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CCN PROTEINS AND CANCER: TWO TO TANGO

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1. ABSTRACT

The CCN genes encode secreted proteins, associated to the extracellular matrix. They are involved in diverse biological processes such as regulation of cell-adhesion, migration, proliferation, differentiation and survival. They play important roles in pregnancy, development, angiogenesis, wound repair and inflammation. Several lines of evidence support a role for CCN genes in fibrotic disorders and tumorigenesis. We will focus our attention in this review on two CCN proteins: CCN1 and CCN3, that appear to exert distinct and opposite effects. Recent data suggest that CCN1 acts as a tumor-promoting factor and a key regulator in cancer progression, while CCN3 exhibits suppressive capabilities. The possible opposite functions of CCN1 and CCN3 in tumorigenesis, and the relevance of the distinct expression profiles of these two genes observed in many cancers are discussed below.

2. INTRODUCTION

In addition to abnormal proliferation, which is a hallmark of cancer cells, multiple sets of characteristics are used to define their phenotypes. The molecular events governing cell growth are complex and involve a large number of intracellular and intercellular signaling pathways. Aberrations in these pathways may underline the uncontrolled growth in cancer as well as abnormal cellular responses. Over the last few decades, interest has focused on alterations of oncogenes, tumor suppressor genes and growth factors that are involved in tumor formation (1).

More recently, a new family of structurally related proteins emerged as biologically significant in the course of several important diseases including cancer. This family of growth regulators designated as CCN currently comprises CCN1 (cysteine-rich 61, Cyr61), CCN2 (connective tissue growth factor, CTGF), CCN3 (nephroblastoma overexpressed, NOV), CCN4 (Wnt-induced secreted proteins, WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3). These conserved proteins consist of four distinct modules that share partial identity with 1) insulin-like growth factor-binding proteins, 2) the Von Willebrand factor type C repeat, 3) a thrombospondin type 1 repeat and 4) a C-terminal domain which contains a heparin binding motif and a cysteine-knot motif that may participate in dimerization and receptor binding. The major translational products (except for CCN5) of most CCN family members are secreted proteins with 343 to 381 amino-acid residues, each containing 38 conserved cysteines (2,3). CCN proteins are considered as matricellular proteins. Consistent with their localization to the extracellular matrix and their similarity to other matrix proteins, they have been shown to be involved in diverse biological activities such as regulation of cell adhesion, migration, proliferation, differentiation, and survival. They may also play important roles in pregnancy, development, angiogenesis, wound repair, fibrotic disorders and inflammation (3-6).

Several lines of evidence support a role for CCN genes in tumorigenesis (7). We will focus our attention in this article on two CCN proteins, CCN1 and CCN3, that appear to exert distinct and opposite effects. Recent data

suggest that CCN1 acts as a tumor-promoting factor while CCN3 shows suppressive capabilities. Both of them seem to be important regulators of cancer progression and metastasis. The apparent opposite effects of CCN1 and CCN3 in tumor formation may illustrate the need for balanced antagonist pathways in the control of normal cell growth and development; the relevance of the distinct expression profiles of these two proteins observed in many different cancers are discussed below.

CCN3 has been discovered as an integration site of the Myeloblastosis Associated Virus (MAV) in one chicken nephroblastoma (8). MAV-induced avian nephroblastomas represent a unique model of the Wilms' tumor, a paedriatric tumor of the kidney affecting approximately one in 10,000 children (9-10). MAV is a tumorigenic retrovirus competent for replication, which does not contain oncogenic sequences from cellular origin. CCN3 was originally designated NOV (Nephroblastoma Ovexpressed) because large quantities of CCN3 mRNA were also found in all other MAV-induced nephroblastomas, whereas in normal post-natal kidney levels of CCN3 mRNA were low (8,9). Since non-acute retroviruses have been reported to induce tumors by integrating in the vinicity of growth regulatory genes in the host genome, one hypothesis to explain the induction of CCN3 transcripts in all the tumors tested was that the CCN3-expression might be stimulated by enhancer sequences localized in the long terminal repeat (LTR) sequences of the virus. The use of Fluorescent In Situ Hybridazation (FISH) analysis and Bacterial Artificial Chromosomes (BACs) permitted us to establish that the CCN3 gene is not a preferential integration site of the MAV virus and that increased levels of CCN3 in tumors likely results from the clonal expansion of target cells that already express high levels of CCN3 (11, our unpublished data). CCN3 was the first member of the CCN family found deregulated during tumorigenesis. This observation raised the possibility of new biological features for this group of growth regulators, extending their functions beyond the normal response to growth stimulation. A link was made between alteration of CCN3 and tumor development in avian nephroblastomas. Furthermore, the use of this system highlighted for the first time the dual potential of a CCN protein: the full-length CCN3 protein acting as a tumor suppressor gene whereas a truncated isoform showed growth activity.

CCN1 was first identified as the product of a growth factor-inducible immediate early gene expressed in fibroblast upon serum-induced growth of starved cells. The expression of CCN1 can be induced by a variety of growth factors, hormones and drug components, including serum, epidermal growth factor (EGF), fibroblast growth factor (FGF2), transforming growth factor beta (TGFbeta), 17 beta-estradiol, muscarinic acetylcholine receptors, and vitamin D3 (12-14). CCN1 is an extracellular matrix-associated angiogenic inducer that functions as a ligand of integrin receptors to promote cell adhesion, migration and proliferation. Purified CCN1 protein has been reported to mediate cell adhesion, stimulate chemotaxis, increase growth factor-induced DNA synthesis, foster cell survival and enhance angiogenesis *in vivo* (6).

3. POSITIVE ASSOCIATION BETWEEN EXPRESSION OF CCN1/CCN3 AND TUMORIGENICITY

Elevated expression of CCN1 has been detected in different types of cancer. More importantly, CCN1 was reported to exert positive effects on tumorigenicity in the case of human breast carcinomas, which are characterized by loss of normal cell proliferation, resulting in epithelial hyperplasia or sclerosing adenosis. After progression to carcinoma, numerous cellular alterations can be identified, including increased expression of oncogenes, such as c-myc, decreased expression of tumor-suppressor genes (e.g. p53, Rb), alteration in cell structure such as an increase in vimentin expression, loss of cell adhesion that may involve E-cadherin and integrins, and increased expression of angiogenic factors (e.g. VEGF, FGF) (15). It is now well established that CCN1 is overexpressed in invasive and metastatic human breast cancer cells. Many experiments were conducted to better document the role of CCN1 in breast cancer, sex steroid regulation and hormone-induced carcinogenesis. In an analysis of primary breast tumors, CCN1, CCN2 and CCN4, but not CCN3, were found to be overexpressed (16). Significant association between CCN1 expression and stage, tumor size, positive lymph nodes, age and estrogen receptor status was found, linking the expression of CCN1 to clinical and pathological parameters. This underlines a role for CCN1 in the progression of breast cancer and suggests that it could serve as a valuable tool for monitoring tumor status. CCN1 mRNA levels are increased in MCF-7 breast cancer cells upon stimulation by estrogens, suggesting that the corresponding protein may be involved in the development of estrogen-mediated breast cancer (16-17). Human breast cancer aggressiveness correlates with the capacity of the cells to bypass estrogen (E_2) requirement for growth, the most common clinical evolution during breast cancer progression, with the concurrent dysregulation of growth factor/growth factor receptor (18). Heregulin (HRG) is a growth factor capable of activating the erB-2/3/4 receptor signaling pathway, and is often related to the invasive breast cancer phenotype (19). It was shown that HRG induces breast cancer progression, consistent with loss of estrogen receptor (ER) function and E_2 response, invasion and metastasis (20). The exact mechanism leading to E_2 resistance and tumorigenicity remains poorly understood. There is now evidence that CCN1 is sufficient to overcome the normal E_2 requirements for breast cancer cell growth (21). In MCF-7 cells, the expression of ER alpha is decreased and forced expression of CCN1 was sufficient to induce cell growth in the absence of E_2. The cells became E_2-independent but continued to respond to this estrogen. This observation may explain why CCN1 expression is regulated in both ER-positive and ER-negative breast cancer cells (21). CCN1 was also described as a gene directly involved in HRG-induced breast cancer aggressiveness as it was highly expressed in MDA-MB-231 cells, a human breast cancer cell line that naturally overexpresses HRG (22). Acquisition of a transformed phenotype is often correlated with the ability of cells to grow in an anchorage-independent fashion. MCF-7 cells expressing CCN1 were able to form colonies on Matrigel in

