CCN5: A critical review of signaling, development and pathophysiology

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Abstract:

CCN5 is a member of the CCN family of proteins. CCN proteins have been implicated in many important cell processes, such as proliferation, migration, adhesion and epithelial-mesenchymal transitions. CCN proteins are also involved in physiological processes, such as embryonic development, angiogenesis, and multiple cancers. In this review I focus on the unique biology of CCN5 in these processes and consider future directions for research.

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1. Introduction:

CCN5 is a member of the CCN family of proteins. Six CCN proteins comprise the CCN family, which are involved in cellular functions such as, proliferation, adhesion, survival. Through these functions, CCN family members have been implicated in important physiologic processes, such as embryonic and skeletal development, angiogenesis and wound healing (Chen & Lau 2009, Holbourn et. al 2009). Disruption of normal CCN family member signaling has been implicated in multiple types of cancer, emphasizing the importance of understanding CCN family proteins.

CCN5 History

CCN5 was discovered by several groups in the 1990's. The first publication including CCN5 came from our laboratory, where CCN5 was found to be regulated by heparin, an inhibitor of smooth muscle cell proliferation (Delmolino et al 1997). Through subtractive hybridization, we compared heparin-treated vascular smooth muscle cells to chrondroitin sulfate treated VSMC, which is similar to heparin, but lacks its anti-proliferative activity. The screen showed 16 upregulated and 25 down regulated genes. One of the upregulated clones was homologous to members of the CCN family. After the discovery of CCN5 we investigated the antiproliferative properties and found that knock down of CCN5 decreased the antiproliferative effect caused by heparin, indicating a role for CCN5 in heparin signaling (Lake and Castellot 2003).

Later, another group cloned Wnt Inducible Secreted Protein 2 (WISP-2) from the wnt-1 transformed mouse mammary epithelial cell line, C57MG (Pennica et al. 1998). Gene

expression was analyzed in wnt-1 transformed C57MG through subtractive hybridization. WISP-2 (CCN5) was overexpressed in the transformed C57MG cells by 5 fold. The CCN5 gene was mapped to human chromosome 20q12-20q13 and was duplicated up to four times in colon cancer cells. However, the expression of WISP-2 was lower in the colon cancer cells, despite the higher copy number. This data suggested that WISP-2 was a regulator in colon cancer and possibly others (Pennica et al. 1998).

CCN5 was also found to be regulated in transformed rat embryonic fibroblasts (REF). REFs were transformed by over expressing oncogenic H-*ras* and knockdown of *p53*. Gene expression profiles showed a decrease in a gene termed rCOP-1. rCOP-1 was shown to have 40% homology with the CCN family, but missing the CT domain. This group also found overexpression of rCOP-1 through a retroviral vector caused the transformed REFs to undergo apoptosis, but had no effect on normal cells, suggesting a tumor suppressor role for rCOP-1 (Zhang et al. 1998).

A fourth group identified CCN5 as connective tissue growth factor like protein (CTGF-L) from primary human osteoblast cells. CTGF-L was found to have significant homology to the CCN family. Expression was found in osteoblasts, fibroblasts, ovary, testes and heart tissue. CTGF-L was found to be highly expressed in osteoblasts forming bone, alkaline phosphatase positive bond marrow cells and chondrocytes. CTGF-L expression promoted osteoblast adhesion and inhibits integrin binding to fibrinogen. CTGF-L also inhibited osteocalcin production in the osteoblast like Ros 17/2.8 cell line,

which is important for bone production. These results indicate that CTGF-L plays an important role in regulation of bone turnover (Kumar et al. 1999).

Based on the recommendations of the International CCN Society, rCOP-a, CTGF-L and WISP-2 are now known as CCN5. These studies laid the foundation for nearly 50 publications on CCN5 in the next decade. CCN5 has been found to play an important role in human development and disease through it's regulation of important cellular processes, such as proliferation, migration, matrix modification and adhesion.

CCN5 Structure

The functions of CCN5 are highly dependent on it's domain structure. CCN proteins all have a homologous modular structure that dictates function. The structure of CCN3 and 5 has been explored through small X-ray diffraction. These two CCN proteins have a long modular domain, that appears to act as a scaffold in the ECM. The elongation of the domains allows for multiple proteins to bind, and thus may modulate signaling through spatial interactions (Holburn et al 2011). Four domains comprise this CCN structure (Figure 1): an N-terminal secretion signal, an insulin like growth factor binding protein (IGFBP), von Willebrand factor type C (VWC), variable hinge region a thrombospondin 1 (TSP1), and in all but CCN5, a cysteine rich carboxyl terminal repeat domain (CT) (Bork 1993). The absent CT domain in CCN5 may be important to its biological function, as the CT domain is involved in binding matrix proteins, integrins and important signaling molecules (Holbourn et. al 2009). Indeed, domain analysis has indicated that each domain may serve a distinct function for the protein, and in certain

cell types there are splice variants that create proteins comprised of the individual CCN domains (Wei et. al 2010). In mouse vascular smooth muscle (VSMC), the VWF domain has been implemented in nuclear localization of CCN5. Disruption of the nuclear localization signal in the VWF domain using site directed mutagenesis abolishes nuclear localization of CCN5 (Wiesman et al 2010). The findings described above as well as domain analysis in other CCN family members implicates and important role for domains and warrants further study.

Although other domains have not been investigated for CCN5, other CCN family member proteins have been shown to have domain specific functions. CCN2 has been shown to induce angiogenesis during breast cancer. Removing the CT domain abrogates this activity, suggesting CCN5 may negatively regulate angiogenesis, since it is missing the CT domain (Chein et al 2011). In the retinal angiogenesis model in mice, the cysteine knot motif in the CT domain of CCN2 bound various angiogenic factors, such as von Willebrand factor, PDGF and VEGF and induced angiogenesis. Mutant CCN2 containing no CT domain was injected into the vitreal compartment and decreased angiogenesis (Pi et al 2012). The inhibitory effect of CT loss on angiogenesis may implicate CCN5 in negatively regulating angiogenesis. Thus, antibodies against the CT domain could become candidate for anti-angiogenic therapies. CCN2 CT domain also has been shown to bind fibronectin through α5β1 integrin and enhanced chrondrocyte adhesion to the extracellular matrix (Hoshijimi et al 2006). CCN5 lacks the CT domain and may play an opposing function in cell adhesion in chrondrocytes. In CCN2, the VWF and IFGBP domains also bound to the

extracellular matrix protein, aggrecan, and increased transcription of aggrecan mRNA (Aoyama et al 2009). Through this research it is clear that the domain structure plays a role in downstream signaling effects of CCN family members, particularly regarding the CT domain. CCN5 has many physiologic functions, such as proliferation, migration and adhesion. Further domain analysis may link functions to locations on the protein, which will aid in dissecting the CCN5 signaling network (Table 1).

