCN1 and TNF α induces a sufficient amount of ROS to trigger a robust and biphasic activation of JNK (Chen et al. 2007). Upon CCN1/TNF α treatment, JNK activation occurs within 15 min. but transiently declines between 1 and 3 h after stimulation, most likely due to NF κ B-induced synthesis of new phosphatases. Eventually CCN1-induced ROS may inhibit the newly synthesized phosphatases and a second phase of JNK activation occurs between 4 and 6 h after stimulation, concomitant with cell death. This second phase of JNK activation, which is not seen in TNF α stimulation alone, is essential for CCN1/TNF α -induced apoptosis.

In CCN1/FasL synergism, CCN1 induces ROS to hyperactivate p38 MAPK, leading to the activation and mitochondrial localization of Bax and consequent cytochrome c release (Juric et al. 2009). Other reports also show that p38 can phosphorylate and inhibit the pro-survival activity of members of the Bcl-family, thereby promoting Bax/Bak activation and mitochondrial cytochrome c release (Torcia et al. 2001; Gomez-Lazaro et al. 2007). However, ROS does not seem to play an important role in CCN1 synergism with TRAIL in PC-3 prostate cancer cells, suggesting cell-type specific differences in the mechanism of apoptotic synergism (Franzen et al. 2009). Interestingly, CCN1 action is a double edge sword in prostate carcinoma cells, since it promotes the proliferation of prostate cells but also enhances the cytotoxicity of TRAIL. Thus, cancer cells may overexpress CCN proteins to promote their proliferation, although this also puts them at risk of higher susceptibility to TRAIL-mediated immune surveillance.

Mechanisms of ROS induction by CCN1

Integrin-mediated cell adhesion to ECM proteins generates ROS, which is required for the adhesion process (Chiarugi et al. 2003). Integrin signaling is known to activate small GTPases such as RAC and RHO, leading to disassembly and repolymerization of F-actin, formation of lamellipodia, and clustering of ligand-bound integrins into focal complexes. RAC can also regulate ROS generation through multiple mechanisms, including 5-lipoxygenase (LOX) (Chiarugi et al. 2003), specific isoforms of NADPH oxidases (NOX)(Chiarugi et al. 2003), and the mitochondria (Werner and Werb 2002)(Fig. 4). Whereas LOX

catalyzes the metabolism of arachidonic acid to leukotrienes, NOX generates free superoxide from molecular oxygen and is the predominant source of ROS in phagocytic cells. Integrin-mediated fibroblast adhesion to CCN1 and CCN2 activates RAC1 (Chen et al. 2001), which induces ROS generation through 5-LOX and mitochondria for apoptotic synergism with TNF α (Chen et al. 2007). CCN1 interaction with integrins $\alpha_v\beta_5$, $\alpha_6\beta_1$, and the HSPG syndecan-4 is required for this process, and this requirement of multiple receptors may help specify the target cells for elimination. Although TNF α itself induces ROS via NOX-dependent mechanisms to mediate apoptosis and necrosis (Chen et al. 2007; Kim et al. 2007; Yazdanpanah et al. 2009), inhibition of NOX has no effect on CCN1/TNF α synergism, showing a remarkable specificity of ROS in these distinct pathways.

A recent study shows that CCN1 can activate neutral sphingomyelinase 1 (nSMase1) (Juric et al. 2009), which generates the lipid second messenger ceramide that can increase ROS through several mechanisms (Won and Singh 2006). In CCN1/FasL synergism, the activation of nSMase1 plays a critical role in the generation of ROS that triggers the hyperactivation of p38, leading to enhanced Bax activation and cytochrome c release. The discovery that matrix proteins can activate nSMase1 is without precedent, and the mechanism of nSMase1 activation through integrin signaling is currently unknown.

