CCN2 expression and localization in melanoma cells

Wei Sha · Andrew Leask

Received: 5 January 2011 / Accepted: 7 March 2011 / Published online: 12 June 2011
© The International CCN Society 2011

Abstract The matricellular protein connective tissue growth factor (CTGF, CCN2) is overexpressed in several forms of cancer and may represent a novel target in anti-cancer therapy. However, whether CCN2 is expressed in melanoma cells is unknown. The highly metastatic murine melanoma cell line B16(F10) was used for our studies. Real time polymerase chain reaction analysis was used to detect mRNA expression of CCN1, CCN2, CCN3 and CCN4 in Western blot and immunofluorescence analyses were used to detect CCN2 protein. Inhibitors of signal transduction cascades were used to probe the mechanism underlying CCN2 expression in B16(F10) cells. CCN2 was expressed in B16(F10) cells, and was reduced by the FAK/src inhibitor PP2 and the MEK/ERK inhibitor U0126 indicating that CCN2 acts downstream of these pathways in B16 (F10) murine melanoma cells. Expression of CCN1, CCN3 and CCN4 was not reduced by PP2 or U0126; in fact, expression of CCN4 mRNA was elevated by PP2 or U0126 treatment. To our surprise, CCN2 protein was detected in the nuclei of B16(F10) cells, and was undetectable in the cytoplasm. CCN2 was expressed in B16(F10) melanoma cells, adding to the list of cancer cells in which CCN2 is expressed. Of the CCN family members tested, only CCN2 is downstream of the highly oncogenic MEK/ERK pathway. CCN2 should be further evaluated for a possible role in melanoma growth and progression.

Keywords CCN2 · CTGF · Melanoma · Ras · ERK

Introduction

It has long been recognized that expression of the CCN family of matricellular proteins, is highly associated with certain cancers (Perbal 2001; Bleau et al. 2005). As an example, pancreatic tumour cells have been shown to possess 59 times the normal amount of connective tissue growth factor (CTGF/CCN2) (Wenger et al. 1999). CCN2 expression appears to be especially high in the tumor stroma (Hartel et al. 2004); however CCN2 is expressed in PANC-1 tumor cells downstream of ras/ERK/ERK (Pickles and Leask 2007) and an antibody against CCN2 reduces the in vivo growth and metastasis of these cells (Aikawa et al. 2006). Moreover, pancreatic tumor cells derived from CCN2 shRNA-expressing clones showed dramatically reduced growth in soft agar and when implanted subcutaneously in nude mice (Bennewith et al. 2009). These results suggest that CCN2 may be a key mediator of pancreatic cancer and may represent a novel therapeutic target for this disorder.

Melanoma is a highly aggressive form of cancer; a tumor thickness approaching 4 mm presents a high risk of metastasis, and a diagnosis of metastatic melanoma has a median survival of 6–9 months (Chin et al., 2006). Surgery cures so-called thin melanomas; but, post-surgery, melanomas recur at regional or distant sites in a significant number of patients with cutaneous melanoma of 2–4 mm thickness. It is therefore of crucial clinical importance, for example, to develop strategies aimed at identifying biochemical markers indicating the potential of early lesions to develop into highly metastatic tumors and to identify appropriate targets for drug intervention. Surprisingly, little information exists on the expression of CCN2 in melanoma. However, one study did use in situ hybridization of tissue sections to show that CCN2 was overexpressed in the stroma in four cases of desmoplastic (i.e. fibrotic) malignant melanoma compared to four cases of

W. Sha · A. Leask (✉)
Department of Dentistry,
Schulich School of Medicine of Dentistry,
Dental Sciences Building, University of Western Ontario,
London, ON, Canada N6A 5C1
e-mail: andrew.leask@schulich.uwo.ca

nondesmoplastic (i.e. non fibrotic) amelanotic malignant melanoma (Kubo et al. 1998).

In this report, we investigate the expression and localization of CCN2 in B16(F10) murine melanoma cells. Our results suggest that, as in other cancers, CCN2 may play a key role in the progression of melanoma.