2. **Cell Physiology**

Proliferation

The function of CCN5 in cell proliferation has been studied in depth in many cell types. For example, in our lab, we have shown that CCN5 mediates the anti-proliferative effects of heparin in VSMC through RNAi knockdown of CCN5. VSMC were infected with RNAi against CCN5 and used for RT-PCR. Results showed a decrease of 77% in CCN5 transcripts compared to scramble RNAi infected cells (Lake et al 2003 -2). In addition, over expressing CCN5 inhibits vascular smooth muscle (VSMC) proliferation in vitro. In the rat carotid artery balloon injury model, CCN5 was expressed in uninjured tissues, but expression was inhibited following injury and only restored after complete wound healing, indicating CCN5 suppresses VSMC proliferation during vascular injury (Lake et al 2003 -1). The inhibitory effect on proliferation has also been found in uterine smooth muscle. Overexpression of CCN5 via an adenoviral construct decreased proliferation of both normal human uterine smooth muscle and leiomyoma cells *in vitro* by 51% and 29% respectively. Human leiomyoma tissues were found to have decreased CCN5 expression when compared to healthy uterine tissue, indicating a role

for CCN5 in disease progression (Mason et al 2004). CCN5 has also been implicated in proliferation of other types of tumors. In the estrogen positive invasive breast cancer cell line, MDA-MB-231, cells over expressing CCN5 via transfection showed less proliferation than control cells. In addition, in the less invasive breast cancer cell line, MCF-7, over expressing CCN5 led to a decrease in BrdU labelled cells, indicating decreased entry to S-phase as a mechanism of decreased proliferation (Fritah et al 2008). CCN5 also plays a role in regulating proliferation in normal cell physiology. In preadipocytes, overexpression of CCN5 inhibited proliferation by 30% (Inadera et al. 2009). In human umbilical vein endothelial cells, a peptide fragment, -TAWGPCSTTCGLGMATRV- corresponding to CCN5's TSP1 domain was found to inhibit proliferation by 75% (Karagiannis & Popel 2007).

However, some studies show an opposite effect of CCN5 on cell proliferation. In MCF-7 breast cancer cells, estrogen was shown to upregulate CCN5, consistent with findings in uterine smooth muscle. However, CCN5 knockdown using anti-sense oligonucleotides caused a decrease in MCF-7 proliferation, indicating a role for CCN5 in breast cancer progression (Banerjee et al 2003). Proliferation of MCF-7 cells through stimulation with epidermal growth factor (EGF) or estrogen also induced CCN5 expression. MCF-7 cells treated with RNAi against CCN5 showed less proliferation compared to control in response to EGF treatment, indicating CCN5 as a downstream mediator of EGF signaling (Banerjee et al 2005). Phorbol ester induced proliferation in MCF-7 cells also was disrupted by RNAi knockdown of CCN5. The proliferative effect of phorbol esters on MCF-7 cells could be replicated by overexpressing CCN5 mRNA

(Sengupta et al 2006). Differences in CCN5 impact on cell proliferation may be due to specific signaling pathways between cell types, or methods used to induce and measure proliferation (Russo & Castellot 2010). More studies need to be done to fully understand the biology of CCN5 in cell proliferation between cell types. Our lab has observed this effect between lung smooth muscle cells and lung epithelial cells. Data suggests that CCN5 inhibits airway smooth muscle cell proliferation, as with vascular smooth muscle. However, RNAi knockdown of CCN5 expression in lung epithelial cells caused an increase in cell proliferation and migration (Castellot lab, unpublished data). This interesting effect suggests that CCN5 may play a role in epithelial-mesenchymal transition, and may explain the contrasting effects seen in cancer cells and mesenchymal tissue.

Migration

CCN5 also can regulate cell migration. Cell migration is an important aspect of many disease processes, such as cancer, atherosclerosis, uterine fibroids, and asthma. Aberrant cell migration proceeds metastasis and can lead to increased cancer aggression. CCN5 has been found to be a invasion suppressor gene in multiple cell types. Overexpressing CCN5 decreases VSMC motility, invasiveness and expression of matrix metalloproteinase 2 (MMP-2), a key protein in matrix degradation and cell invasion (Lake & Castellot 2003). In addition, CCN5 knockdown VSMC showed increased invasion in a Matrigel transwell assay (Lake et al 2003). CCN5 has also been shown to inhibit motility in uterine smooth muscle. Overexpressing CCN5 using an

adenoviral vector inhibited both normal uterine smooth muscle and leiomyoma cell migration during a scratch wound assay by more than 50% (Mason et al 2004).

Similarly in breast cancer, silencing CCN5 expression in MCF-7 cells increased expression of MMP-2 and MMP-9 (Banerjee et al 2008). CCN5 expression has been shown to be correlated with breast cancer aggression. The more invasive breast cancer cell line, MCF-7, expressed lower levels of CCN5 compared to the less aggressive cell line, MDA-MB-231 (Fritah et al 2008). Supporting this, RNAi knockdown of CCN5 in VSMC increased motility during scratch wound assays. Overexpressing CCN5 in MDA-MB-231 cells decreased invasiveness in a Matrigel transwell assay by two fold. Motility in scratch wound assays was also inhibited by CCN5 overexpression. Knockdown of CCN5 using shRNA in MCF-7 cells increased invasiveness in transwell assays (Fritah 2008). In MDA-MB-231 breast cancer cells, CCN5 expression suppressed miR-10b, which is upregulated in breast cancer and positively regulates cell migration and invasion. Silencing CCN5 upregulates TWIST1, a miR-10b activator, through activation of hypoxia inducible factor 1 (HIF-1)-JNK signaling, leading to increased cell invasion (Haque et al 2011). CCN5 also acts as a tumor suppressor through activation of p53. P53 is a master tumor suppressor gene and is inactivated in many types of cancer. MCF-7 p53 mutant invasiveness was abrogated by adding recombinant CCN5 protein. Invasive p53 mutant MCF-7 cells showed less expression of CCN5 compared to wild type p53 cells, indicating a role for CCN5 in p53's function as a tumor suppressor gene (Dhar et al 2008-1). The TSP1 domain of CCN5 has been shown to decrease invasiveness of endothelial cells *in vitro*,

indicating again that specific domains of CCN5 have unique functions (Karagiannis and Popel 2007). However, data from our lab has shown full-length CCN5 expression inhibits VSMC proliferation and migration, but allows for normal endothelial cell regeneration in a mouse model for vascular injury (Russo & Castellot 2010). This suggests that the in vitro result using the isolated TSP1 domain may not reflect the physiological function of CCN5 in the intact animal. Further studies are needed to address this issue..

