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PROSPECTS

Functional Properties and Intracellular Signaling of CCN1/Cyr61

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Abstract CCN1/Cyr61 is a member of the protein family that can be promptly induced by growth factors. CCN1/Cyr61 promotes cell proliferation, adhesion, and differentiation. It plays important roles in angiogenesis and extracellular matrix production. In addition, CCN1/Cyr61 has many potential functions in tumorigenesis, development, embryo implantation, as well as formation of endometriotic lesions. Expression of CCN1/Cyr61 is regulated by a variety of agents including cytokines, growth factors, steroid hormones, and some drugs. These inducers regulate the transcription of CCN1/Cyr61 through several signaling transduction pathways. CCN1/Cyr61 is able to interact either with the cell itself or the surrounding cells through an autocrine–paracrine mechanism. It has been reported that CCN1/Cyr61 exerts its functions via interacting with at least five integrins as well as heparan sulfate proteoglycan. By activating Wnt, NF-kappaB, or tyrosine kinase signaling pathways, CCN1/Cyr61 is not only able to control the growth of epithelial cells and fibroblasts, but also induce or suppress apoptosis in a cell type-specific manner. J. Cell. Biochem. 100: 1337–1345, 2007.

Key words: CCN1/Cyr61; angiogenesis; embryogenesis; tumorigenesis; apoptosis; signal transduction

CCN1/Cyr61 as a member of growth factor-inducible immediate-early genes belongs to CCN family. Other members of the CCN family include CCN2 (connective tissue growth factor, CTGF), CCN3 (nephroblastoma-overexpressed, NOV), CCN4 (Wnt-inducible secreted protein-1, WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3). The CCN1/Cyr61 protein is composed of four conserved modular domains that share sequence similarities to insulin-like growth factor-binding proteins, von Willebrand factor type C repeats, thrombospondin type 1 repeats, and carboxylterminal region containing cysteine knot domains, respectively [Rachfal and Brigstock, 2005]. Due to containing these specific modular domains, CCN proteins have various biological activities and functions.

CCN1/Cyr61 either localizes intracellularly or associates with extracellular matrix and cell surfaces. Studies by Tamura et al. [2001] reveal that CCN1/Cyr61 is found both in the cytoplasm and nucleus of cultured bladder smooth muscle cells. The nuclear localization of CCN1/Cyr61 is very surprising, since the primary structure of CCN1/Cyr61 does not include a classic nuclear localization sequence, like those transcription factors, that allows them to translocate to the nucleus. However, a growing list of such polypeptides lack of nuclear localization sequence, including NOV, epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiogenin, and parathyroid hormone-related peptide, have been found in the nucleus. They are probably correlated with transcriptional regulation, and/or mRNA transport [Tamura et al., 2001]. Thus, the detection of CCN1/Cyr61 in the nuclei opens new prospects as to the multifunctional roles of this protein.

CCN1/Cyr61 regulates cell proliferation, adhesion, migration, differentiation, apoptosis, growth arrest and extracellular matrix production. Therefore, CCN1/Cyr61 plays potential

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roles in development, chondrogenesis, angiogenesis, tumorigenesis, wound healing, and vascular diseases.

CCN1/Cyr61 AND EMBRYOGENESIS

During embryogenesis, CCN1/Cyr61 is expressed at sites of neovascularization and mesenchymal condensation and can be detected in vessels such as the dorsal aorta, bronchial arch, and umbilical artery. It may help to promote uterine vessel growth toward the embryo [O'Brien and Lau, 1992]. CCN1/Cyr61 is produced by trophoblastic giant cells and trophoblasts of the ectoplacental cone and facilitates placental neovascularization in the mouse placenta [Rachfal and Brigstock, 2005]. An essential role of CCN1/Cyr61 during embryogenesis has been shown in knock-out mice. CCN1/Cyr61-null animals suffer developmental failure and embryonic death due to vascular defects in the placenta [Mo et al., 2002]. The *Xenopus laevis* homolog of CCN1/Cyr61, Xcyr61, has been shown to be required for normal gastrulation movements, which is at least mediated in part through the adhesive properties of Xcyr61 and its related ability to modulate the assembly of the ECM [Latinkic et al., 2003].