In vivo evidence of the synergism

Since *Ccn1*- and *Ccn2*-null mice are embryonic and perinatal lethal, respectively, they cannot be used to address the roles of CCNs in TNF α or Fas-mediated apoptosis in vivo. To circumvent this problem, knockin mice were created in which the *Ccn1* genomic locus was replaced by an allele encoding DM, a CCN1 mutant that is disrupted in two $\alpha_6\beta_1$ -HSPG binding sites located in the carboxy-terminal domain (Chen et al. 2000, 2007). The resultant CCN1-DM protein is still active in promoting α_v integrin-mediated cellular activities (Leu et al. 2004), but is unable to induce ROS accumulation or synergize with TNF α or FasL to promote fibroblast apoptosis (Chen et al. 2007; Juric et al. 2009). Unlike *Ccn1*-null mice, *Ccn1^{dm/dm}* mice are viable, fertile, and show no obvious morphological or behavioral defects. When injected subcutaneously with a small bolus of soluble TNF α to induce dermal apoptosis, these mutant mice showed >60% reduction in apoptotic cells compared to wild-type mice, consistent with CCN1/TNF α synergism in vivo (Chen et al. 2007).

To study TNF cytokine-mediated cytotoxicity in vivo, *Ccn1^{dm/dm}* mice were tested in three different models of hepatotoxin-induced apoptosis: intravenous delivery of

concanavalin A (ConA), intravenous injection of an agonistic monoclonal antibody that activates Fas (clone Jo2), and intragastric administration of alcohol. ConA induces robust TNF α synthesis in macrophages and T cells and leads to massive TNF α -dependent hepatocyte apoptosis, which is completely abrogated by neutralizing antibodies against TNF α or by genetic ablation of TNFR1 and TNFR2 (Trautwein et al. 1998; Wolf et al. 2001). To examine Fas-mediated apoptosis, the monoclonal antibody Jo2 recognizes and activates the Fas receptor to induce Fas-mediated apoptosis, a process that is annihilated by genetic disruption of Fas, demonstrating the specificity for Fas (Ogasawara et al. 1993). In addition, ethanol gavage in mice mimics binge drinking and results in FasL-induced hepatocyte apoptosis that is ablated by neutralizing antibodies against FasL (Zhou et al. 2001). In all three experimental models, *Ccn1^{dm/dm}* mice consistently show >60% reduction in hepatocyte apoptosis compared to wild-type mice (Fig. 5) (Juric et al. 2009; Chen et al. 2007). These results show that CCN1 is a physiological regulator of TNF α and Fas-mediated apoptosis in vivo, and suggest an important role for CCN1 in the pathogenesis of toxin-induced hepatitis. However, these findings do not exclude the participation of other factors such as IFN γ , which may regulate TNF cytokine cytotoxicity in certain contexts.

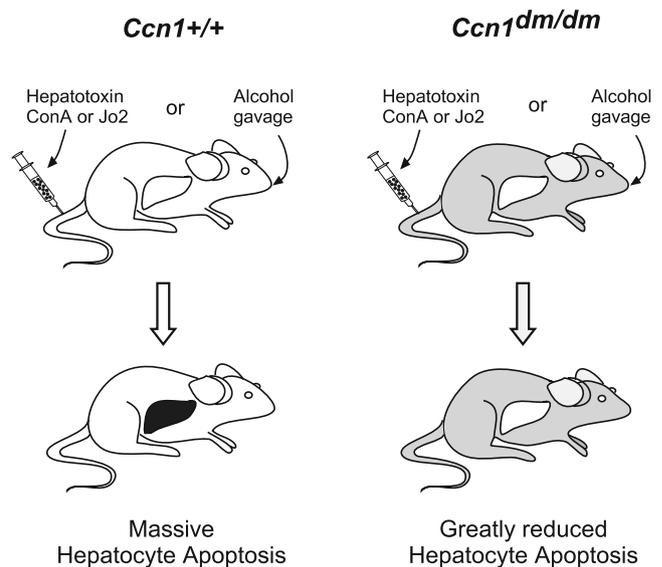


Fig. 5 CCN1 is critical for TNF α and Fas-mediated apoptosis in vivo. *Ccn1^{dm/dm}* knockin mice express the CCN1 mutant DM, which is disrupted in the binding sites for $\alpha_6\beta_1$ and HSPGs and is therefore unable to induce ROS or apoptosis. TNF α -induced apoptosis was tested by either direct subcutaneous injection of TNF α , or by treatment with ConA, which induces TNF α production from macrophages (Chen et al. 2007). Fas-mediated apoptosis was tested by tail-vein injection of the agonistic mAb, Jo2, or by a gavage of ethanol. In each scenario, apoptosis is reduced by 60–70% in *Ccn1^{dm/dm}* mice compared to wild type mice, indicating that CCN1 is critical for optimal TNF α and Fas-mediated apoptosis in vivo