In a study of 122 breast tumor samples, CCN5 was expressed at higher levels in the moderate and more invasive, groups of tumors (Davies et al. 2007) that have a poor prognosis. CCN5 appears to behave differently in different tumor types, as in a similar sample of 94 colorectal cancer tissue samples, CCN5 was down regulated compared to normal tissue samples (Davies et al. 2010). The opposing role of CCN5 in regulation of cell migration and invasion may be due to differences in cell signaling in different types of cancer. These differences must be investigated for CCN5 to be considered for a drug target in disease.

Adhesion

The role of CCN5 in cell adhesion has not been studied extensively as other members of the CCN family have. Three different osteoblastic cell lines, primary human osteoblasts, osteosarcoma MG63 and Ros 17/2.8, all adhered to immobilized CCN5 in a dose dependent manor. Recent studies in our lab have indicated CCN5 is able to block the ability of αVβ3 and αIIβ3 integrins to fibrin. Through this mechanism, CCN5

may regulate podosome adhesion in SMC (unpublished data, Ron Meyers). More studies are needed to assess the role of CCN5 in adhesion, particularly in the invasive breast cancer lines in which it controls proliferation and migration.

Epithelial-Mesenchymal Transition

CCN5 is involved in proliferation, migration and adhesion of a variety of cell lines. These processes are involved in endothelial-mesenchymal transitions (EMT) in which an epithelial cell gradually loses epithelial characteristics, such as E-cadherin expression, and takes on a more mesenchymal, or fibroblast like, phenotype. This switch has been implicated in multiple cancers, particularly during the switch from non-invasive to invasive. In pancreatic cancer cells, CCN5 recombinant protein was able to induce a mesenchymal to epithelial transition based on expression of the epithelial cell marker, vitmenin (Dhar et al 2007-2). Furthermore, pancreatic cancer cells displaying EMT showed no expression of CCN5 (Dhar et al 2007-2). In the poorly differentiated breast cancer cell line, MDA-MB-231, CCN5 expression correlated with a transcriptional regulator of breast cell differentiation markers, and more differentiated breast cancer cell lines showed higher expression of CCN5. In addition, overexpression of CCN5 in MCF-7 cells created a more differentiated phenotype as measured by morphological changes and increased expression of cytokeratin, a marker of breast epithelial differentiation (Fritah et al 2008).

Recently, microarray expression studies in MCF-7 demonstrated CCN5 represses genes involved in the EMT. Specifically, Sabbah and colleagues found CCN5 is

recruited to the TGFB receptor II promoter and represses transcription (Sabbah et al 2011). TGFB is important in promoting the EMT in cancer. This data provides a potential mechanism by which CCN5 prevents EMT and acts as a tumor suppressor. In the neuroblastoma line, neuro2a, stable transfectants overexpressing CCN5 showed greater neurite growth and differential compared to control cells (Ohkawa et al 2011). No other studies have been done concerning CCN5 in the nervous system, but its role in promoting differentiation is consistent with other cell lines.

However, again, in other cell types, CCN5 seems to have an opposite effect. In mesechymal stem cells derived human femoral bone marrow, CCN5, as well as CCN1, 2 expression declined during adipocyte differentiation (Shutze et al 2005). Similarly, CCN5 was shown to be a downstream target of wnt signaling in adipocytes. During adipocyte differentiation, CCN5 expression declined. However, during de-differentiation as induced by tumor necrosis factor α did not increase CCN5 expression. During differentiation in adipocytes, there may be changes to CCN5 DNA to prevent expression, or the results could be due to differences in signaling pathways in de-differentiation (Inadera et al 2008). Collectively, CCN5's role in proliferation, migration and adhesion modulate the EMT seen in multiple cancer cell lines and during stem cell differentiation. CCN5's regulation of these processes emphasizes its role as a tumor suppressor and warrants further investigation.

3. Cell Signaling

Wnt

CCN5 is a Wnt regulated gene (Pennica et al. 1998). Wnt proteins bind to the cell surface receptor, frizzled, which inhibits glycogen synthase kinase (GSK-3B) and casein kinase 1 (CK1). Inhibition of these proteins stabilized the pool of beta catenin in the cytosol and allows some beta-catenin molecules to translocate to the nucleus. In the nucleus, B-catenin interacts with the transcription factor TCF/LEF and induces gene expression. Wnt signaling is important in development and cancer, and can be overexpressed to give cells oncogenic properties. Overexpressing wnt-1 in C57MG caused overexpression of CCN5 as well (Pennica et al. 1998). Adenovirus infected mouse pluripotent progenitor cells, C3H10T1/2, overexpressing Wnt3A showed a 2.5 fold increase in CCN5 expression (Si et al. 2006). Similarly, in synovial fibroblasts, up regulating wnt signaling through transfection with stable B-catenin caused an increase in CCN5 transcription. In addition, estrogen was able to enhance CCN5 expression synergistically with increased wnt signaling (Tanaka et al. 2005).

Physiological activators of the Wnt pathway also have been shown to increase CCN5 expression. During mesenchymal stem cell differentiation, expression of Wnt-3A induced expression of CCN5 and CCN1 (Si et al 2006). Mechanical loading of bone is also known to induce the Wnt pathway. MC3T3-E1 cells treated with the GSK-3β inhibitor and mechanical loading showed a synergistic effect on CCN5 expression, increasing it 7 fold over control (Robinson et al 2006). The The Hepatitis C viral (HCV) core protein is known to modulate the Wnt pathway as well. HCV core proteins increases transcriptional activity induced by Wnt3A, through enhancement of Tcf dependent transcription and β-catenin stabilization. (Liu et al 2011). HCV core protein