As to the relationship between CCN1/Cyr61 and embryogenesis, we also find that CCN1/Cyr61 mRNAs occurs specifically on the unclosed endometrial luminal epithelium surrounding the embryo in the mouse uterus on Day 4 (2400 h) of pregnancy (day 1 = day of vaginal plug) (Fig. 1A), when attachment occurs. CCN1/Cyr61 mRNAs are still localized specifically on day 5 of pregnancy. Interestingly, there is no CCN1/Cyr61 mRNA detected in the mouse uterus before Day 4 and even in the morning of Day 4 (0900 h), as well as at the inter-implantation sites of uterus on Day 5 (Fig. 1B) [Chen et al., 2006]. Studies by Mo et al. [2002] reveal that phenotypes of CCN1/Cyr61 mutants bear intriguing similarities to those of mice lacking integrin α_v, which is consistent with the hypothesis that CCN1/Cyr61 may act as a physiological ligand of integrin α_v during embryo implantation. We presume that CCN1/Cyr61 binds to integrin $\alpha_v\beta_3$ after being induced by the blastocyst and then mediates the primary interaction between blastocyst and endometrium.

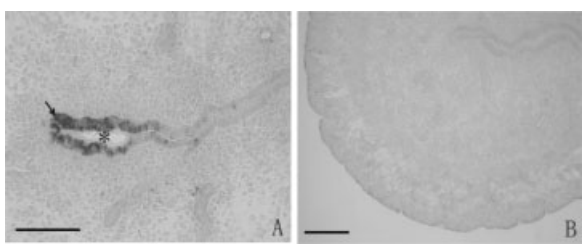

Fig. 1. In situ hybridization of CCN1/Cyr61 mRNA in pregnant mouse uterus. **A**: CCN1/Cyr61 can be detected on the luminal epithelium surrounding the implanting blastocyst at the implantation site on Day 5 morning of pregnancy in mouse uterus. All the sections were counter-stained with 1% methyl green. The positive signal was visualized as a dark brown color. **B**: There is no detectable signal at the inter-implantation site on Day 5 morning. In situ hybridization for CCN1/Cyr61 was repeated at least three times with the uteri from three different animals at each stage. Arrow: luminal epithelium; * implanting blastocyst; Bar = 60 μm.

The expression pattern of CCN1/Cyr61 in human uterus is different from that in the mouse. The level of CCN1/Cyr61 is higher in proliferative phase than in secretory phase of menstrual cycle. Studies by Absenger et al. [2004] reveal that women with endometriosis have a significantly higher amount of CCN1/Cyr61 and integrin $\alpha_v\beta_3$ in their endometrium. There are more CCN1/Cyr61 proteins expressed in ectopic endometriotic lesions than in eutopic endometria. Baboon model of induced endometriosis is used for further study. The CCN1/Cyr61 transcript is extensively upregulated in the eutopic endometrium from all baboons with induced endometriosis. Ectopic endometriotic lesions show a further increase in CCN1/Cyr61 mRNA, with highest expression found in red lesions [Gashaw et al., 2006]. From these encouraging results, it is reasonable to predict that CCN1/Cyr61 might become a useful marker for endometriosis. Endometriosis is closely related to infertility. However, whether the successful pregnancy in human needs the proper expression of CCN1/Cyr61 is still unclear.

CCN1/Cyr61 AND ANGIOGENESIS

CCN1/Cyr61 is detected to express on the vessel wall of the developmental circulatory system and regulates the proliferation of vessel endothelial cells and angiogenesis [Brigstock, 2003]. CCN1/Cyr61 can be both direct and indirect angiogenesis inducer.