Future questions

The results summarized above indicate that CCN matricellular proteins are contextual regulators of several TNF family cytokines, dictating or promoting their cytotoxicity. However, many other questions still remain. Since cell adhesion to other ECM proteins also generates ROS, what might be the underlying mechanism that sets CCNs apart in being able to synergize with TNFs? Do the remaining members of the CCN and TNF families interact, and do CCNs regulate TNF family cytokine functions other than apoptosis? Beyond fibroblasts and hepatocytes, which cell types are susceptible to CCN/TNF synergism?

Since CCNs and TNF α are coexpressed at sites of inflammation, it is tempting to speculate that CCNs may help to terminate the inflammatory responses initiated by TNF α by unmasking its apoptotic function. Inasmuch as chronic inflammation and elevated TNF α levels contribute to the morbidity of many diseases, CCN/TNF interaction may play an important role in the loss of functional cells during disease progression. For example, in acute and chronic diseases of the liver, such as hepatitis, fibrosis, and fulminant liver failure caused by viral infection, alcohol abuse, and hepatotoxin exposure, elevated levels of TNF α are observed and in some cases TNF α -dependent apoptosis correlate with disease severity (Muto et al. 1988; Czaja et al. 1989; Bird et al. 1990; Gonzalez-Amaro et al. 1994; Blazka et al. 1995; Shibata et al. 2008). Beyond hepatic pathology, other contexts in which CCN/TNF-mediated apoptosis may play a role include placental inflammation and cardiomyopathy, since CCN expression and marked TNF α -dependent apoptosis occur concurrently (Haider and Knofler 2009; Yaoita and Maruyama 2008). The possibility that CCN/TNF synergism may play important roles in the pathogenesis of various diseases mentioned above is intriguing and clearly merits further investigation.