was overexpressed in the hepatocellular cancer cell line, Huh-7. Microarray expression analysis showed an increase in Wnt-1 and subsequently CCN5 expression (Fukutomi et al. 2005). CCN5 protein also increased when Huh-7 cells were infected with Wnt-3A adenovirus. Cells co-infected with adenoviral Wnt-3A and HCV core protein showed a 1.8 fold increase in CCN5 expression compared to adenoviral Wnt-3a infected cells alone (Liu et al 2011). Childhood adrenocorticoid tumors (ACT) also involve over activation of the Wnt signaling pathway. ACT tissue samples were sampled from 62 patients. Gene expression of CCN5 was increased in ACT samples compared to normal tissue. In addition, immunohistochemistry staining in ACT tissue slices showed increased accumulation of CCN5 protein in 82% of samples (Letica et al 2011). Salivary gland tumor (SGT) progression involves stabilization and nuclear localization of B-catenin and salivary gland development relies on the Wnt signaling pathway (Ferrazzo et al 2009, Hai 2011). Microarray analysis of gene expression in SGT derived cell lines showed a four fold increase in CCN5 expression compared to several other tumor types. However, CCN5 expression was down regulated compared to normal SG cells, indicating loss of CCN5 expression may play a role in SGT progression (Kouzu et al 2006).

CCN5 expression appears to have different effects in depending on the tumor type or signaling pathway. In SMC and perhaps normal SG, CCN5 acts as a tumor suppressor, inhibiting cell proliferation, migration and adhesion (Russo & Castellot 2009, Kouzu et al 2006). However, in multiple cancers, CCN5 has been shown to be overexpressed with Wnt activation and present in greater levels compared to healthy tissue. As proto-

oncogenes are activated, frequently the cell responds by increasing expression of tumor suppressor genes, such as p53, which could be the case with overexpression of CCN5 in the Wnt signaling pathway. More research is needed to establish the effects of CCN5 expression in cancer cells.

Small signaling molecules which modulate the Wnt pathway also can induce CCN5 expression. Protein Kinase A (PKA) is an important regulatory protein in the Wnt pathway. PKA phosphorylates GSK-3β, inactivating it, and allows for stabilization of β-catenin (Suzuki et al 2008). PKA activation has been shown to increase CCN5. Treatment of MCF-7 cells with the PKA activator, [cholera toxin plus 3-isobutyl-1-methylxanthine (CT/IBMX) increased CCN5 expression (Inadera 2003). PKC is a negative regulator of B-catenin stability, blocking the Wnt signaling pathway (Gwak et al 2006). In MCF-7 cells, the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibited estrogen induced CCN5 expression, and activators of PKC, such as phorbol esters, increased CCN5 expression, again implicating CCN5 as a downstream target of wnt signaling (Inadera 2003, Sengupta et al 2006). The PKA regulatory subunit type 1A is frequently mutated in primary pigmented nodular adrenocorticoid disease (PPNAD), leading to over activation of PKA. Comparison of a PPNAD cell line to normal adrenocoricoid cells showed an decrease in miR-449, which targets CCN5, leading to an overall increase in CCN5 in PPNAD cells. Inhibiting PKA increased miR-449, and decreased CCN5 expression (Iliopoulos et al 2009).

In addition to PKA, large tumor suppressors (LAT1/2) can also modulate CCN5 expression. LAT1/2 are implicated in several different types of cancer, including leukemia, lung, prostate and breast cancers. LAT1/2 regulate key proteins in the Wnt pathway and have been shown to downregulate CCN5 in Hela cells using a whole genome microarray (Visser and Yang 2010).

Estrogen

Estrogen signaling was first discovered to modulate CCN5 in estrogen responsive breast cancer (Banjeree et al 2001, Inadera et al 2000). Since then, estrogen has been found to synergistically increase the effect of Wnt signaling on CCN5 (Tanaka et al 2005). In a physiological setting, rheumatoid arthritis fibroblasts isolated from human synovial tissue overexpressing non-degradable β-catenin (s/d β-catenin) showed increased CCN5 expression. 17-β-Estradiol treatment with over expression of s/d β-catenin increased CCN5 expression over 13 fold greater than the s/a B-catenin expressing cells along (Tanaka et al 2005). More research needs to be done to completely understand the relationship between estrogen, Wnt and CCN5.

However, the relationship between CCN5 and estrogen alone has been researched extensively. The effect of estrogen on CCN5 primarily is attributed to ER-α signaling. ER negative breast cancer epithelium cells overexpressing ER-α induced CCN5 expression after overexpression of ER-α. In addition, estrogen signaling increased both CCN5 expression and protein levels in MCF-7 cells, which could be abrogated by anti-sense oligonucleotides against ER-α (Banjeree et al 2003). *In vivo*, 17β-estradiol has

to induce tumor formation in athymic nude mice in xenographs. During solid tumor formation, CCN5 was increased in MCF-7 tumors. The partial ER-α antagonist, tamoxifen, inhibited tumor growth and CCN5 expression, implicating CCN5 in the mechanism of tumor formation (Ray et al. 2006). However, tamoxifen has been shown to be a partial agonist/antagonist for ER-α and a complete antagonist for ER-β. This leaves room for study of ER-β in the mechanism of estradiol regulation of CCN5 expression.

Estrogen has been shown to regulate CCN5 transcription in MCF-7 cells through an estrogen responsive element between -581 and -569 upstream of the estrogen inducible transcription start site (Fritah et al 2006). Estradiol increased CCN5 reporter gene expression, and this effect could be abrogated by anti-estrogens ICI-182-780 and 4-hydroxytamoxifaen. Chromatin immunoprecipitation showed an estradiol dependent recruitment of ER-A to the estrogen responsive element. CREB binding protein was also found to translocate to the estrogen responsive element in CCN5 (Fritah et al 2006). This data suggests a ER-α and CREB dependent mechanism for CCN5 expression in MCF-7 cells. ER-α has been shown to interact with the transcription co-factors CLIM and RLIM, which regulate the LIM-homeodomain transcription factors (Johnsen et al 2009). Silencing CLIM or RLIM with siRNA in MCF-7 cells decreased estrogen induced expression of CCN5. In addition, treatment with estrogen induced RLIM and CLIM recruitment to the CCN5 promoter, indicating a direct role for these transcription factors in CCN5 expression (Johnsen et al 2009). Estrogen may also help to stabilize CCN5 mRNA. Estrogen induced expression of CCN5 in MCF-7 cells is

accomplished through both transcriptional activation and transcript stabilization (Banjeree et al 2003).