Potent proangiogenic properties of CCN1/Cyr61 were demonstrated in a rat cornea model and in a rabbit ischemic hindlimb model. Purified CCN1/Cyr61 protein stimulates

directed migration of human microvascular endothelial cells in culture through an $\alpha_v\beta_3$-dependent pathway and induces neovascularization in rat corneas in vivo. Both of these activities were blocked by specific anti-CCN1/Cyr61 antibodies [Babic et al., 1998]. In the rabbit ischemic hindlimb model, Cyr61 gene transfer to rabbits appears potent in stimulating limb revascularization, thereby promoting great improvement in tissue perfusion in the ischemic limb [Fataccioli et al., 2002].

Cyr61 can also regulate the expression of genes involved in angiogenesis and matrix remodeling, including vascular endothelial growth factor A (VEGF-A), VEGF-C, type I collagen, matrix metalloproteinase 1 (MMP1), MMP3, and tissue inhibitors of metalloproteases (TIMPs) [Chen et al., 2001].

The essential nature of CCN1/Cyr61 as a regulator of vascular development has been established through gene targeting in mice. CCN1/Cyr61-null mice suffer embryonic death due to vascular defects including undervascularization of the placental labyrinth and loss of vascular integrity in the embryo [Mo et al., 2002].

CCN1/Cyr61 plays potential roles in vascular diseases such as atherosclerosis and restenosis. Marked CCN1/Cyr61 protein expression was noted in cardiomyocytes of patients with end-stage ischemic cardiomyopathy but was almost absent in nonfailing human myocardium hypertrophic growth [Hilfiker-Kleiner et al., 2004]. These findings of CCN1/Cyr61 as a potent proangiogenic factor to drive sprouting of new vessels could be a promising therapeutic candidate for treating severe peripheral ischemic diseases.

CCN1/Cyr61 AND TUMORIGENESIS

Increasing evidences demonstrate that aberrant expression of CCN1/Cyr61 is linked to tumorigenesis. Earlier reports [Xie et al., 2001b; Tsai et al., 2002; Lin et al., 2004] reveal that elevated CCN1/Cyr61 level is associated with advanced breast adenocarcinoma, pancreatic cancer, and gliomas. Overexpressed CCN1/Cyr61 in MCF-12A normal breast cells can induce tumor formation in nude mice [Tsai et al., 2002]. Moreover, overexpressed CCN1/Cyr61 in MCF-7 breast cancer cells promotes the invasion of these cells when being transplanted into mice [Xie et al., 2001a]. In addition, an increased expression of CCN1/Cyr61 is also detected in rhabdomyosarcoma, colon adenosarcoma, papilloma of bladder, and multiforms of glioblastoma. Several types of pediatric tumors contain a high level of CCN1/Cyr61, such as angiofibroma, malignant fibrous histiocytoma, infantile myofibromatosis, and malignant hemangiopericytoma [Perbal, 2001]. In contrast, earlier reports [Pilarsky et al., 1998; Tong et al., 2001; Sampath et al., 2001a; Croci et al., 2004] reveal that downregulation of CCN1/Cyr61 expression is noted in prostate cancer, uterine leiomyoma, rhabdomyosarcoma, embryonic rhabdomyosarcoma, and non-small-cell lung carcinoma. The expression of CCN1/Cyr61 in neuroblastoma and carcinoma of prostate has a clearly negative correlation with the malignancy phenotype [Pilarsky et al., 1998]. CCN1/Cyr61 is regarded as an inhibitor in non-small-cell lung cancer [Chien et al., 2004]. Obviously, CCN1/Cyr61 plays different roles in different types of tumors, suggesting sophisticated functions of CCN1/Cyr61 are strictly depending on cellular context.