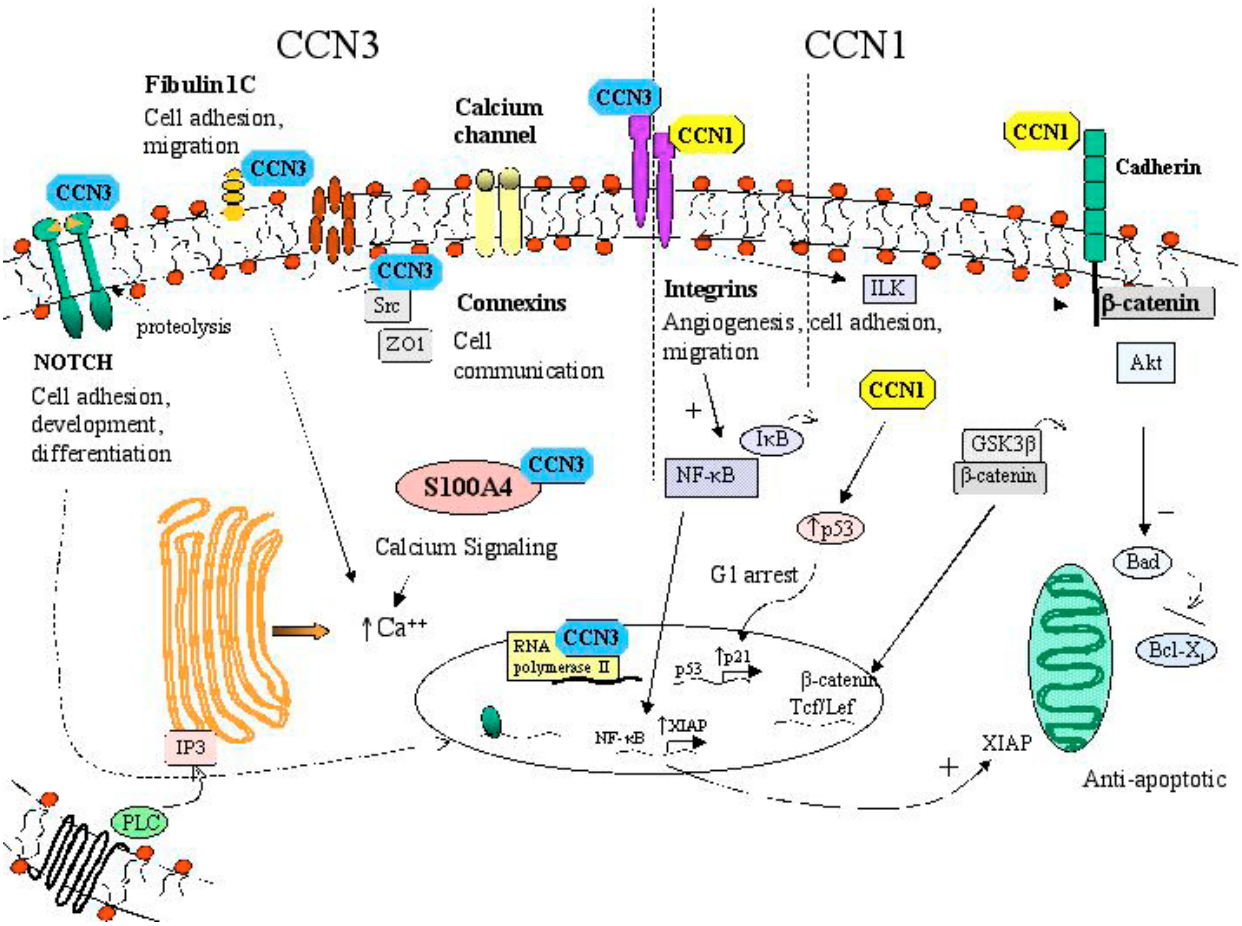

Figure 1. Possible signaling pathways activated by CCN1 and CCN3. CCN3 protein interacts with proteins located in the ECM (like integrins and fibulin 1C), at the plasma membrane (like Notch receptor and connexins), and in the cytoplasm (like S100A4). All these proteins are now known to be correlated to cancerogenesis processes. CCN3 modulates intracellular concentration of ion Ca^{2+}, suggesting a role in calcium signaling. In addition, CCN3 was shown to interact with the transcription machinery, suggesting a direct regulation of gene expression. Extracellular CCN3 promotes cell- adhesion and migration through direct binding to various integrin receptors. CCN1 protein also supports cell adhesion and migration through interaction with integrins receptors, but the intracellular signaling pathways that are activated seem to be quite different. One of these, leading to the increase in proliferation and tumorigenesis, includes the activation of the integrins/NF-kappaB/XIAP signaling pathway. CCN1 might also contribute to tumorigenesis through activation of integrin-linked kinase mediated beta-catenin-TCF/LEF and Akt signaling pathway. In some cancer lines in which CCN1 reduces the proliferation rate, cell cycle is blocked in G1 phase, resulting from an upregulation of p53 tumor suppressor gene and its transcriptional target $p21^{WAFI/CIPI}$.

anchorage-independent manner, without the presence of E_2 (23). Furthermore, in ovariectomized athymic nude mice, MCF-7 cells expressing CCN1 were tumorigenic, and the tumors presented increased vascularization and overexpression of VEGF, similar to human invasive carcinomas.

In an other set of experiments, increased expression of CCN1 both at the mRNA and protein levels was observed in a large panel of primary breast tumors (50% of cases) that express the progesterone receptor (PR) (24). The use of neutralizing antibody to PR and specific PR inhibitors served to demonstrate that the induction of CCN1 expression by progestin results from a transcriptional regulation by PR. In addition, EGF and CCN1 exert synergistic effects on cell growth. These results suggest that CCN1 might serve as a mediator of progesterone activity in enhancing growth-factor-driven tumor growth in breast cancer.

These observations established a functional relationship between the expression of CCN1 and tumorigenicity. In primary human breast carcinoma, high levels of CCN1 were associated to advanced stages of tumoral development. Invasive breast cancer cells express high levels of CCN1, while lower levels were found in those with less pronounced tumorigenic potential (e.g. MCF-7 cells): in contrast, normal cells show barely detectable levels. High levels of CCN1 expression positively correlate with HRG overexpression and inversely correlate with ER expression (20).