Cross talk between the estrogen and CCN5 signal transduction pathways has been investigated as well. Estrogen signaling can be modified by protein kinase A/C activity. CCN5 expression was increased following treatment with CT/BMX and TPA alone. Treatment with CT/BMX with estrogen stimulated greater expression than estrogen alone, indicating a positive regulatory effect of PKC on CCN5 expression. Treatment with PKA inhibited estrogen induced expression of CCN5, where as TPA enhanced another estrogen responsive gene, pS2. This data suggests differences in estrogen regulation of CCN5 compared to other estrogen responsive genes (Inadera 2003).

Epidermal growth factor also interacts with the estrogen signaling pathway. Activation of EGFR can lead to further activation of ER in a non-estrogen dependent pathway (Levin et al 2003). EGF signaling leads to an increase in proliferation of MCF-7 breast cancer cells, but this can be abrogated by silencing CCN5. In addition, EGF induced CCN5 expression in a dose dependent manor that increased synergistically with addition of estrogen in MCF-7 cells. Silencing or inhibiting EGFR, inhibiting P13K with wortmainin, or inhibiting ERs with ICI-182-780 all inhibited EGF induced expression of CCN5 (Banerjee et al 2005). The IGF pathways also activates ER through phosphorylation in a non- estrogen dependent manor. Estrogen signaling can also activate the IGF pathway, leading to bi-directional crosstalk (Batella et al 2012). In MCF-7 cells, IGF is a potent mitogen and treatment with IGF increased CCN5

expression. SiRNA knockdown of CCN5 abrogated the mitogen effect of IGF. This IGF induced CCN5 expression is also regulated by estrogen. The pure anti-estrogen, ICI-182,780 inhibited the IGF induced expression of CCN5 as well as proliferation, implicating membrane ERs as a participant in this signaling pathway (Dhar et al 2007). Tip30 is a putative tumor suppressor transcription factor, which negatively regulates estrogen induced transcription of c-myc, a potent proto-oncogene (Jiang et al 2004). Tip-30 also negative regulates IGF and CCN5 expression, possibly through repression of ER-α transcription. Tip30 knock out in mammary epithelial cells promotes proliferation and immortalization. Knock down of either CCN5 or IGF-1 inhibited this effect (Pecha et al 2007). This data suggests Tip30 may regulate breast cancer progression through estrogen signaling and ultimately CCN5 and IGF-1 expression.

Progesterone also regulates CCN5 expression. In MCF-7 cells progesterone treatment caused a transient increase in CCN5 mRNA which could be clocked by the progesterone antagonist RU38468. However, when treated with progesterone and estrogen in combination, progesterone inhibited CCN5 expression (Banerjee et al 2003). Estrogen and progesterone are important regulators of uterine smooth muscle. Our lab has extensively investigated CCN5 signaling in uterine smooth muscle and demonstrated its regulation by estrogen and progesterone. During the proestrous phase of the reproductive cycle, estrogen levels are high and CCN5 is expressed five fold higher than in metestrous females, which express low levels of estrogen (Mason et al 2004). Estrogen and progesterone treatment increased CCN5 expression in uterine smooth muscle from OVX rats. Interestingly, estrogen plus progesterone treatment

increased CCN5 expression greater than either treatment alone (Mason et al 2004). CCN5 signaling appears to be highly dependent on the cell type and pathology of the tissue. For example, CCN5 expression in relation to progesterone differs between uterine smooth muscle and the MCF-7 breast cancer line. Additionally, in uterine smooth muscle, CCN5 has anti-proliferative effects, whereas in breast cancer, CCN5 appears to act as a mitogen. In both lines, CCN5 is induced by estrogen. In cancer cells, it is possible that signaling pathways have been changed and thus alter CCN5 expression. Investigation into CCN5 signaling and physiological effects will be needed to establish possible treatments involving CCN5.

Transforming Growth Factor-β

Transforming growth factor β (TGFβ) is an important cytokine implicated in cancer progression, angiogenesis, as well as cellular functions such as survival, migration, and proliferation. In mouse osteoblasts, TGFβ-1 increased CCN5 expression and protein level in a time dependent manor (Parisi et al 2007). Kallikrein-related peptidase 12 (KLK12) is a serine protease implicated in multiple aspects of cancer progression and has been shown to cleave latent TGFβ to its active form. Through a degradomic approach, KLK12 was shown to target CCN5 for proteolysis through the TSP domain, and in turn increase release of TGF-β, FGF-2, VEGF, and BMP2. *In* vitro CCN5 bound TGFβ-1 and cleavage by KLK12 decreased TGFβ-1 binding, releasing active TGFβ-1 . In human umbilical endothelial cells (HUVECs), survival after serum starvation was decreased by addition of KLK12 cleaved CCN5 compared to addition of full length CCN5, indicating different roles for full length or cleaved CCN5 (Guillon-Munos et al

2011). This is in agreement with data from our lab in VSMC, showing different variants of CCN5 may have different functions *in vivo* (Wei et al 2009). KLK12 may mediate processing of CCN5 to individual domain fragments through proteolysis, which may have different functions on cell physiology (Guillon-Munos et al 2011). CCN5 has been found to promote osteocyte differentiation through matrix deposition later temporally in osteoblast development. This signaling was mediated by increased phosphorylation of Smad 1/3/8 as well as members of the MAPK family (Kawaki et al 2011). CCN5 signaling through Smads to promote osteocyte differentiation shows yet another link between the CCN5 and TGFb pathways.

TGFβ-1 has also been linked to CCN5 in a physiologic setting. Liver biopsies from patients with HVC induced fibrosis were sectioned and stained for TGFβ-1 and CCN5. Synthesis of TGFβ-1 was increased during liver injury, followed by an increase in CCN5 production, according to the degree of fibrosis (Tache et al 2011). HVC core proteins have also been shown to increase CCN5 expression through the wnt pathway, indicating possible cross talk between TGFβ, CCN5 and wnt in hepatitis (Tache et al 2011, Lui et al 2011, Fukutomi et al. 2005).