CCN1/Cyr61 AND APOPTOSIS

CCN1/Cyr61 can either induce or suppress apoptosis in a cell type-specific manner. Generally, CCN1/Cyr61 adhesion to endothelial cells promotes cell survival, whereas CCN1/Cyr61 adhesion to fibroblasts induces apoptosis. CCN1/Cyr61 plays an important role in resistance to chemotherapeutic agent-induced apoptosis in human breast cancer MCF-7 cells [Lin et al., 2004]. A direct evidence of CCN1/Cyr61 in inhibiting apoptosis is revealed by Said et al. [Said and Koeffler, 2004] that in human endometrial cancer cells AN3CA transfected with CCN1/Cyr61, apoptosis factors including Bax and Bad are elevated. Activation of Bax elicits release of cytochrome C which leads to apoptosis. Upregulation of Bad is able to inhibit anti-apoptotic activity of Bcl-2 and Bcl-xL. CCN1/Cyr61 triggers the activation of Bax, which renders release of cytochrome C, activation of caspase-9 and -3, and finally leads to cell apoptosis [Todorovicc et al., 2005]. Thus, the increased apoptosis is associated with elevated expression of the pro-apoptotic proteins such as Bax and Bad, depolarization of mitochondrial membrane, activation of caspases, and inhibition of anti-apoptosis proteins (Bcl-2). A second

pathway involved in apoptosis is mediated by the interaction between TNF receptors and their ligands, which leads to a cascade of activation of caspases [Said and Koeffler, 2004]. AN3CA cells transfected with CCN1/Cyr61 have an increased level of tumor necrosis factor receptor-associated ligand (TRAIL). TRAIL interacts with the TNF receptors known as DR-4 and -5 to activate caspase-8 and finally activates the downstream effector caspase-3 and induces apoptosis.

CCN1/Cyr61 AND CHONDROGENESIS

CCN1/Cyr61 is expressed in hypertrophic chondrocytes at the growth plate, and the fracture callus in rats. CCN1/Cyr61 appears in human bone at sites of bone remodeling [Hadjiargyrou et al., 2000]. In human osteoblasts, CCN1/Cyr61 is regulated by a variety of bone-relevant growth factors [Schutze et al., 1998]. The expression of CCN1/Cyr61 during mouse embryogenesis is tightly correlated with the vasculature development and mesenchymal condensation as they differentiate into chondrocytes. CCN1/Cyr61 promotes collagen and cartilage production in limb bud culture and stimulates chondrogenesis, mitogenesis, and adhesion in limb mesenchymal cells. These findings indicate that CCN1/Cyr61 plays an important role in cartilage cell differentiation and chondrogenesis. The presence of CCN1/Cyr61 may help to induce angiogenesis as a prelude to replacement of cartilage by bone.

PATHWAYS INVOLVED IN CCN1/Cyr61 INDUCTION

CCN1/Cyr61 can be induced by over 30 agents. It is stimulated rapidly by growth factors like EGF [Sampath et al., 2002], PDGF, VEGF, basic fibroblast growth factor (bFGF), and transforming growth factor-β (TGF-β) [Abe and Sato, 2001; Parisi et al., 2006]. It is also inducible by chemokines such as interleukin-1 (IL-1), IL-2, IL-6 [Sampath et al., 2001b; Mo et al., 2002; Hammacher et al., 2005]. In addition, earlier reports reveal that serum [O'Brien et al., 1990], cortisol or BMP-2 [Parisi et al., 2006], 12-*O*-tetradecanoylphorbol 13-acetate, estrogen, vitamin D_3, tumor necrosis factor-α (TNF-α) [Sampath et al., 2001b], Flavolutan or R5020 [Sampath et al., 2002], tamoxifen [Rivera-Gonzalez et al., 1998], choleratoxin or phorbol [Brigstock, 2003], and fibrin [Pendurthi et al., 2000] are all agonists of CCN1/Cyr61 expression. Even mechanical stretch can induce the expression of CCN1/Cyr61 [Tamura et al., 2001]. Many agents have been used to identify the upstream signaling molecules involved in CCN1/Cyr61 gene induction.

The signaling events involved in CCN1/Cyr61 gene induction in smooth muscle cell and endothelial cell are clarified into two pathways, RhoA GTPase and p38 MAP kinase pathways. When Sphingosine 1-Phosphate (S1P) binds to a G protein-coupled receptor called endothelial differentiation gene receptor, activated RhoA and p38 upregulate the AP-1 and cAMP response element binding protein (CREB) through mitogen- and stress-activated protein kinase (MSK). MSK1 has a much higher affinity for CREB, and has a primary role in regulating both CREB and CREB kinase activity. Phosphorylation of CREB stimulates CCN1/Cyr61 promoter activity, resulting in transcription of CCN1/Cyr61 [Han et al., 2003].