Evidences that CCN1 acts as a pro-proliferating and tumorigenic regulator was also reported in other types

of cancers. Indeed, CCN1 was shown to be highly expressed in primary gliomas, both in cell lines derived from high-grade gliomas and in the more tumorigenic astrocytomas (25). As seen with breast cancer tumors, a correlation was found between CCN1 expression and tumor grade, pathology, gender, age at diagnostic and patient survival. This observation provides a link between the expression of a CCN1 gene and clinical and pathological parameters. The detection of CCN1 at the time of diagnosis is of prognostic significance and suggests that it may play a role in tumor progression (25). *In vitro*, stable expression of CCN1 in gliomas enhances anchorage-independent cell growth in soft agar, accompanied by a faster cell migration (25). Finally, CCN1-expressing glioma cells formed more tumors than control cells when injected in nude mice. These tumors were larger and more vascularized. Elevated expression of CCN1 has also been detected in human benign prostatic hyperplasia (26). However, in another study, expression of CCN1 was reduced in 50% of tumor samples from patients with prostate cancer (27). Purified CCN1 protein was reported to induce cell proliferation, invasion and motility *ex vivo*, an observation which correlates with the expression of CCN1 in benign prostatic hyperplasia (28). In epithelial and stromal prostatic cells, lysophosphatidic acid (LPA) was shown to increase the expression of CCN1 through the activation of G protein-coupled receptors. It is noteworthy that LPA, which has been detected in body fluids, can act as a hormone and growth factor-like lipid (28). Prostate hyperplasic cells showed a strong CCN3-labelling in the lumen, suggesting that CCN3 was excreted in the seminal fluid. Acinar epithelial cells exhibited a CCN3-labelling in the cytoplasm (29). CCN3 expression was also detected in the cytoplasm of cell lines derived from metastasis to bone (PC3), brain (DU145) and lymph node (LNCap). Elevated expression of CCN1 has been detected in other types of tumors such as pancreatic cancers, bladder papilloma, colon adenocarcinoma, melanoma, medulloblastoma, pediatric tumors as well as fibrosarcoma cells (30,3).

CCN3 expression was correlated to high proliferative tumors, cancers with bad prognosis and metastatis in renal cell carcinomas (RCC), osteosarcomas, Ewing's sarcomas, and prostate cancers. In RCCs, which represent 85-90% of the kidney tumors, a higher level of CCN3 protein was detected in the culture medium of cells from fast growing tumors. An inverse relationship was observed between the amount of CCN3 secretion by cancer cells in culture and i) the time required to establish a tumor *in vivo* after injection of these cells in nude mice, and ii) for the tumor to reach a given size (31). Thus, different levels of CCN3 expression were associated to the various grades of these tumors. CCN3 proteins expression is also linked to cellular differentiation. Under normal conditions, CCN3 has been associated with mesenchymal cells to undergo cartilage, muscle and neuronal differentiation (3). After pathological stimuli, cells can undergo physiologic and morphologic cellular adaptations to preserve their viability, thereby changing their functions. These adaptations may involve modifications in cellular growth rate, size, and lead to hyperplasia and hypertrophy. Approximately 60% of osteosarcomas were found positive for CCN3 expression, and an inverse relationship has been drawn between the level of CCN3 expression and alkaline phosphatase, which is an early marker of osteablatic differentiation (32). Since alkaline phosphatase expression is correlated to the loss of aggressiveness of osteosarcoma cells, the CCN3 expression could be associated with osteoblast proliferation, and therefore could be considered as a marker of bad prognosis in these tumors (32). In the case of Ewing's sarcomas, the expression of CCN3 in primary tumors correlated with a higher risk to develop metastasis (32). CCN3 expression was detected in 30% of Ewing's sarcoma tissue samples. It was not detected in primary tumors of patients that did not develop metastasis. In contrast, 50% of the primary tumors that did exhibit lung and/ or bone metastasis showed a high level of CCN3 protein (32).

4. CCN1 AND CCN3 ACTING AS ANTI-PROLIFERATIVE AGENTS

CCN3 is the prototype of anti-proliferative CCN proteins. In cancers such as gliomas, chondrosarcomas, neuroblastomas, Wilms' tumors, and rhabdomyosarcomas, CCN3 expression was associated with differentiation and good prognosis. The first evidence showing the correlation between CCN3 expression and good prognosis came from the analysis of gliomas (33). Glioma and astrocytoma cells that expressed the highest amounts of CCN3 were those that exhibited the least aggressiveness, suggesting an inverse relationship between tumorigenicity and CCN3 expression. In the human G59 glioblastoma cell line, forced expression of human CCN3 produced an inhibition of proliferation *in vitro*, as compared to untransfected parental cells (34). After injection in athymic nude mice, G59 transfected cells induced only few tumors with a very small size, while parental G59 cells gave rise to many large tumors (34). In addition, 70-80% of mice injected with cells expressing low amount of CCN3 developed tumors whereas only 30% of mice injected with cells expressing high level of CCN3 developed tumors. In neuroblastomas with good prognosis and no N-Myc amplification[1], a strong CCN3 labelling was detected in the cytoplasm of differentiated ganglion cells (3), whereas in tumors with bad prognosis, CCN3 staining was low to moderate within the small tumor cells. In TC71 Ewing's sarcoma cell line, forced expression of CCN3 inhibited cell proliferation *ex vivo* and the ability of these cells to grow without anchorage (35-36). Similar results were obtained with an inducible system, therefore establishing a direct involvement of the CCN3 protein in the regulation of proliferation. In addition, athymic nude mice injected with TC71-CCN3 cells did not develop tumors. These results are in line with those obtained with glioblastomas cells. The expression of CCN3 in human Jeg3 choriocarcinoma cells also resulted in an inhibition of cell proliferation and tumorigenicity therefore indicating that these two effects of CCN3 were not restricted to defined cell types. The overall reduction in proliferation produced by CCN3 in different cell lines raised the possibilities that CCN3 i) slows down the cell cycle, ii) triggers apoptosis, or iii) confers less adhesiveness. No difference in the cell cycle distribution was observed between parental G59 cells and G59 expressing CCN3, and Ewing's sarcoma cells expressing

CCN3 are less adhesive than the TC71 parental cells (35,36). CCN3 expression is associated with differentiation in rhabdomyosarcoma and cartilage tumors. Immunohistochemistry, Western blot analysis, and Real-time PCR performed on enchondromas and various grades of chondrosarcomas revealed that CCN3 expression varied with the grade of the cartilage lesions (37). The highest levels were detected in pre-malignant enchondromas and low-grade chondrosarcomas. In Wilms' tumors, CCN3 expression was associated with striated heterotypic muscular differentiation of the blastemal cells and in rhabdomyosarsomas, the largest quantities of CCN3 were detected in the most differentiated cells (38). These few examples established that CCN3 expression is correlated to tumor differentiation and good prognosis, however it ~~is~~ likely participates in different cellular process depending on cell type. Deregulation of the CCN3 expression is not specific to solid tumors. CCN3 expression was also down-regulated in an inducible murine model of chronic myeloid leukemia (CML) and in CML patients at different stages of the illness. Interestingly, the levels of CCN3 expression returned to normal in patients undergoing remission (39, Gilmour et al. un published). In uterine leiomyoma, the most common tumor of the reproductive tract, CCN1 was also shown to be down-regulated both at the mRNA and protein levels, whereas high expression was found in adjacent myometrical smooth muscle cells (40). It was suggested that CCN1 dysregulation might result from the ability of E_2 and FGF2 to sustain the growth of uterine smooth muscle. CCN1 was described as anti-proliferative and tumorigenic agent in non-small cell lung cancer (NSCLC) where its expression was markedly decreased in human lung tumor samples (41). In contrast to the situation encountered in normal breast cell, CCN1 was highly expressed in normal lung tissue samples while it was down-regulated in NSCLCs from the same individuals. Similar observations were made in various cells line derived from lung tumors. Transfection of CCN1 in non-producing cell lines resulted in a reduced number of cells able to grow without anchorage (41).

5. POSSIBLE MECHANISMS RESPONSIBLE FOR CHANGES IN PROLIFERATION AND TUMORIGENICITY

The above examples established that CCN3 and CCN1 appeared to exert different biological functions depending upon the cellular context, tissues and tumors. This situation suggested the activation of completely different signaling pathways, in part resulting from the ability of CCN proteins to interact with/or control the transcription and activity of partners involved in complex signaling pathway (42-43).

5.1. CCN proteins interact with different signaling partners

A significant body of evidence using two-hybrid system, GST-pull down, *in vitro* and *in vivo* co-immunoprecipition showed that CCN3 protein physically interacted with proteins located in the ECM (like integrins and fibulin 1C), at the plasma membrane (like Notch receptor and connexins), and in the cytoplasm (like S100A4). These proteins included receptors, channels and signaling molecules including transcription factors.