Acknowledgement We thank Joon-Il Jun and Vladislava Juric for helpful comments. This work was supported by grants from the NIH (CA46565, GM78492, and HL81390) to L.F.L.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3:745–756
- Almeida EA, Ilic D, Han Q, Hauck CR, Jin F, Kawakatsu H, Schlaepfer DD, Damsky CH (2000) Matrix survival signaling: from fibronectin via focal adhesion kinase to c-Jun NH(2)-terminal kinase. *J Cell Biol* 149:741–754
- Battegay EJ, Raines EW, Colbert T, Ross R (1995) TNF-alpha stimulation of fibroblast proliferation. Dependence on platelet-derived growth factor (PDGF) secretion and alteration of PDGF receptor expression. *J Immunol* 154:6040–6047
- Bertazza L, Mocellin S (2008) Tumor necrosis factor (TNF) biology and cell death. *Front Biosci* 13:2736–2743
- Bird GL, Sheron N, Goka AK, Alexander GJ, Williams RS (1990) Increased plasma tumor necrosis factor in severe alcoholic hepatitis. *Ann Intern Med* 112:917–920
- Blazka ME, Wilmer JL, Holladay SD, Wilson RE, Luster MI (1995) Role of proinflammatory cytokines in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol* 133:43–52
- Bornstein P (1995) Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J Cell Biol* 130:503–506
- Chang L, Kamata H, Solinas G, Luo JL, Maeda S, Venuprasad K, Liu YC, Karin M (2006) The E3 ubiquitin ligase itch couples JNK activation to TNF α -induced cell death by inducing c-FLIP(L) turnover. *Cell* 124:601–613
- Chen C-C, Lau LF (2009) Functions and mechanisms of action of CCN matricellular proteins. *Int J Biochem Cell Biol* 41:771–783
- Chen N, Chen CC, Lau LF (2000) Adhesion of human skin fibroblasts to Cyr61 is mediated through integrin $\alpha 6 \beta 1$ and cell surface heparan sulfate proteoglycans. *J Biol Chem* 275:24953–24961
- Chen C-C, Chen N, Lau LF (2001) The angiogenic factors Cyr61 and CTGF induce adhesive signaling in primary human skin fibroblasts. *J Biol Chem* 276:10443–10452
- Chen C-C, Young JL, Monzon RI, Chen N, Todorovic V, Lau LF (2007) Cytotoxicity of TNF α is regulated by integrin-mediated matrix signaling. *EMBO J* 26:1257–1267
- Cheresh DA, Stupack DG (2008) Regulation of angiogenesis: apoptotic cues from the ECM. *Oncogene* 27:6285–6298
- Chiarugi P, Giannoni E (2008) Anoikis: a necessary death program for anchorage-dependent cells. *Biochem Pharmacol* 76:1352–1364
- Chiarugi P, Pani G, Giannoni E, Taddei L, Colavitti R, Raugei G, Symons M, Borrello S, Galeotti T, Ramponi G (2003) Reactive oxygen species as essential mediators of cell adhesion: the oxidative inhibition of a FAK tyrosine phosphatase is required for cell adhesion. *J Cell Biol* 161:933–944
- Czaja MJ, Flanders KC, Biempica L, Klein C, Zern MA, Weiner FR (1989) Expression of tumor necrosis factor-alpha and transforming growth factor-beta 1 in acute liver injury. *Growth Factors* 1:219–226
- Franzen CA, Chen CC, Todorovic V, Juric V, Monzon RI, Lau LF (2009) The matrix protein CCN1 is critical for prostate carcinoma cell proliferation and TRAIL-induced apoptosis. *Mol Cancer Res* 7:1045–1055
- Gomez-Lazaro M, Galindo MF, Melero-Fernandez de Mera RM, Fernandez-Gomez FJ, Concannon CG, Segura MF, Comella JX, Prehn JH, Jordan J (2007) Reactive oxygen species and p38 mitogen-activated protein kinase activate Bax to induce mitochondrial cytochrome c release and apoptosis in response to malonate. *Mol Pharmacol* 71:736–743
- Gonzalez-Amaro R, Garcia-Monzon C, Garcia-Buey L, Moreno-Otero R, Alonso JL, Yague E, Pivel JP, Lopez-Cabrera M, Fernandez-Ruiz E, Sanchez-Madrid F (1994) Induction of tumor necrosis factor alpha production by human hepatocytes in chronic viral hepatitis. *J Exp Med* 179:841–848
- Haider S, Knofler M (2009) Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium. *Placenta* 30:111–123
- Holbourn KP, Acharya KR, Perbal B (2008) The CCN family of proteins: structure-function relationships. *Trends Biochem Sci* 33:461–473
- Juric V, Chen CC, Lau LF (2009) Fas-mediated apoptosis is regulated by the extracellular matrix protein CCN1 (CYR61) in vitro and in vivo. *Mol Cell Biol* 29:3266–3279

- Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M (2005) Reactive oxygen species promote TNF α -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120:649–661
- Kim YS, Morgan MJ, Choksi S, Liu ZG (2007) TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. *Mol Cell* 26:675–687
- Kireeva ML, Lam SCT, Lau LF (1998) Adhesion of human umbilical vein endothelial cells to the immediate-early gene product Cyr61 is mediated through integrin $\alpha\beta 3$. *J Biol Chem* 273:3090–3096
- Lau LF, Lam SC (1999) The CCN family of angiogenic regulators: the integrin connection. *Exp Cell Res* 248:44–57
- Leask A, Abraham DJ (2006) All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci* 119:4803–4810
- Leu S-J, Chen N, Chen C-C, Todorovic V, Bai T, Juric V, Liu Y, Yan G, Lam SCT, Lau LF (2004) Targeted mutagenesis of the matricellular protein CCN1 (CYR61): selective inactivation of integrin $\alpha 6\beta 1$ -heparan sulfate proteoglycan coreceptor-mediated cellular activities. *J Biol Chem* 279:44177–44187
- Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104:487–501
- Marastoni S, Ligresti G, Lorenzon E, Colombatti A, Mongiat M (2008) Extracellular matrix: a matter of life and death. *Connect Tissue Res* 49:203–206
- Meng TC, Fukada T, Tonks NK (2002) Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. *Mol Cell* 9:387–399
- Mongiat M, Ligresti G, Marastoni S, Lorenzon E, Doliana R, Colombatti A (2007) Regulation of the extrinsic apoptotic pathway by the extracellular matrix glycoprotein EMILIN2. *Mol Cell Biol* 27:7176–7187
- Muppidi JR, Tschopp J, Siegel RM (2004) Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity* 21:461–465
- Muto Y, Nouri-Aria KT, Meager A, Alexander GJ, Eddleston AL, Williams R (1988) Enhanced tumour necrosis factor and interleukin-1 in fulminant hepatic failure. *Lancet* 2:72–74
- Ogasawara J, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S (1993) Lethal effect of the anti-Fas antibody in mice. *Nature* 364:806–809
- Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, Jayawardena S, De Smaele E, Cong R, Beaumont C, Torti FM, Torti SV, Franzoso G (2004) Ferritin heavy chain upregulation by NF- κ B inhibits TNF α -induced apoptosis by suppressing reactive oxygen species. *Cell* 119:529–542
- Shibata H, Yoshioka Y, Ohkawa A, Abe Y, Nomura T, Mukai Y, Nakagawa S, Tani M, Ohta T, Mayumi T, Kamada H, Tsunoda S, Tsutsumi Y (2008) The therapeutic effect of TNFR1-selective antagonistic mutant TNF- α in murine hepatitis models. *Cytokine* 44:229–233
- Todorovic V, Chen C-C, Hay N, Lau LF (2005) The matrix protein CCN1 (CYR61) induces apoptosis in fibroblasts. *J Cell Biol* 171:559–568
- Torcia M, De CG, Nencioni L, Ammendola S, Labardi D, Lucibello M, Rosini P, Marlier LN, Bonini P, Dello SP, Palamara AT, Zambrano N, Russo T, Garaci E, Cozzolino F (2001) Nerve growth factor inhibits apoptosis in memory B lymphocytes via inactivation of p38 MAPK, prevention of Bcl-2 phosphorylation, and cytochrome c release. *J Biol Chem* 276:39027–39036
- Trautwein C, Rakemann T, Brenner DA, Streetz K, Licato L, Manns MP, Tiegs G (1998) Concanavalin A-induced liver cell damage: activation of intracellular pathways triggered by tumor necrosis factor in mice. *Gastroenterology* 114:1035–1045
- Varfolomeev EE, Ashkenazi A (2004) Tumor necrosis factor: an apoptosis JuNKie? *Cell* 116:491–497
- Werner E, Werb Z (2002) Integrins engage mitochondrial function for signal transduction by a mechanism dependent on Rho GTPases. *J Cell Biol* 158:357–368
- Wolf D, Hallmann R, Sass G, Sixt M, Kusters S, Fregien B, Trautwein C, Tiegs G (2001) TNF- α -induced expression of adhesion molecules in the liver is under the control of TNFR1-relevance for concanavalin A-induced hepatitis. *J Immunol* 166:1300–1307
- Won JS, Singh I (2006) Sphingolipid signaling and redox regulation. *Free Radic Biol Med* 40:1875–1888
- Yaoita H, Maruyama Y (2008) Intervention for apoptosis in cardiomyopathy. *Heart Fail Rev* 13:181–191
- Yazdanpanah B, Wiegmann K, Tchikov V, Krut O, Pongratz C, Schramm M, Kleinriders A, Wunderlich T, Kashkar H, Utermohlen O, Bruning JC, Schutze S, Kronke M (2009) Riboflavin kinase couples TNF receptor 1 to NADPH oxidase. *Nature* 460:1159–1163
- Zhou Z, Sun X, Kang YJ (2001) Ethanol-induced apoptosis in mouse liver: Fas- and cytochrome c-mediated caspase-3 activation pathway. *Am J Pathol* 159:329–338