Prostaglandins and their receptors are found to regulate CCN1/Cyr61 expression in HEK 293/EBNA cells through Rho and MAP kinase pathways as well. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), an agonist of prostaglandin FP receptor, stimulates FP receptor and triggers G_q protein-coupled receptor activation, which includes intracellular Ca^{2+} release, IP turnover, and protein kinase C activation. Butaprost is a synthetic prostaglandin analog that interacts with EP_2 receptor. EP_2-mediated CCN1/Cyr61 expression is via Rho and MAP kinase pathways. Butaprost triggers G_s protein-coupled receptor activation mechanism, which involves activation of adenylate cyclase and initiation of the cAMP pathway with resultant activation of protein kinase A [Liang et al., 2003].

CCN1/Cyr61 is regulated by the canonical Wnt signal and plays an important role in Wnt3A-induced osteoblast differentiation [Si et al., 2006]. When mesenchymal stem cells were treated with Wnt3A, three members of the CCN family, CCN1/Cyr61, CCN2/CTGF, and CCN5/WISP2, were most significantly upregulated. CCN1/Cyr61 is a direct target of canonical Wnt/β-catenin signaling in mesenchymal stem cells.

Pressure overload, ischemia, and neurohormonal factors, such as Ang II or α1-adrenergic

stimuli, induced CCN1 expression in ventricular cardiomyocytes isolated from 1- to 3-day-old rats. Ang II, signaling via the angiotensin type 1 (AT1) receptor and PKC-dependent mechanisms, and α1-adrenergic stimulation with phenylephrine induce myocardial expression of CCN1 [Hilfiker-Kleiner et al., 2004].

Stretch enhanced CCN1 expression in bladder smooth muscle cells via the activation of the mechanosensitive transcription factor, early growth response gene product 1(Egr-1), and involves the protein kinase C/PI3K/Rho-kinase signaling pathway [Tamura et al., 2001; Grote et al., 2004]. Furthermore, studies by Yamaguchi et al. [2002] reveal that stretch-dependent Egr-1 activation enhances the expression of membrane type 1 matrix metalloproteinase in rat microvascular endothelial cells with a significant impact on angiogenesis by affecting the activity of matrix metalloproteinase-2.

CCN1/Cyr61-MEDIATED SIGNAL TRANSDUCTION PATHWAYS

CCN1/Cyr61 has been shown to exert a range of diverse functions, by binding to cell surface at least through five integrins, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_6\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_M\beta_2$, and heparan sulfate proteoglycans (HSPGs) [Leu et al., 2003]. HSPGs act as coreceptors [Chen et al., 2001]. The utilization of integrins by Cyr61 is cell type- and function-specific. For example, in fibroblasts, CCN1/Cyr61 induces cell adhesion by binding to $\alpha_6\beta_1$ and HSPG, induces cell migration by interacting with $\alpha_v\beta_5$, and induces cell proliferation by binding to integrin $\alpha_v\beta_3$ [Chen et al., 2001]. Cell adhesion to Cyr61 in platelets and monocytes is mediated through integrins $\alpha_{IIb}\beta_3$ and $\alpha_M\beta_2$, respectively [Jedsadayanmata et al., 1999; Grzeszkiewicz et al., 2002; Schober et al., 2002]. Engagement of CCN1/Cyr61 with its receptors results in activation of several signal transduction pathways and initiation of the transcription of target genes. CCN1/Cyr61-mediated signal transduction pathways are summarized in Figure 2.