5.1.1. Interaction with extracellular matrix proteins and receptors

CCN1 and CCN3 promote cell adhesion and migration through direct binding to various integrin receptors and to heparan sulfate proteoglycans (HSPG) (see below for more details) (44). Briefly, in HUVECs, adhesion promoted by CCN3 involves integrins $alpha_V beta_3$, $alpha_5 beta_1$, $alpha_6 beta_1$ and migration process the integrins $alpha_V beta_3$ and $alpha_5 beta_1$. Integrin $alpha_M beta_2$ is the major adhesion receptor mediating monocyte adhesion, and $alpha_{MI}$ domain binds specifically to CCN1 (45). As previously described, CCN1 induced colony formation on matrigel. This phenomenon is blocked by anti-integrin $alpha_5 beta_3$ antibody, suggesting that the effect of CCN1 involves this integrin (21-22). More recently, a novel and functionally important binding site for the integrin $alpha_5 beta_3$ was identified in the domain II of soluble CCN1 (46). Overexpression of CCN1 also results in enhanced levels of integrins $beta_1$, which is also known to interact with CCN1.

CCN3 physically interacts with fibulin 1C, another ECM component which has been reported to interact with fibronectin, laminin and fibrinogen (47-48). Emerging clues indicate that fibulin 1C functions may be involved in cell adhesion and migration (49). In addition to ECM proteins, CCN3 interacts with the extracellular domain of the transmembrane Notch1 protein and activates downstream effectors of the Notch signaling pathway (50). Notch signaling is crucial during embryonnic development, by maintaining self-renewal of some progenitors and inducing differentiation of other. Notch is known to mediate cell adhesion, suggesting that regulation of cell adhesion by CCN3 may in part involve the Notch pathway. Futhermore, Notch signaling is now known to either promote tumorigenesis or act as a tumor suppressor (51-52).

5.1.2. Interaction with cell communication and calcium signaling proteins

Confocal analysis, GST pull-down, *in vitro* and *in vivo* coimmunoprecipitation revealed that CCN3 physically interacts with connexins, involved in gap junction intercellular communication. Gap junctions are transmembrane channels that allow the diffusion of small molecules like ions, carbohydrates, amino acids, nucleotides between adjacent cells. A growing body of evidence highlights the importance of the deregulation of gap junctions functions in cancer development (53-56). The Increased growth rate of tumor cells might be a direct consequence of the reduction in gap junctions functions. It was shown that forced expression of Cx43 cDNAs, but not Cx40, suppressed the growth and/or tumorigenicity of many transformed cells (57-58). The C-terminal part of Cx43 was shown to be involved in the inhibition of cell proliferation (58). Interestingly, CCN3 was up-regulated in Jeg3-Cx43 cells and in C6-Cx43 cells (36,57). Results suggested that the slower growth rate of cells expressing Cx43 might result from the up-regulation of CCN3. CCN3

was found to physically interact with the C-terminal part of Cx43, but not with Cx40 or Cx32 (58-59). Confocal analysis showed that parental C6 cells and Jeg3 cells exhibited a nuclear CCN3-labelling, while in Cx43-expressing cells staining was detected in the cytoplasm and at the plasma membrane where it co-localized with Cx43 (36,58-59). It is tempting to postulate that physical interaction between the CCN3 and Cx43 proteins triggers the localization of CCN3 at the plasma membrane of cancer cells and restores, at least in part, functional gap junctions. It was suggested that inhibition of proliferation might involve the interaction between the C-terminal parts of Cx43 with CCN3. This interaction could for example affect the interaction of the Cx43 tail with its other parterns ZO-1, a- and b-tubulin as well as c-Src. In addition to the channel function of connexins, a role in transcriptional and cytoskeletal regulation has been reported (60-65). Today, we don't know if this mechanism leading to the reduction of growth is unique to CCN3, since other CCN proteins, like CCN1, have been found up-regulated in Cx43-expressing cells (66). Two-hybrid system and GST pull-down also revealed an interaction between CCN3 and S100A4, which belongs to the S100 proteins group, one of the largest subfamilies of EF-hand proteins (67-68). The S100A4 proteins are thought to modulate the propagation of calcium signals. The human S100A4 gene is often rearranged in cancers, either by deletions, translocations or amplifications. High amounts of S100A4 are detected in metastasis, but S100A4 itself is unable to induce tumorigenesis. To date, S100A4 is known to be involved in cell motility, cell adhesion, cell cycle progression, and angiogenesis. Inasmuch as CCN3 shares some of these functions with S100A4, it is tempting to postulate that the CCN3 role in cell physiology is due in part to direct binding to S100A4. This interaction may also participate in the transient intracellular concentration of calcium that is induced in glioblastoma cells by the application of CCN3 protein (67). Both the entry and the mobilization of the internal calcium stores were induced by CCN3 and the inhibition of voltage dependent channels was ineffective, suggesting that the effects of CCN3 involve store operated calcium channels. Binding of CCN3 to S100A4 could also be of prime interest in the context of connexins since they can modulate the propagation of ions signal, suggesting a network communication, with possible implication in tumor progression and metastasis.

5.1.3. Interaction with the transcriptional machinery

It was also established that CCN3 interacts with the rbp7 subunit of the RNA polymerase II. In HeLa and 143 osteosarcoma cell line, the CCN3 labelling in the nucleus co-localises with the transcriptional machinery but not with the replication machinery (3). Furthermore, the C-terminal module of CCN3 has been found to bind a specific sequence in the promoter of the plasminogen activator inhibitor type-2 (PAI-2) (69). Current studies performed in our laboratory suggest that CCN3 truncated forms may indeed activate transcription (Planque *et al.* in preparation). Taken together, these results suggest that nuclear forms of CCN3 may directly regulate genes transcription.

5.2. Regulation of gene transcription

The mechanisms activated by CCN1 are quite different than those triggered by CCN3. One of these, leading to modifications in proliferation and tumorigenesis, include the activation of the integrins/NF-kappaB/XIAP signaling pathway, which is known to be involved in resistance to chemotherapeutic agent-induced apoptosis (70). In cells overexpressing CCN1, an increase in NF-kappaB activity was detected, and the inhibitor IkappaB renders the cells more susceptible to these anti-cancer drugs (71). Supporting a role for CCN1 and NF-kappaB, the expression of the anti-apoptotic gene XIAP, which is regulated by NF-kappaB, is increased in CYR61-overexpressing cells, thus explaining their observed resistance to apoptosis. CCN1 might contribute to tumorigenesis through activation of integrin-linked kinase mediated beta-catenin-TCF/LEF and Akt signaling pathway (70). Beta-catenin is a transcription cofactor with T cell factor/lymphoid enhancer factor TCF/LEF playing important roles in cell adhesion and intracellular signal transduction. Under normal conditions, beta-catenin is maintained at low levels through degradation of cytoplasmic beta-catenin, which is in excess to its binding sites such as the cadherin at the plasma membrane. It is also targeted for ubiquitination and degradation in the proteasome after phosphorylation by casein kinase I and glycogen synthase-3beta (GSK-3beta) (72). Expression of CCN1 in glioma cells causes inhibition of GSK-3beta activity, leading to beta-catenin accumulation and translocation into the nucleus as a complex with the transcription factor TCF/LEF that regulates target gene expression (70). Akt represents another mechanism of b-catenin stabilization. In gliomas cells, expression of CCN1, results in the stimulation of beta-catenin-TCF/LEF signaling pathway. CCN1 can activate Akt and inhibit the apoptotic effector Bad, resulting in its cytosolic sequestration which prevents its binding to the survival factor Bcl-X_L. Phosphorylation of Bad by Akt is a possible mechanism by which CCN1 delivers a survival signal, leading to the inhibition of apoptosis. Moreover, it was shown that glioma cells expressing CCN1 could activate the PI3k pathway through ILK (70).