CCN5 also regulates TGFβ, creating a bi-directional cross talk between the pathways. Recently, our lab has shown that CCN5 localizes to the nucleus in VSMC, although the function of nuclear localization was unknown (Weisman et al 2010). Sabbah and colleagues investigated the nuclear localization and function of CCN5 in breast carcinoma. Using MCF-7 cells, CCN5 was shown to localize to the nucleus using

immunofluorescence and subcellular fractionation. Tissue sections taken from breast cancer patients also showed CCN5 expression in the nuclei. Domain analysis showed several possible DNA binding domains in CCN5 in all three structural domains and the signal sequence. Silencing CCN5 in MCF-7 cells created an increase in TGFβ signaling and TGFβ expression, and CCN5 expression repressed TGFβ signaling through binding to the TGFβ promoter in combination with the histone deaceteylase, HDAC (Sabbah et al 2011). Here, CCN5 is acting as a tumor suppressor, repressing the TGFβ pathway that promotes EMT in breast cancer cells. These findings are in agreement with the existing data regarding CCN5's control of EMT in cancer cells. However, repression of EMT is considered a tumor supressor function. Yet in the Wnt signaling pathway in the same types of cells, CCN5 appears to function as a proto-oncogene, promoting cell growth and proliferation. The biphasic nature of CCN5 seems to depend on the signaling pathway in question and the upstream activation. The upstream control and downstream targets of CCN5 are a complex network (Figure 2). More research into the cross talk between signaling pathways is needed to fully elucidate the role of CCN5 in cancer.

4. **Development**

The role of CCN5 in development has been investigated in both adult and fetal tissue. CCN5 knockout or CCN5 overexpressing mice die before the gastrulation stage and do not implant properly into the uterus. This data suggests a crucial role for CCN5 in embryonic development (Russo & Castellot 2010). The lethality of CCN5 transgenic mice has made the study of development difficult. In the future, research will need

conditional knockouts or heterozygous mice to investigate the role of CCN5 in development. Despite these challenges, CCN5 expression in embryonic and adult tissues (Gray et al 2007, Jones et al 2007).

CCN5 is expressed early in development in most embryonic tissues, with expression becoming increasingly restricted throughout development in the mouse. CCN5 is expressed in tissues of all three germ layers, ecto, endo and mesoderm. Embryonic organs showed expression of CCN5 through immunohistochemistry from developmental stage E9-E12. After E12 the expression of CCN5 became tissue specific. CCN5 is expressed in the endothelium and smooth muscle of arteries and veins, which is consistent with the biological activity of CCN5 found in VSMC *in vitro* and *in vivo.* CCN5 is also expressed the the heart, which has yet to be investigated on a cellular level. Human embryonic tissue sections also showed CCN5 expression in the myocardium, endothelium and smooth muscle of arteries and veins. CCN5 is also expressed in the mouse in GD14 and GD16 in the bronchial epithelium and mesenchymal cells. In the human fetal lung, CCN5 was expressed at low, but uniform levels at 5 months of development. The mouse musculoskeletal system also expressed CCN5 beginning at G14 in chrondrocytes during ossification. However, the hyaline cartilage in the resting zones did not express CCN5, suggesting a role for CCN5 in cell proliferation during bone formation (Jones et al 2007). This is consistent with findings at the cellular level, which found CCN5 expression during osteocyte proliferation (Kumar et al. 1999). However, in human fetal tissue, CCN5 expression was found at low levels at the cellular levels and was not expressed at all in osteocytes or osteoclasts (Jones et al 2007). The

intestinal epithelium of the mouse highly expressed CCN5 at day E12 and gradually was reduced to low levels by G16. In intestinal smooth muscle, CCN5 was expressed at low levels during E12, moderate levels in GD14 and intermediate levels in GD16.

Overall, it is clear that CCN5 has an important role in development in many types of tissue in both the embryo and adult. CCN5 expression patterns change over time, and thus could be a source for deregulation in adult pathology.

5. Pathophysiology

Angiogenesis

Due to the signaling pathways involved in CCN5 function, it is possible that CCN5 plays a role in angiogenesis. Other CCN family members, specifically CCN2, have been shown to play a key role in developmental and pathologic angiogenesis. Diabetic retinopathy is one pathology of deregulated angiogenesis, and retinal angiogenesis is an important model for studying angiogenesis. In the retinal vasculature of neonate mice, CCN2 overexpression was shown to promote vascular growth and repair through migration of endothelial cells, pericytes, and astrocytes. Endothelial cells and pericytes were found to be the main sources of CCN2 during angiogenesis (Pi et al 2011). CCN2 has also been shown to induce angiogenesis in both normal retinal development, ischemia induced angiogenesis and during lung cancer (Pi et al 2012). Interestingly, CCN2 induces angiogenesis during breast carcinoma, but this activity is abolished by

deleting the CT domain (Chien et al 2011). This suggests that CCN5, lacking the CT domain, may negatively regulate angiogenesis. CCN5 localized around blood vessels in human epidermal tissue, and is significantly down regulated during wound healing, which is highly dependent on angiogenesis (Rittié et al 2011). This also suggests a negative role for CCN5 in controlling angiogenesis. CCN5 has been shown to have divergent roles in tumor genesis depending on the signaling pathway and cell type in question, and appears to have oncogenic properties in multiple types of cancer. The possible negative regulation of angiogenesis is yet another factor in the dichotomy of CCN5.

Due to the inhibitory effect of CCN5 on smooth muscle proliferation, and the role of other CCN5 family members in angiogenesis, and CCN5 localization to the nucleus, I thought it may be possible for CCN5 to regulate transcription of angiogenic factors in VSMC. If CCN5 inhibits VSMC proliferation, it is possible that it also regulates the overall contractile phenotype, which has been known to mechanically and chemically alter endothelial cell proliferation. Microarray data from our lab suggests that CCN5 may regulate important proteins in angiogenesis, such as VEGF or the Ang/Tie proteins in VSMC. Angiogenesis is an important factor for multiple physiologic processes, such as vascular development, wound healing, tumor growth, and diabetic retinopathy, and should be investigated in the context of CCN5 signaling.

Cancer

Breast Cancer:

Much research has implicated CCN5 in breast cancer progression. Although CCN5 acts as an inhibitor of proliferation in smooth muscle, in multiple breast cancers CCN5 induces proliferation, migration and is correlated with poor prognosis (Banerjee et al. 2008, Banerjee et al. 2003, Davies et al. 2007). CCN5 transcripts were analyzed from normal human breast tissue and breast tumors. CCN5 expression was low in normal breast tissue but showed increased expression in the breast tumors. Furthermore, CCN5 was positively correlated with aggressiveness and negatively correlated with patient outcome (Davies et al. 2007). However, Banjeree (2008) found that CCN5 expression was biphasic, with low CCN5 expression in healthy tissues, increased expression in non-invasive breast cancers, and then decreased expression in poorly differentiated, invasive tumors. Again, the paradoxical effects of CCN5 may be due to differences in tumor type, or the specific signaling pathways that are altered, as described in the CCN5 signaling pathways above.

