The International Journal of Biochemistry & Cell Biology 41 (2009) 771–783

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Review

Functions and mechanisms of action of CCN matricellular proteins

Chih-Chiun Chen, Lester F. Lau*

Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago College of Medicine, Chicago, IL 60607, United States

ARTICLE INFO

Article history:
Received 13 May 2008
Received in revised form 25 July 2008
Accepted 25 July 2008
Available online 15 August 2008

Keywords:
Angiogenesis
Cancer
Cardiovascular disease
Chondrogenesis
Fibrosis
Integrin
TNFα
Wound healing

ABSTRACT

Members of the CCN (CYR61/CTGF/NOV) family have emerged as dynamically expressed, extracellular matrix-associated proteins that play critical roles in cardiovascular and skeletal development, injury repair, fibrotic diseases and cancer. The synthesis of CCN proteins is highly inducible by serum growth factors, cytokines, and environmental stresses such as hypoxia, UV exposure, and mechanical stretch. Consisting of six secreted proteins in vertebrate species, CCNs are typically comprised of four conserved cysteine-rich modular domains. They function primarily through direct binding to specific integrin receptors and heparan sulfate proteoglycans, thereby triggering signal transduction events that culminate in the regulation of cell adhesion, migration, proliferation, gene expression, differentiation, and survival. CCN proteins can also modulate the activities of several growth factors and cytokines, including TGF-β, TNFα, VEGF, BMPs, and Wnt proteins, and may thereby regulate a broad array of biological processes. Recent studies have uncovered novel CCN activities unexpected for matricellular proteins, including their ability to induce apoptosis as cell adhesion substrates, to dictate the cytotoxicity of inflammatory cytokines such as TNFα, and to promote hematopoietic stem cell self-renewal. As potent regulators of angiogenesis and chondrogenesis, CCNs are essential for successful cardiovascular and skeletal development during embryogenesis. In the adult, the expression of CCN proteins is associated with injury repair and inflammation, and has been proposed as diagnostic or prognostic markers for diabetic nephropathy, hepatic fibrosis, systemic sclerosis, and several types of cancer. Targeting CCN signaling pathways may hold promise as a strategy of rational therapeutic design.

Contents

Abbreviations: AVSD, atrioventricular septal defects; BMP, bone morphogenetic protein; ECM, extracellular matrix; FAK, focal adhesion kinase; HIF-1α, hypoxia-inducible factor-1α; HSPG, heparan sulfate proteoglycan; LRP, lipoprotein receptor-related protein; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; ROS, reactive oxygen species; SRE, serum response element; TGF-β, transforming growth factor β; TNFα, tumor necrosis factor α; TPA, 12-*O*-tetradecanoyl-phorbol 13-acetate; VSMCs, vascular smooth muscle cells.

* Corresponding author. Tel.: +1 312 996 6978.
E-mail address: LFLau@uic.edu (L.F. Lau).

1357-2725/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2008.07.025

1. Introduction

Far from being an inert scaffolding for the organization of cells into tissues, the extracellular matrix (ECM) is now recognized as a dynamic and multifunctional regulator of cell behavior (Aszodi et al., 2006). The ECM can bind and modulate the bioavailability and activity of growth factors, cytokines, chemokines, and extracellular enzymes. In addition, ECM proteins can directly interact with cell surface receptors to trigger the activation of signal transduction cascades, thereby regulating diverse cellular functions. A subset of ECM proteins, known as matricellular proteins, is dynamically expressed and does not serve obvious structural roles in the matrix (Bornstein and Sage, 2002). Rather, they function primarily to modulate cellular responses to other environmental factors. Known matricellular proteins include thrombospondins, SPARC, hevin, osteopontin, tenascin C and X, and members of the CCN family. Recent studies have shown that CCN proteins are essential regulators of embryonic development, and in the adult they play critical roles in inflammation, injury repair, fibrotic diseases, and cancer.