5.3. Regulation of the cell cycle

Transfection of CCN1 in non-producing lung cell lines reduced their proliferation rate, which is intimately linked to regulation of the cell cycle. This is well in line with the G1 arrest observed in CCN1 stable transfectants, which results from an upregulation of p53 tumor suppressor gene and its transcriptional target p21WAF. An increase in pocket pRB2/p130 and a decrease in cyclin-dependent kinase 2 activity were also detected (41). Expression of CCN1 in NSCLC was reported to induce an upregulation and nuclear localization of beta-catenin, followed by an increase in the proto-oncogene c-myc which is known to stabilize the p53 protein leading to cell cycle arrest and apoptosis (73).

5.4. Overall signaling pathways activated by CCN1 and CCN3

The major signaling pathways activated by CCN1 and CCN3 that may lead to changes in growth rate and tumorigenicity are highlighted in figure 1. The multimodular structure of the CCN proteins provides the basis for multiple interactions and for combinational events. All these signaling networks reflect the relevance

of the diverse biological events occurring ducting cancer process: some are common to the two CCN proteins while others appear to be antagonist. Moreover, the mechanisms are expected to vary greatly among tissues and cell types from cellular generation to death since CCN1 and CCN3 have the capability to exert either positive or negative effect on growth. Common signaling pathways activated by CCN1 and CCN3 are grouped in the dotted frame of the figure 1. They include, in part, the binding to integrins receptor. It has been proposed that interaction of CCN proteins with integrins accounts for most of their biological properties both in normal and pathological conditions (6,44-45). Most importantly, this interaction aims for the acquisition of a vascular network and blood supply with the ability of the cells to migrate and attach for tumor expansion (5). Gap junctions functions and exchange of ions between adjacent cells are also key factors in the control and coordination of cell growth (54). By its interactions with connexins and S100A4 proteins, CCN3 is now known to play important roles in cell communication and CCN1 might also be of prime interest in this context (58-59,67). CCN3 modulates intracellular concentration of ion Ca^{2+}, suggesting a role in calcium signaling. It is worth noting that some cancer cells exhibit a nuclear CCN3 labeling (58-59,74). In addition, CCN3 was shown to interact with a sub-unit of the RNA polymerase II in a two-hybrid assay in yeast, and to co-localize with the transcription machinery (3), suggesting a direct regulation of gene expression by CCN3 (47, Planque *et al.* in preparation). Finally, CCN3 activates the NOTCH1 receptor resulting in differentiation and development and interacts with Fibulin 1C to increase cell adhesion and migration (48,50). The intracellular signaling pathways activated by CCN1 appear to be quite different than those triggered by CCN3. One of these, reflecting its positive effects on proliferation and tumorigenesis, include the activation of the integrins/NF-kappaB/XIAP signaling pathway, which is known to be involved in resistance to chemotherapeutic agent-induced apoptosis (70-71). On another hand, CCN1 might contribute to tumorigenesis through activation of integrin-linked kinase mediated b-catenin-TCF/LEF and Akt signaling pathway (70). These mechanisms induce the survival of the cancer cells by inhibiting the mitochondrial apoptotic pathway. In some cancer lines in which CCN1 reduces the proliferation rate of the cells, cell cycle is blocked in G1 phase, resulting from an upregulation of p53 tumor suppressor gene and its transcriptional target $p21^{WAFI/CIPI}$ (73). To date, no cell cycle deregulation has been described in cancer cells in which CCN3 inhibits proliferation.

6. ROLE OF CCN1 AND CCN3 IN ANGIOGENESIS, CELL ADHESION AND MIGRATION

It is well recognized that angiogenesis (the acquisition of a vascular network and blood supply) is required for the expansion of solid tumors. This phenomenon involves the concerted action of several angiogenic factors. CCN1 is considered as an angiogenic factor since elevated expression of CCN1 was associated with a higher degree of angiogenesis during processes such as wound repair and tumor growth. CCN1 is acting in part indirectly by modifying the structure or stability of the ECM (75,13). Expression of CCN1 regulates vascular development associated with impaired VEGF-C expression, suggesting that CCN1-regulated expression of VEGF-C plays a role in vessel bifurcation (76). *In vivo*, CCN1 null-mice suffer embryonic death resulting from failure in chorioallantoic fusion and placental vascular insufficiency and compromized vessel integrity. In gastric adenocarcinoma, transfection of CCN1 triggers larger and better vascularized tumors when injected to recipient nude mice (31). Cells expressing CCN1, such as MCF-7 breast cancer cells or gastric adenocarcinoma cells transfected with CCN1, show greater tumorigenicity than non-expressing cells. This may be attributed, at least in part, to the angiogenic properties of CCN1 as larger and highly vascularized tumors where formed with increased numbers of endothelial cells (14). In addition to the modulation of expression of growth factor, CCN1 was reported to facilitate angiogenesis through the induction of expression of ECM-degrading metalloproteases and the decrease in expression of their inhibitors (77). After induction of angiogenesis, cells have a greater ability to migrate and proliferate, resulting in higher metastatic potential. MCF-7 cells stably expressing CCN1 showed significant increase in migration, suggesting a greater invasive potential of these tumor cells (20). A distinct property of CCN1 towards cell growth is the induction of cell adhesion. CCN1 was shown to trigger HUVEC (human umbilical vein endothelial cells) cell adhesion, and promoted spreading, thus showing that it could be involved in cytoskeletal rearrangement (78). Purified CCN1 protein was also shown to promote cell adhesion and spreading of prostatic cells (30). Inasmuch as CCN3 is associated with tumors exhibiting high metastatic potential, numerous studies were performed to understand the function of CCN3 in cell adhesion, motility and invasion in several cancer models and cell lines. Results were slightly different, suggesting that underlying mechanisms may be cell-specific. In endothelial cells, purified recombinant CCN3 protein promoted cell- adhesion and survival in a dose-dependent manner, and induced cell migration (45). HUVECs maintained in serum-free medium were undergoing apoptosis. The cells survived when applying CCN3 protein in the medium and preincubation of CCN3 protein with affinity purified anti-CCN3 antibodies abolished HUVECs survival. Mechanistically, CCN3- promoted adhesion was involving cell surface receptors including integrins a_Vb_3, a_5b_1, a_6b_1 and HSPG. CCN3 stimulated migration through integrins a_Vb_3 and a_5b_1 pathways, but not through a_6b_1 pathway. CCN3 was shown to physically interact with integrin receptors *in vitro*, suggesting that CCN3 functions in cell- adhesion and migration might be due to direct binding of secreted CCN3 to integrins receptors. The ability of CCN3 to stimulate endothelial cells- adhesion, survival and migration allowed to postulate that CCN3 is a new angiogenic inducer. In rat corneal micropocket assay, CCN3 protein was shown to stimulate pro-angiogenic activities (45). Neo-vascularisation was not observed when pre-incubating the CCN3 protein with anti-CCN3 antibodies. Interestingly, tumors induced by C6 glioma cells expressing CCN3 did not exhibit a higher level of vascularisation, in agreement with their reduce

tumorigenicity (37). These results suggest that in cancers in which CCN3 reduced the proliferation rate of tumor cells and their tumorigenicity, it might not behave as an angiogenic inducer. Since the late stages in tumor development require vascularization of the tumor, this is of prime interest. In line with these observations, it was previously shown that CCN3 might interfere with tumor maintenance rather than establishment of tumor state (36). Human glioblastomas cells (G59) and Ewing's sarcoma cells (TC71) which stably express CCN3, promoted greater adhesion and migration as compared to control cells (34-35). In the G59 cells, CCN3 promoted cell adhesion on poly-Lysin, laminin and fibronectin, but adhesion of the parental cell line was not affected by purified recombinant CCN3 protein coated on wells. These results suggest that CCN3 might not be an adhesion molecule *per se*, but might rather regulate adhesion proteins. In the Ewing's system, parental and CCN3 expressing cells exhibited the same ability to attach to laminin and fibronectin but cells that do produce CCN3 were less adhesive on collagen I and IV. Mechanistically, this reduction in adhesion might be due to a decreased expression of integrin $alpha_2beta_1$. In G59 cells, migration seemed to depend on platelet derived growth factor receptor-alpha (PDGFR-alpha) signaling: forced expression of CCN3 increased PDGFR-alpha and metalloproteinase-3 (MMP3), which is a downstream target of the PDGFR-alpha pathway. However, in TC71 cells expressing CCN3, MMP-9 expression was found to be down-regulated and the insulin like growth factor receptor I (IGFRI) pathway was activated (Benini et al. unpublished).