Skin Cancer:

CCN5 is highly expressed in both epidermal and dermal human epithelium (Quan et al 2009). CCN5 is expressed primarily in epithelial keratinocytes, and in the dermis, it is secreted by fibroblasts, blood cells, hair follicles and sweat glands (Rittié et al 2011). During treatment of human skin with UV light, CCN5 was down regulated by 50%, 24 hours after exposure. CCN5 protein levels were also down regulated during wound healing of human forearm epidermal tissue by 80% (Rittié et al 2011). CCN5's antiproliferative activity may be inhibited during UV exposure or wound healing, leading to the hyper proliferative effects seen in both conditions (Quan et al 2009, Rittié et al

2011). However, CCN5 acts as a tumor suppressor or proto-oncogene depending on the cell type and signaling pathway. It could be that CCN5 acts a proliferative factor in epithelial cancer, and the DNA damage response is inactivating it as a mechanism of halting cell cycle progression. More functional studies on CCN5 during epithelial cancer is needed to answer these questions.

Pancreatic Adenocarcinoma, Hepatocellular Carcinoma, and Colon Carcinoma: Pancreatic carcinoma shows significantly decreased levels of CCN5 compared to normal tissue (Dhar et al 2007). CCN5 loss was associated with loss of p53, and recombinant CCN5 decreased markers of mesechymal transition. CCN5 is important in development and is known to control differentiation in multiple cell types. Here, CCN5 decreases mesenchymal markers, further implicating it in controlling EMT during carcinoma. During hepatocellular carcinoma, CCN5 had the opposite effect of increasing cell proliferation through activation of the wnt pathway in heptocytes treated with Hepatitis C viral core proteins. The wnt pathway is also altered in colon cancer. CCN5 DNA was amplified in colon carcinoma, but transcript levels were decreased compared to healthy colon tissue (Pennica et al 1998). Again, this emphasizes the differential role for CCN5 in different cell types.

Smooth Muscle

Uterine Smooth Muscle:

Human leiomyomas are benign neoplastic growths that occur in the smooth muscle of the uterus. Although they are benign, leiomyoma causes serious problems in women's

reproductive health, and currently surgery is the only option for treatment. CCN5 is down regulated in human leiomyoma tissue compared to surrounding normal tissue (Mason et al 2004). Recently data from our lab has shown CCN5 inhibits the ability of human organiods and rat *tsc1* mutant cells to form fibroid like tumors in NOD-SCID mice (unpublished data, Castellot lab).

Vascular Smooth Muscle:

Pathologies of VSMC are a significant contributor to cardiovascular disease. Arterial restenosis and atherosclerosis are both diseases involving increased VSMC proliferation, which leads to vessel occlusion. In a rat model of restenosis, CCN5 expression is down regulated after injury, similar to the down regulation of CCN5 during wound healing (Lake et al 2003, Rittié et al 2011). These data suggest that CCN5 has an anti-proliferative effect in VSMC and epithelial tissue, and this activity is decreased during both normal and pathologic functions. During a rat carotid injury model, CCN5 overexpression inhibited proliferation, motility and neointima invasion. These effects were also seen in culture of primary VSMC (Lake & Castellot 2003). Endothelial cells were not effected by CCN5 overexpression in VSMC. This specificity for VSMC may be helpful in designing therapeutics for the excessive proliferation observed in restenosis and atherosclerosis without damaging the endothelium.

Airway Smooth Muscle:

Airway remodeling during asthma is a major contributor to irreversible changes in the lung during chronic asthma. Airway remodeling depends on the proliferation and

migration of airway smooth muscle cells (ASMC), which restrict the bronchioles. Although there are treatments for asthma that prevent symptoms, there are currently few treatments available for the permanent damage caused by airway remodeling. In preliminary data from our lab, we have shown that CCN5 inhibits proliferation and motility of human and mouse airway ASMC. Other CCN family members have also shown to play a role in airway remodeling. CCN6 induced proliferation of lung fibroblasts through integrin β1 during pulmonary fibrosis (Batmukh et al 2011). This indicates that CCN5 and other CCN family members may play opposing role in airway remodeling and fibrosis. As CCN5 lacks the CT domain, it is possible that this and other contradicting functions between CCN family members are due to the CT domain.

6. **Conclusion**

CCN5, like its other family members, is an important protein in many pathological and physiologic processes. CCN5 has a clear role in development, cancer, epithelial response to UV, and smooth muscle remodeling. CCN5 may also be involved in other pathologies such as wound healing or angiogenesis during tumor growth. Many tools have been developed to study CCN5, such as RNAi, constructs for over expression or knock down, as well as antibodies against specific domains (Wei et al 2009). During my project I also looked at the function of individual domains in CCN5's physiologic effects. Our lab has developed adenoviral vectors expressing all combinations of CCN5 domains plus the CT domain. Further investigation into domain specific function may yield therapeutic targets. An additional step towards understanding CCN5 will be creating recombinant CCN5 which can be added extracellularly to cells and tissue.

CCN5 interaction with growth factors, ECM components and proteases and may facilitate cross talk by acting as a scaffold protein. Further study of these interactions and the interactions between CCN family members themselves will reveal important information about the CCN5 signaling network. Although much research has been done, it is clear there are still many areas open for investigation. CCN5 is important in many physiologic processes and further investigation may yield new drug therapies for the many functions of CCN5.

Figure 1. CCN5 family member structure

The following figure depicts the domains of the six CCN family member proteins. Four domains comprise this CCN structure: an N-terminal secretion signal, an insulin like growth factor binding protein (IGFBP), von Willebrand factor type C (VWC), variable hinge region a thrombospondin 1 (TSP1), and in all but CCN5, a cysteine rich carboxyl terminal repeat domain (CT). The absent CT domain in CCN5 may be important to its biological function, as the CT domain is involved in binding matrix proteins, integrins and important signaling molecules.

Table 1. CCN family member domain analysis

The following table summarizes the known functions of domains in the CCN family in multiple cell types. The CT domain has been implicated in cell migration, proliferation and adhesion. Interestingly, CCN5 lacks this domain and may have opposite effects in some cell types. No analysis has been done thus far on CCN1, 4, and 6, leaving open areas for research.