Members of the CCN family were first identified as secreted proteins whose synthesis was induced by mitogenic growth factors or oncogenes, or deregulated in transformed cells. The first three members described – *C*YR61 (cysteine-rich 61; CCN1) (O'Brien et al., 1990), *C*TGF (connective tissue growth factor; CCN2) (Bradham et al., 1991), and *N*OV (nephroblastoma overexpressed; CCN3) (Joliot et al., 1992) – provided the acronym for the CCN family. CCN4 (WISP1), CCN5 (WISP2), and CCN6 (WISP3) were subsequently identified as Wnt-inducible secreted proteins (Pennica et al., 1998), and together they comprise the family of six homologous, cysteine-rich proteins in vertebrates. CCN proteins share a modular structure, with an N-terminal secretory peptide followed by four conserved domains with sequence homologies to insulin-like growth factor binding proteins (IGFBPs), von Willebrand factor type C repeat (vWC), thrombospondin type I repeat (TSP), and a carboxyl-terminal (CT) domain that contains a cysteine knot motif (Bork, 1993) (Fig. 1). Each structural module is encoded by a separate conserved exon, suggesting that CCN genes are products of exon shuffling (Brigstock, 1999; Lau and Lam, 1999). The N-terminal and C-terminal halves of the proteins are connected by a hinge region that is not conserved and is particularly sensitive to proteolysis (Kireeva et al., 1996; Dean et al., 2007). Since CCN proteins have acquired multiple names reflecting the various circumstances of their identification, a unified nomenclature has been proposed by international consensus to rename these proteins as CCN1–6 in order to minimize confusion (Brigstock et al., 2003). For example, the name CTGF (connective tissue growth factor) originally given to CCN2 implies activities and a mechanism of action akin to those of classical growth factors, a notion that has not been supported by experimental evidence to date.

Early studies on CCN proteins proceeded along two divergent paths: one advanced the idea that CCN proteins are polypeptide growth factors (Bradham et al., 1991; Frazier et al., 1996), while the other demonstrated their roles as ECM-associated cell adhesion molecules (Yang and Lau, 1991; Kireeva et al., 1996). The latter perspective envisions CCNs as matricellular proteins, which function primarily to modify cellular responses to other environmental factors and stimuli through interaction with cell adhesion receptors (Lau and Lam, 1999). The collective work from many laboratories in the CCN community now supports this view (Lau and Lam, 2005; Rachfal and Brigstock, 2005; Leask and Abraham, 2006; Yeger and Perbal, 2007). It should be noted that the purification of biologically active CCN proteins has presented a particular challenge, presumably due to the unusually high number of cysteine residues (~10%). The difficulty in purifying CCN proteins of high quality and the lack of unified biochemical and functional assays that define their specific activities have impeded progress in this field. It is possible that variabilities in results from different laboratories, where they exist, might be in part due to differences in methods of protein preparation.

Analyses of CCN functions are now extending the boundaries of known ECM functions. For example, CCN proteins can induce apoptosis as cell adhesion substrates, dictate the cytotoxicity of tumor necrosis factor α (TNFα), and play an essential role in hematopoietic stem cell self-renewal. In this review, we summarize the current information on CCN functions and action mechanisms, and endeavor to unravel the common threads that may underlie their seemingly disparate roles in various contexts. A recent monograph on the CCN family provides an informative resource on research in this area (Perbal and Takigawa, 2005).

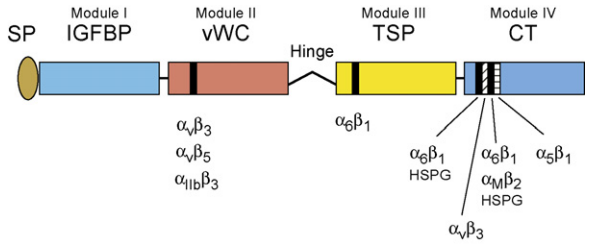

Fig. 1. Schematics of CCN protein structure and localization of their integrin binding sites. The six CCN proteins include CCN1 (CYR61), CCN2 (CTGF), CCN3 (NOV), CCN4 (WISP-1, ELM1), CCN5 (WISP-2, COP-1), and CCN6 (WISP-3). They share significant structural homology, including an N-terminal secretory signal peptide (SP), followed by modular domains (illustrated in different colors) with sequence homologies to insulin-like growth factor binding protein (IGFBP, module I), von Willebrand factor type C repeat (vWC, module II), thrombospondin type 1 repeat (TSP, module III), and a cysteine knot containing carboxyl domain (CT, module IV). Throughout the four modules are 38 cysteine residues that are highly conserved. CCN5 uniquely lacks the CT domain but conserves domains I–III. A protease-sensitive hinge region with no sequence homology among the CCN proteins separate domains II and III. Specific binding sites (black and hatched bars) for several integrins and HSPGs have been identified for CCN1 and CCN2 (Chen et al., 2000; N. Chen et al., 2004; Leu et al., 2003, 2004; Gao and Brigstock, 2004, 2006).