7. SUMMARY AND PERSPECTIVES

There is a growing body of evidence to support the idea that CCN1 and CCN3 play a role in the cancer processes. Depending upon the tumors and cellular context, these proteins are associated to positive or negative effects on tumorigenicity. In most cases, CCN1 seems to increase proliferation and metastatic capabilities. Hence, many tumors show high levels of CCN1 expression. Furthermore, CCN1-negative tumor cell lines tend to be less tumorigenetic than those do express the protein. This was confirmed by *in vivo* experiments in immunodeficient mice. Tumorigenicity of human tumor cells overexpressing CCN1 is higher than that of non-expressing cells. In a few cases, increased CCN3 expression was also associated to high proliferative tumors. The overexpression of CCN3 detected in certain types of tumors appeared paradoxical for a negative growth regulator. However, because tumors contain an increased number of developed structures and heterotypic tissues, negative regulatory factors are also required for differentiation. It is therefore not so heretical that a gene involved in normal differentiation process is aberrantly expressed in highly differentiated tumors. In contrary to CCN1, CCN3 is mostly found to negatively regulate cell proliferation. It is down-regulated in numerous tumors and forced expression in cancer cell lines reduces growth rate. In nude mice, cancer cells stably expressing CCN3 showed reduced ability to induce tumors. Recent studies suggest that, in addition to cancer types and activation of different signaling pathway, the dual biological properties observed for one protein could also result from the fact that full length secreted CCN proteins can show antiproliferative activities, whereas some truncated isoforms are likely to stimulate proliferation and behave as oncogenes (43). The expression of CCN3 is altered in many tumors and high amounts of the protein could be associated with either differentiation (Wilms' tumor, glioblastoma, neuroblastoma, rhabdomyosarcoma and chondrosarcoma) or with increased proliferation and metastases (Ewing's sarcoma, prostate cancer and renal cell carcinoma).

This review underlines some distinct and opposite effects of CCN1 and CCN3 on proliferation and tumorigenesis. Usually, when elevated expression of one CCN protein is detected in a tumor, the detection of the other is under undetectable level. This hypothesis is reinforced by the inverted regulation of these two genes observed during mechanical stress: under stress conditions, CCN1 expression is highly up-regulated while the mRNA levels of CCN3 is low but increases after the relaxation period (Schild C, 2004). Moreover, in normal tissues, the expression of CCN proteins is tightly regulated during development. They show specific and differential pattern of expression, suggesting that each member of the CCN family exhibits its own features (3). It is thus expected that a high regulation of the pattern of expression of CCN genes also varies during the course of tumor formation and development. We may expect to find an inverse profile of expression of CCN1 and CCN3 in cancers. Hence, CCN3 is required at late stages of the chondrogenesis/osteogenesis differentiation process, whereas CCN1 was required at early stages. It is possible that CCN3 counterbalances the stimulatory effects of the immediate early CCN1: one forcing a step on the way to growth stimulation and the other moving back for growth inhibition, a choreography for two to tango. The differential expression of CCN genes could thus be temporally regulated to elicit specific functions depending on the cellular context. Whether deregulation of CCN genes in cancer represents a cause or a consequence still remains to be established. Stemming from all of these data, CCN proteins might serve as valid targets for new therapeutic strategies of molecular medicine to fight against cancers. CCN proteins may constitute amenable tools for early diagnosis, typing and therapy of cancers and the detection of CCN proteins in tumor samples or biological fluids might help to target treatment.

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9. REFERENCES

1. Hahn W.C & R.A.Weinberg: Modelling the molecular circuitry of cancer [Review]. *Nat Rev Cancer* 2, 331-341 (2002)

2. Perbal B: CCN proteins: multifunctional signalling regulators [Review]. *Lancet* 363, 62-64 (2004)

3. Perbal B: NOV (nephroblastoma overexpressed gene) and the CCN family of genes :structural and functional issues [Review]. *Mol Pathol* 54, 57-79 (2001)

4. Takigawa M: CTGF/Hcs24 as a multifunctional growth factor for fibroblasts, chondrocytes and vascular endothelial cells. *Drug News Perspect* 16, 11-21 (2003)

5. Brigstock D.R: The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family [Review]. *Endocr Rev* 20, 189-206 (1999)

6. Lau L.F. & Lam S.C: The CCN family of angiogenic regulators: the integrin connection [Review]. *Exp Cell Res* 248, 44-57 (1999)

7. Planque N. & B Perbal: A structural approach to the role of CCN proteins in tumorigenesis [Review]. *Cancer Cell International* 3, 1-15 (2003)

8. Joliot V, C. Martinerie, G. Dambrine, G. Plassiart, M. Brisac, J. Crochet & B.Perbal: Proviral rearrangements and overexpression of a new cellular gene (nov) in myeloblastosis-associated virus type 1-induced nephroblastomas. *Mol Cell Biol* 12, 10-21 (1992)

9. Perbal B: Contribution of MAV-1-induced nephroblastoma to the study of genes involved in human Wilms' tumour development.. *Critical Reviews in Oncogenesis*, 589-613 (1994)

10. Beckwith J.B, N.B. Kiviat & J.F. Bonadio: Nephrogenic rests, nephroblastomatosis, and the pathogenesis of Wilms' tumour. *Pediatric Pathology* 10, 1-36 (1990)

11. Coullin P, C.L. Li, L. Ziercher, C. Auffray, A. Bernheim, R. Zoorob & B. Perbal: Assignment of the chicken NOV gene (alias CCN3) to chromosome 2q34-->q36: Conserved and compared synteny between avian, mouse and human. *Cytogenet Genome Res* 97, 140C (2002)

12. Teber I, R. Kohling, E.J. Speckmann, A. Barnekow & J. Kremerskothen: Muscarinic acetylcholine receptor stimulation induces expression of the activity-regulated cytoskeleton-associated gene (ARC). *Brain Res Mol Brain Res* 121, 131-136 (2004)

13. Brigstock D.R: The CCN family: a new stimulus package [Review]. *J Endocrinol* 178, 169-175 (2003)

14. Brigstock D.R: Regulation of angiogenesis and endothelial cell function by connective tissue growth factor (CTGF) and cysteine-rich 61 (CYR61). *Angiogenesis* 5, 153-165 (2002)

15. Shackney S.E, J.F. Silverman: Molecular evolutionary patterns in breast cancer. *Adv Anat Pathol* 10, 278-290 (2003)

16. Xie D, K. Nakachi, H. Wang, R. Elashoff & H.P. Koeffler: Elevated levels of connective tissue growth factor, WISP-1, and CYR61 in primary breast cancers associated with more advanced features. *Cancer Res* 61, 8917-8923 (2001)

17. Xie D, C.W. Miller, J. O'Kelly, K. Nakachi, A. Sakashita, J.W. Said, J. Gornbein & H.P. Koeffler: Breast cancer. Cyr61 is overexpressed, estrogen-inducible, and associated with more advanced disease. *J Biol Chem* 276, 14187-14194 (2001)

18. Nicholson R.I & J.M. Gee: Oestrogen and growth factor cross-talk and endocrine insensitivity and acquired resistance in breast cancer. *Br J Cancer* 82, 501-513 (2000)