Wnt Pathway

CCN1/Cyr61 functions through β-catenin-mediated Wnt signal transduction pathway in the non-small-cell lung cancer cells and H520-Cyr61 cells transfected with Cyr61 [Said and Koeffler, 2004]. β-catenin is regulated by adenomatous polyposis coli (APC), axin and glycogen synthase kinase-3beta (GSK-3β). When there's no specified signal, β-catenin is phosphorylated by GSK-3β and degraded by ubiquitination and proteosome pathway [Sadot et al., 2000]. When CCN1/Cyr61 binds to integrin receptor, kinase activity of GSK-3β is inhibited by phosphorylation and β-catenin accumulates and translocates into the nucleus. In the nucleus, β-catenin binds to the T-cell factor/lymphocyte-enhancing factor (TCF/LEF) to form a complex which serves as a transcriptional regulator that promotes the expression of c-myc. c-myc upregulates p53, p21, and p130 sequentially. p130 cooperates with p21 to suppress cellular growth [Tong et al., 2004]. Taken together, in H520-Cyr61 cells, CCN1/Cyr61 triggers nuclear translocation of β-catenin and functions through catenin/TCF-LEF/c-myc/p53/ p21/p130 pathway, resulting in growth arrest.

While studies by Xie et al. [2004] reveal that forced expression of CCN1/Cyr61 in glioma cells upregulates transcription of cyclin D1 instead of c-myc. Overexpression of CCN1/Cyr61 also results in the phosphorylation of GSK-3β and accumulation and nuclear translocation of β-catenin, leading to activation of the β-catenin-mediated Wnt pathway. And it is certified that these effects are most likely through the activation of integrin-linked kinase (ILK). Overexpressed CCN1/Cyr61 increases expression of integrins β_1 and β_3. ILK interacts directly with the cytoplasmic domain of the integrins β_1 and β_3, and its kinase activity is modulated by interactions with the CCN1/Cyr61 leading to its activation [Delcommenne et al., 1998]. Furthermore, ILK can upregulate cyclin D1, also through phosphorylation and inactivation of GSK-3β [D'Amico et al., 2000]. Thus, in human gliomas cells U343-CCN1/Cyr61, CCN1/Cyr61 functions through ILK/catenin/TCF-LEF/cyclin D1 pathway.

NF-κB Pathway

Overexpressed CCN1/Cyr61 significantly increases the resistance of the breast cancer cell MCF-7 to doxorubicin, paclitaxel, and β-lapachone through NF-κB pathway and confers the cells capacity of anti-apoptosis [Lin et al., 2004]. After CCN1/Cyr61 binding to $\alpha_v\beta_3$ or $\alpha_v\beta_5$, NF-κB is activated by shedding of repressible protein from it through