CCN family member	Domain	Function	Citation
CCN2			
3T3	CT	+ proliferation	Brigstock et al 1997
Fibroblasts	CT	+ proliferation	Grotendorst & Duncan 2005
Chrondrocyte	CT	binds fibronectin/ integrin α5β1 + adhesion	Hoshijimi et al 2006
MCF-7	CT	+ angiogenesis	Chien et al 2011
Retinal model of angiogenesis	CT	+ angiogenesis binds VEGF	Pi et al 2012
In vitro	CT, TSP	binds VEGF	Holbourn et al 2011
Chrondrocyte	VWF, IFGBP	bind aggrecan + aggrecan transcription	Aoyama et al 2009
Myofibroblast	N-Term	+ differentiation + collagen secretion	Grotendorst & Duncan 2005
CCN 3			

CCN family member	Domain	Function	Citation
Mesenchymal stem cells	CT	- differentiation	Bohlig et al 2008
Glioblastoma 59	CT	cytoplasmic localization	Planque et al 2006
CCN5			
VSMC	VWF	nuclear localization	Weissman et al 2010
HUVECs	TSP	cleavage by KLK12 and release of active TGFβ	Guillon-Munos et al 2011

Figure 2. CCN5 Signaling in Cancer

CCN5 has been shown to have different signaling patterns in different cell types. This figure summarizes the cell signaling literature from cancer cell lines, primarily MCF-7 cells, but also including PPNAD, mouse mammary epithelia, and HUVECs. Signaling may be different in mesenchymal cells, as in cancer and epithelial tissue CCN5 appears to have a positive effect on proliferation and function as a proto-oncogene, and in mesenchymal cells CCN5 induces growth arrest. Table 2 summarizes the studies that contributed the the CCN5 signaling diagram.

Table 2. CCN Signaling in Mesenchymal, Cancer, and Epithelial Cells

The following table summarizes the known signaling pathways of CCN5 in mesenchymal, cancer and epithelial cells. Studies highlighted in white were included in the signaling diagram in Figure 2. Studies highlighted in light gray were not included in Figure 2. Estrogen, activators of the wnt pathway, HCV core proteins were all shown to increase CCN5 expression in multiple cell types. In addition, multiple cancer biopsies showed increased expression of CCN5. This is contrary to the growth arrest effects seen in mesenchymal cells. More studies are needed to elucidate the differences in signaling pathways of CCN5 in epithelial, mesenchymal and cancer cells.

Cell Type	Signaling	Effect	Citation
Mesenchymal Cells			
CH3H 10T1/2	over express Wnt3a	increase in CCN5 expression	Si et al 2006
Synovial Fibroblasts	over express stable β-catenin	increase in CCN5 expression	Tanaka et al 2005
	over express stable β-catenin plus estrogen	greater increase in CCN5 expression compared to β-catenin alone	Tanaka et al 2005
Mesenchymal stem cells	mechanical loading of bone	increase in CCN5 expression	Robinson et al 2006
	mechanical loading of bone plus GSK3β inhibitor	increase in CCN5 expression	Robinson et al 2006

Cell Type	Signaling	Effect	Citation
Rhematoid Arthritis Fibroblasts	over express stable β-catenin	increase in CCN5 expression	Tanaka et al 2005
Mouse osteoblast	differentiation in culture through ascorbate treatment	increase in CCN5 expression	Kawaki et al 2011
Uterine smooth muscle	estrogen and progesterone alone or in combination	increase in CCN5 expression	Mason et al 2004
Hepatic Cells			
Huh-7	overexpression of HCV core proteins	increase in CCN5 expression	Lui et al 2011
	Wnt3a plus HCV overexpression	greater increase in CCN5 expression than either alone	Lui et al 2011
Huh-7	overexpression of HCV core proteins	increase in CCN5 expression	Fukutomi et al 2005
Breast Cancer			
MCF-7	CT/IBMX (PKA activator)	increase in CCN5 expression	Inadera 2003
MCF-7	TPA (PKA activator)	increase in CCN5 expression	Sengupta et al 2006
	phorbol ester (PKA activator)	increase in CCN5 expression	Inadera 2003, Sengupta et al 2006

Cell Type	Signaling	Effect	Citation
MCF-7	siRNA against ERα	decreased CCN5 expression	Banjeree et al 2003
	estradiol treatment	increase in CCN5 expression	Banjeree et al 2003
MCF-7	tamoxifen	decreased CCN5 expression	Ray et al 2006
MCF-7	estradiol treatment	increased in CCN5 expression specifically through ERE at -581 and -569 upstream	Fritah et al 2006
MCF-7	ICI-182-780	decreased CCN5 expression	Fritah et al 2006
MCF-7	siRNA against CLIM or RLIM	decreased estrogen induced expression of CCN5	Johnsen et al 2009
MCF-7	estradiol treatment	induced recruitment of CLIM and RLIM to CCN5 promoter and increased transcription	Johnsen et al 2009
MCF-7	estradiol treatment	increased CCN5 mRNA through stabilization	Banjeree et al 2003
MCF-7	EGF treatment	increase in CCN5 expression	Banjeree et al 2005
MCF-7	Wortmanin treatment	decreased EGF induced CCN5 expression	Banjeree et al 2005
MCF-7	ICI-182-780	decreased EGF induced CCN5 expression	Banjeree et al 2005

Cell Type	Signaling	Effect	Citation
MCF-7	siRNA against EGFR	decreased EGF induced CCN5 expression	Banjeree et al 2005
MCF-7	IGF treatment	increase in CCN5 expression	Dhar et al 2007
MCF-7	Progesterone	increase in CCN5 expression	Banjeree et al 2003
	RU38468 (progesterone antagonist)	decrease in CCN5 expression compared to other tumors	Banjeree et al 2003
MCF-7	CCN5 expression	decreased TGFβ signaling through CCN5 binding to the promoter in conjunction with HDAC1	Sabbah et al 2011
HeLa	LAT1/2 siRNA	increase in CCN5 expression	Visser & Yang 2010
Other Cancers			
Human ACT samples	primary tumor samples	increase in CCN5 expression compared to normal tissue	Letica et al 2011
Human SGT samples	primary tumor samples	increase in CCN5 expression compared to other tumors	Kouzo et al 2006
	primary tumor samples	decrease in CCN5 expression compared to other tumors	Kouzo et al 2006
PPNAD cells	H89 (PKA inhibitor)	increased miR-449 and decreased CCN5 expression	Iliopoulos et al 2009
Epithelial/Endothelial			

Cell Type	Signaling	Effect	Citation
Mouse mammary tissue	Tip30 deletion	increase in CCN5 expression	Pecha et al 2007
HUVEC	KLK12 treatment	increased active TGFβ-1 release through CCN5 cleavage	Guillion-Munos et al 2011

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