19. Atlas E, M. Cardillo, I. Mehmi, H. Zahedkargaran, C. Tang & R. Lupu: Heregulin is sufficient for the promotion of tumorigenicity and metastasis of breast cancer cells in vivo. *Mol Cancer Res* 1, 165-175 (2003)

20. Menendez J.A, I. Mehmi, D.W. Griggs & R.Lupu: The angiogenic factor CYR61 in breast cancer: molecular pathology and therapeutic perspectives [Review]. *Endocr Relat Cancer* 10, 141-152 (2003)

21. Tsai M.S, D.F. Bogart, P. Li, I. Mehmi & R. Lupu: Expression and regulation of Cyr61 in human breast cancer cell lines. *Oncogene* 21, 964-973 (2002)

22. Tsai M.S, A.E. Hornby, J. Lakins & R.Lupu: Expression and function of CYR61, an angiogenic factor, in breast cancer cell lines and tumor biopsies. *Cancer Res* 60, 5603-5607 (2000)

23. Tsai M.S, D.F. Bogart, J.M. Castaneda, P. Li, I. Mehmi & R. Lupu: Cyr61 promotes breast tumorigenesis and cancer progression. *Oncogene* 21, 8178-8185 (2002)

24. Sampath D, R.C. Winneker & Z. Zhang: The angiogenic factor Cyr61 is induced by the progestin R5020 and is necessary for mammary adenocarcinoma cell growth. *Endocrine* 18, 147-159 (2002)

25. Xie D, D. Yin, H.J. Wang, G.T. Liu, R. Elashoff, K. Black & H.P. Koeffler: Levels of expression of CYR61 and CTGF are prognostic for tumor progression and survival of individuals with gliomas. *Clin Cancer Res* 10, 2072-2081 (2004)

26. Daaka Y: Mitogenic action of LPA in prostate. *Biochim Biophys Acta* 1582, 265–269 (2002)

27. Pilarsky C.P, U. Schmidt, C. Eissrich, J. Stade, S.E. Froschermaier, M. Haase, G. Faller, T.W. Kirchner & M.P.

Wirth: Expression of the extracellular matrix signaling molecule Cyr61 is downregulated in prostate cancer. *Prostate* 36, 85-91 (1998)

28. Sakamoto S, M. Yokoyama, X. Zhang, K. Prakash, K. Nagao, T. Hatanaka, R.H. Getzenberg & Y. Kakehi: Increased expression of CYR61, an extracellular matrix signaling protein, in human benign prostatic hyperplasia and its regulation by lysophosphatidic acid. *Endocrinology* 145, 2929-2940 (2004)

29. Maillard M, B. Cadot, R.Y. Ball, K. Sethia, D. Edwards, B. Perbal & R. Tatoud: Differential expression of novH proto-oncogene in human prostate cell lines and tissues. *Mol Pathol* 54, 275-280 (2001)

30. Babic A.M, M.L. Kireeva, T.V. Kolesnikova & L.F. Lau: CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. *Proc Natl Acad Sci U S A* 95, 6355-6360 (1998)

31. Glukhova L, E. Angevin, C. Lavialle, B. Cadot, M. Terrier, J. Lacombe, B. Perbal, A. Bernheim & A.F. Goguel: Specific Genomic Alterations Associated With Poor Prognosis In High Grade Renal Cell Carcinomas. *Cancer Genet Cytogenet* 130, 105-110 (2001)

32. Manara M.C, B. Perbal, S. Benini, R. Strammiello, V. Cerisano, S. Perdichizzi, M. Serra, A. Astolfi, F. Bertoni, J. Alami, H. Yeger, P. Picci & K. Scotlandi: The expression of ccn3(nov) gene in musculoskeletal tumors. *Am J Pathol* 160, 849-59 (2002)

33. Wen-Xin L., C. Martinerie, W. Zumkeller, M. Westphal & B. Perbal: Differential expression of novH and CTGF in human glioma cell lines. *J Clin Pathol : Mol Pathol* 49, 91-97 (1996)

34. Gupta N, H. Wang, T.L. McLeod, C.C. Naus, S. Kyurkchiev, S. Advani, J. Yu, B. Perbal & R.R.Weichselbaum: Inhibition of glioma cell growth and tumourigenic potential by CCN3 (NOV). *Mol Pathol* 54, 293-299 (2001)

35. Scotlandi K, S. Benini, M.C. Manara, V. Cerisano, S. Perdichizzi, R. Strammiello & B. Perbal: Biological roles of NOVH in Ewing's sarcoma cells [Abstract]. *Mol Pathol* 56, 65-75 (2003)

36. Benini S, B. Perbal, D. Zambelli, M.P. Colombo, M.C. Manara, M. Serra, M. Parenza, V. Martinez, P. Picci & K. Scotlandi: In Ewing' sarcoma CCN3(NOV) inhibits proliferation while promoting migration and invasion of the same cell type. *Submitted for publication*

37. Yu C, A.T. Le, H. Yeger, B. Perbal & B. Alman: NOV (CCN3) regulation in the growth plate and CCN family member expression in cartilage neoplasia. *J pathol* 201, 609-615 (2003)

38. Chevalier G, H. Yeger, C. Martinerie, M. Laurent, J. Alami, P. Schofield & B. Perbal: NovH: Differential Expression in Developing Kidney and Wilms' Tumors. *Amer Journal of Pathol* 152, 1563-1575 (1998)

39. Gilmour L, S. Price, A.D. Whetton, B. Perbal, A.E. Irvine: Deregulation of Nov expression in chronic myeloid leukemia [Abstract]. *Mol Pathol* 56, 65-75 (2003)

40. Sampath D, Y. Zhu, R.C. Winneker, Z. Zhang: Aberrant expression of Cyr61, a member of the CCN (CTGF/ Cyr61/ Cef10/ NOVH) family, and dysregulation by 17 beta-estradiol and basic fibroblast growth factor in human uterine leiomyomas. *J Clin Endocrinol Metab* 86, 1707-1715 (2001)

41. Tong X, D. Xie, J. O'Kelly, C.W. Miller, C. Muller-Tidow & H.P. Koeffler: Cyr61, a member of CCN family, is a tumor suppressor in non-small cell lung cancer. *J Biol Chem* 276, 47709-47714 (2001)

42. Planque N. & B. Perbal: The CCN Proteins, a new family of cell growth regulators [Review]. In: Recent research developments in human pathology. Eds: Transworld Research Network 1, 333-369 (2003)

43. Perbal B, D.R. Brigstock & L. Lau: Report of the second international workshop on the CCN family of genes. *J Clin Pathol: Mol Pathol* 56, 80-85 (2003)

44. Lin C.G, S.J. Leu, N. Chen, C.M. Tebeau, S.X. Lin, C.Y. Yeung & L.F. Lau: CCN3 (NOV) is a novel angiogenic regulator of the CCN protein family. *J Biol Chem* 278, 24200-24208 (2003)

45. Schober J.M, L.F. Lau, T.P. Ugarova & S.C. Lam: Identification of a novel integrin alphaMbeta2 binding site in CCN1 (CYR61), a matricellular protein expressed in healing wounds and atherosclerotic lesions. *J Biol Chem* 278, 25808-25815 (2003)

46. Chen N, S.J. Leu, V. Todorovic, S.C. Lam & L.F.Lau: Identification of a novel integrin alpha vbeta 3 binding site in CCN1 (CYR61) critical for pro-angiogenic activities in vascular endothelial cells. *J Biol Chem* Aug 17 (2004)

47. Argraves W.S, H. Tran, W.H. Burgess & K. Dickerson: Fibulin is an extracellular matrix and plasma glycoprotein with repeated domain structure. *J Cell Biol* 111, 3155-3164 (1990)

48. Perbal B, C. Martinerie, R. Sainson, M. Werner, B. He & B. Roizman: The C-terminal domain of the regulatory protein NOVH is sufficient to promote interaction with fibulin 1C: A clue for a role of NOVH in cell-adhesion signaling. *Proc Natl Acad Sci USA* 96, 869–874 (1999)

49. Brooke J S, Cha JH, Eidels L. Latent transforming growth factor ß-binding protein-3 and fibulin-1C interact with the extracellular domain of the heparin-binding EGF-like growth factor precursor BMC Cell Biology, 3, 2 (2002).