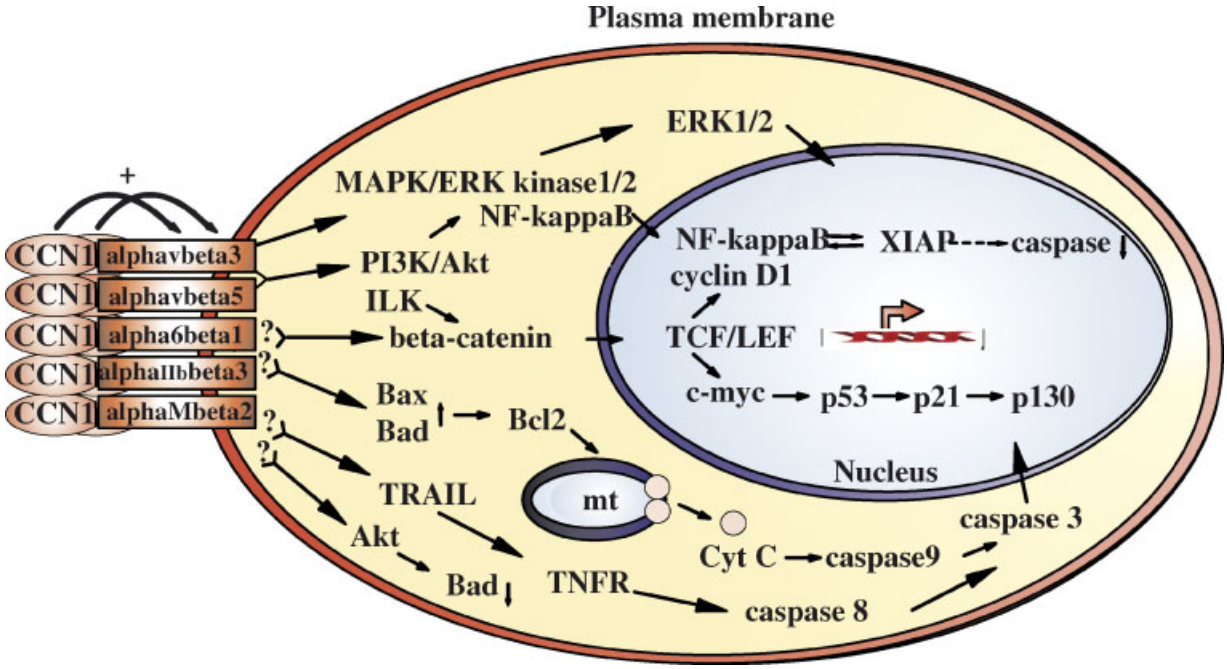

Fig. 2. A schematic representation of CCN1/Cyr61-mediated signaling transduction pathways. **1**. In human breast cancer cell MCF-7, overexpressed CCN1/Cyr61 upregulates $\alpha_v\beta_3$ and binds to it to activate ERK1/ERK2 MAPK, thus promotes cell proliferation and survival. **2**. In MCF-7, Cyr6 binds to $\alpha_v\beta_3/\alpha_v\beta_5$, activates PI3K/Akt, NF-κB is activated and translocates into the nucleus, which initiates the transcription of anti-apoptosis protein XIAP. There exists a positive loop between XIAP and NF-κB. **3**. In the H520-CCN1/Cyr61 cells, CCN1/Cyr61 activates β-catenin to translocate into the nucleus and form a complex with TCF/LEF, induces the expression of c-myc/p53/p21/p130 sequentially which causes growth arrest. While in the gliomas cells, β-catenin and TCF/LEF activates cyclin D1 instead of c-myc. **4**. In AN3CA cells transfected with CCN1/Cyr61, CCN1/Cyr61 upregulates pro-apoptosis Bax and Bad. Cyt C releases from the membrane of the mitochondrion, which activates caspase-induced DNase to result in cell death. **5**. In the AN3CA cells, TRAIL interacts with TNF receptors to activate caspase-8 and -3 to cause cell apoptosis. **6**. In gliomas cells and pulmonary epithelial cells, CCN1/Cyr61 can activate Akt and inhibit the apoptotic effector Bad by its phosphorylation. * Question marks indicate that the receptors have not been experimentally determined. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

phosphorylation. Activated NF-κB enters the nucleus and forms a loop structure to contact DNA. Then it stimulates the transcription of anti-apoptosis protein X-linked inhibitor of apoptosis protein (XIAP). XIAP, as an inhibitor of caspases, can prevent apoptosis in a variety of cell systems in response to cytotoxic stresses, including anti-cancer drugs. XIAP can stimulate NF-κB activation by increasing nuclear translocation of the p65 subunit [Lin et al., 2004]. This implies a positive loop between XIAP and NF-κB, and this may function coordinately to protect cell from apoptosis triggered by chemotherapeutic agents.

CCN1/Cyr61 may contribute to the malignant progression of gastric cancer by promoting cancer cell motility/invasion through upregulation of the functional cyclooxygenase-2 (COX-2) via $\alpha_v\beta_3$/NF-κB-dependent pathway. Studies by Lin et al. [2004] reveal that the overexpressed CCN1/Cyr61 in AGS cells significantly increases the expression of COX-2. A blocking antibody of $\alpha_v\beta_3$ is able to suppress CCN1/Cyr61-mediated NF-κB activation, COX-2 gene expression, and cell invasion. However, the induction of XIAP in CCN1/Cyr61-expressing gastric cancer cells can not be detected, which indicates that CCN1/Cyr61-induced NF-κB activation in different cell types may lead to distinct downstream genes.