50. Sakamoto K, S. Yamaguchi, R. Ando, A. Miyawaki, Y. Kabasawa, M. Takagi, C.L. Li, B. Perbal & K. Katsube:

The Nephroblastoma overexpressed gene (NOV/ccn3) protein associates with Notch1 extracellular domain and inhibits myoblast differentiation via Notch signaling pathway. *J Biol Chem* 277, 29399-29405 (2002)

51. Radtke F & K.Raj: The role of Notch in tumorigenesis: oncogene or tumour suppressor? [Review]. *Nat Rev Cancer* 3, 756-767 (2003)

52. Weng A.P. & J.C. Aster: Multiple niches for Notch in cancer: context is everything [Review]. *Curr Opin Genet Dev* 14, 48-54 (2004)

53. Yamasaki H. & C.C. Naus: Role of connexin genes in growth control [Review]. *Carcinogenesis* 17, 1199-1213 (1996)

54. Naus C.C: Gap junctions and tumour progression [Review]. *Can J Physiol Pharmacol* 80, 136-141 (2002)

55. Mesnil M: Connexins and cancer [Review]. *Biol Cell* 94, 493-500 (2002)

56. Mesnil M: [Gap junctions and cancer: implications and perspectives] [Review in french] *Med Sci (Paris)* 20, 197-206 (2004)

57. Zhu D, S. Caveney, G.M. Kidder & C.C. Naus: Transfection of C6 glioma cells with connexin 43 cDNA: analysis of expression, intercellular coupling, and cell proliferation. *Proc Natl Acad Sci U S A* 88, 1883-1887 (1991)

58. Gellhaus A, X. Dong, S. Propson, K. Maass, L. Klein-Hitpass, M. Kibschull, O. Traub, K. Willecke, B. Perbal, S.J. Lye & E. Winterhager: Connexin43 interacts with NOV: A possible mechanism for negative regulation of cell growth in choriocarcinoma cells. *J Biol Chem* In press (2004)

59. Fu C.T, J.F. Bechberger, M.A. Ozog, B. Perbal & C.C. Naus: CCN3 (NOV) interacts with connexin43 in C6 glioma cells: Possible mechanism of connexin-mediated growth suppression. *J Biol Chem* In press (2004)

60. Giepmans B.N & W.H. Moolenar: The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. *Curr Biol* 8, 931-934 (1998)

61. Toyofuku T., M. Yabuki, K. Otsu, T. Kazuya, M. Hori & M. Tada: Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. *J Biol Chem* 273, 12725-17731 (1998)

62. Giepmans B.N, I. Verlaan, T. Hengeveld, H. Janssen, J. Calafat, M.M. Falk & W.H. Moolenar: Gap junction protein connexin-43 interacts directly with microtubules. *Curr Biol* 11, 1364-1368 (2001)

63. Giepmans B.N, T. Hengeveld, F.R. Postma & W.H. Moolenar: Interaction of c-Src with gap junction protein connexin-43. Role in the regulation of cell-cell communication. *J Biol Chem* 276, 8544-8549 (2001)

64. Lauf U, B.N. Giepmans, P. Lopez, S. Braconnot, S.C. Chen & M.M. Falk: Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. *Proc Natl Acad Sci U S A* 99, 10446-10451 (2002)

65. Giepmans B.N: Gap junctions and connexin-interacting proteins. *Cardiovasc Res* 62, 233-45 (2004)

66. McLoed T.L, J.F. Bechberger & C.C. Naus: Determination of a potential role of the CCN family of growth regulators in connexin43 transfected C6 glioma cells. *Cell Comm Adhes* 8, 441-445 (2001)

67. Li C.L, V. Martinez, B. He, A. Lombet & B. Perbal: Role of the CCN3 (Nov) protein in calcium signaling. *Mol Pathol* 55, 250-61 (2002)

68. Lombet A, N. Planque, A.M. Bleau, C.L. Li & B. Perbal: CCN3 and calcium signaling [Review]. *Cell Commun Signal* 1, 1-10 (2003)

69. Mahony D, B Kalionis, T.M. Antalis: Plasminogen activator inhibitor type-2 (PAI-2) gene transcription requires a novel NF-kappaB-like transcriptional regulatory motif. *Eur J Biochem* 263, 765-72 (1999)

70. Xie D, D. Yin, X. Tong, J. O'Kelly, A. Mori, C. Miller, K. Black, D. Gui, J.W. Said & Koeffler H.P: Cyr61 is overexpressed in gliomas and involved in integrin-linked kinase-mediated Akt and beta-catenin-TCF/Lef signaling pathways. *Cancer Res* 64, 1987-1996 (2004)

71. Lin M.T, C.C. Chang, S.T. Chen, H.L. Chang, J.L. Su, Y.P. Chau & M.L. Kuo: Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF-kappaB-dependent XIAP up-regulation. *J Biol Chem* 279, 24015-24023 (2004)

72. Nelson W.J & R.Nusse: Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 303, 1483-1487 (2004)

73. Tong X, J. O'Kelly, D. Xie, A. Mori, N. Lemp, R. McKenna, C.W. Miller & H.P. Koeffler: Cyr61 suppresses the growth of non-small-cell lung cancer cells via the beta-catenin-c-myc-p53 pathway. *Oncogene* 1-9 (2004)

74. Perbal B: Nuclear localization of NOV protein: a potential role for nov in the regulation of gene expression. *Mol Pahol* 52, 84-91 (1999)

75. Walker T.M, P.R. Van Ginkel, R.L. Gee, H. Ahmadi, L. Subramanian, B.R. Ksander, L.F. Meisner, D.M. Albert & A.S. Polans: Expression of angiogenic factors Cyr61 and tissue factor in uveal melanoma. *Arch Ophthalmol* 120, 1719-1725 (2002)

76. Mo F.E, A.G. Muntean, C.C. Chen, D.B. Stolz, S.C. Watkins & L.F. Lau: CYR61 (CCN1) is essential for

placental development and vascular integrity. *Mol Cell Biol* 22, 8709-8720 (2002)

77. Chen C.C, N. Chen & L.F. Lau: The angiogenic factors Cyr61 and connective tissue growth factor induce adhesive signaling in primary human skin fibroblasts. *J Biol Chem* 276, 10443-10452 (2001)

78. Kireeva M.L, F.E. Mo, G.P. Yang & L.F. Lau: Cyr61, a product of a growth factor-inducible immediate-early gene, promotes cell proliferation, migration, and adhesion. *Mol Cell Biol* 16, 1326-1334 (1996)

Footnote: [1] N-Myc amplification is correlated to bad prognosis. The more the N-Myc gene is amplified, the less the diagnostic is good.

Abbreviations: MAV: Myeloblastosis Associated Virus; ECM: extracellular matrix; HSPG: heparan sulfate proteoglycans; MMP: metalloproteinase; GJIC: gap junction intercellular communication; Cx: connexin; EGF: epidermal growth factor; FGF: fibroblast growth factor; TGFbeta: transforming growth factor beta; VEGF: vascular endothelial growth factor; E_2: estrogen; HRG: Heregulin; ER: estrogen receptor; PR: progesterone receptor; PDGFR-alpha: platelet derived growth factor receptor alpha; IGFRI: insulin-like growth factor receptor I; HUVEC: human umbilical vein endothelial cell; RCC: renal cell carcinoma; NSCLC: non-small cell lung cancer.

Key Words: CCN protein, CCN3-NOV, CCN1-Cyr61, cancer, tumorigenesis, cell growth regulator, Gene, Review

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