Tyrosine Kinase Pathway

Estrogen, progesterone, bone morphogenetic protein (BMP), TGF-β, and hyperglycemia stimulate CCN family proteins to bind to integrins and initiate focal adhesion kinase (FAK) and p42/p44 mitogen-activated protein kinases (MAPK) signaling pathway. This leads to a series of physiological reaction in mesangial cell, epithelial cell, trophoblastic cell, decidual cell, endothelial cell, and chondroblast, and regulate certain processes such as angiogenesis, tumor growth, placentation, embryo implantation, and chondrosteosis [Brigstock, 2003].

In breast cancer cells, CCN1/Cyr61 promotes cell proliferation through ERK1/2 MAPK pathway. A "CCN1/Cyr61-$\alpha_v\beta_3$ autocrine loop" is identified in these cells in which overexpressed CCN1/Cyr61 significantly increases the level of $\alpha_v\beta_3$ up to over 200 times. CCN1/Cyr61-induced activation of ERK1/2 MAPK can be blocked by the administration of functional inhibitor of $\alpha_v\beta_3$. Moreover, overexpressed CCN1/Cyr61 can render resistance to taxol-induced cytotoxicity in the MCF-7 cells. Remarkably, inhibition of $\alpha_v\beta_3$ is able to convert the CCN1/Cyr61-induced taxol-resistant phenotype into a hypersensitive one. Targeting $\alpha_v\beta_3$ may simultaneously prevent breast cancer angiogenesis, growth, and chemo-resistance [Menendez et al., 2005]. In all, CCN1/Cyr61 is sufficient to promote breast cancer cell proliferation, cell survival, and taxol resistance through a $\alpha_v\beta_3$-activated ERK1/2 MAPK signaling pathway.

In fibroblasts, CCN1/Cyr61 promotes the morphological changing through FAK/MAPK pathway. Fibroblast adhesion to CCN1/Cyr61 is mediated through $\alpha_6\beta_1$ and HSPGs, leading to the formation of integrin $\alpha_6\beta_1$-containing focal complexes, cytoskeleton reorganization, and cell spreading with lamellipodia and filopdia [Chen et al., 2001]. These morphological changes are accompanied by signaling events including the activation of FAK, a tyrosine kinase that plays a critical role in integrin signaling, paxcillin (a substrate for FAK) as well as MAPKs [Pendurthi et al., 2000].

Akt Pathway

In pulmonary epithelial cells, overexpressed CCN1/Cyr61 provides cytoprotection in hyperoxia-induced cell death via Akt signaling pathway [Jin et al., 2005]. In glioma cells, CCN1/Cyr61 can enhance tumorigenicity through Akt signaling and activating integrin-linked kinase (ILK) to stimulate β-catenin-TCF/LEF [Xie et al., 2004]. First, forced expression of CCN1/Cyr61 in the glioma cells activates phosphatidylinositol 3 kinase (PI3K) pathway, resulting in the phosphorylation of Akt and anti-apoptotic protein Bad. Therefore, Akt is activated by phosphorylation whereas Bad can be inhibited by phosphorylation, leading to increased cell survival. Menendez et al. [2003] have demonstrated that the resistance to paclitaxel-induced cell death in CCN1/Cyr61-overexpressed MCF-7 cells is related to PI3K/Akt pathway. In agreement to this study, studies by Lin et al. [2004] also reveal that the PI3K/Akt/NF-κB signaling pathway is required for the anti-apoptotic effect of CCN1/Cyr61 in MCF-7 cells.

CONCLUSION

In this work, we have summarized recent findings in CCN1/Cyr61 functions and CCN1/Cyr61-mediated signaling transduction pathways. CCN1/Cyr61 is widely involved in a lot of cell activities and at least mediates embryo implantation, angiogenesis, tumorigenesis, and apoptosis. The biological properties of CCN1/Cyr61 are dependent on their interacting molecules, as they can be positive or negative effectors. Diverse functions of CCN1/Cyr61 are resulted from their extreme ability to bind and activate certain integrin subtypes in a context-specific and cell-specific manner. At present, the signaling pathways that are involved in gene expression of CCN1/Cyr61 and CCN1/Cyr61-mediated signal transduction pathways are being vividly studied, yet to be fully defined, because of the complex crosstalk of intracellular molecules.

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