Materials and methods

Cell culture and western blotting B16(F10) murine melanoma cells were purchased (ATCC) and cultured in high glucose Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Invitrogen; Burlington, Ontario). Cells were cultured until confluence prior to harvesting for mRNA or protein extraction. Protein was divided into two portions: one for extraction of total protein [using ice-cold TG lysis buffer (20 mM Tris- HCl [pH 8], 1% Triton X-100, 10% glycerol, 137 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 50 mM NaF, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, 10 μg of aprotinin ml⁻¹)] and the other for extraction of nuclear or cytoplasmic proteins using a commercial kit (Pierce). 1 ml conditional culture media was collected from every treatment to total protein were concentrated using Heparin Sepharose CL-6B(GE Healthcare) as previously described (Chen et al. 2001). The concentration of protein was detected (Pierce) and equal amounts of protein (100 μg) were subjected to SDS/PAGE on a 4–12% gradient gel (Invitrogen). Gels were then blotted onto nitrocellulose (BioRad) and CCN2 was detected using a mouse anti-CCN2 antibody (1:200 dilution, Santa Cruz) followed by a HRP-conjugated anti-mouse antibody (1:5000; Jackson), a chemiluminescent detection kit (Pierce) and X-Ray film (Kodak). Alternatively, anti-Sp1 (1:200; Santa Cruz) was used. When indicated, cells were transfected with CTGF (CCN2) shRNA Lentiviral or control shRNA Lentiviral Particles (Sanza Cruz) using polybrene and selected for using puromycin as described by the manufacturer (Santa Cruz).

Real time RT-PCR B16(F10) murine melanoma cells were grown in high glucose DMEM until 75% confluence and then treated for 24 h with focal adhesion kinase/src inhibitor (FAK/SRC) PP2, p38 MAP Kinase inhibitor SB203580, MEK/ERK inhibitor U0126, protein kinase C (PKC) inhibitor bisindolmaleimide I (all Calbiochem, La Jolla, CA, USA) at a concentration of 10 μM, except SB203580 which was used at 30 μM. DMSO was used as a control. RNA was harvested using Trizol (Invitrogen) and used for Real-Time RT-PCR. 25 ng of RNA was reverse transcribed and amplified using TaqMan Assays on Demand (Applied Biosystems) in a 15 μl reaction containing primers for mouse CCN1-4 (Assays on Demand; Applied

Biosystems) and 6-carboxyfluorescein labelled TaqMan MGB probe. Reverse Transcriptase One-Step Mastermix was added to samples and the ABI Prism 7900 HT sequence detector (Applied Biosystems) was used according to manufacturer's instructions to detect amplified sequences. Samples were run in triplicate, expression values were standardized to control values from GAPDH primers using the $\Delta\Delta C_t$ method (Livak and Schmittgen 2001). Statistical analysis was done using one way ANOVA and Turkey's post-hoc test on GraphPad.

Immunofluorescence analysis Cells were cultured on glass coverslips until 75% confluence, fixed in 4% paraformaldehyde and subjected to indirect immunofluorescence analysis with an Rabbit anti-CCN2 antibody (Abcam) and a Texas Red-conjugated Goat anti-Rabbit antibody (Jackson ImmunoResearch). Cells were stained with DAPI-containing mounting media (Molecular Probes) and images were detected using a Zeiss Axiophot and Northern Eclipse software (Empix).

Results

CCN2 mRNA and protein expression in B16(F10) murine melanoma cells is inhibited by PP2 and U0126

In order to determine which of the CCN family members were expressed in melanoma cells, B16(F10) murine melanoma cells were cultured until 50% or >75% confluence as indicated. Total RNA was harvested and real time PCR analysis was used to detect CCN1, CCN2, CCN3 and CCN4 mRNA expression in B16(F10) murine melanoma cells. We found that CCN1, CCN2, CCN3 and CCN4 mRNAs were

Fig. 1 Expression of the CCN family members in B 16(F10) murine melanoma cells. Expression of CCN1, CCN2, CCN3 and CCN4 mRNAs were detected by real-time PCR and relative expression to GAPDH was assessed as described in [Methods](#). Experiments were conducted on three independent occasions, each in triplicate. Data represent the average and standard deviations resulting from the three independent experiments. Cells at 50% and greater than 75% confluency were assessed

Fig. 2 Inhibiting MEK and FAK/src decreased CCN2 mRNA expression in B16(F10) murine melanoma cells. Cells were incubated for 24 h with inhibitors of signal transduction cascades. RNA was harvested and subjected to real time RT-PCR and was normalised with GAPDH. The experiment was performed thrice. Data represent the average and standard deviation resulting from the three independent experiments. Cells were incubated for 24 h with inhibitors of signal transduction cascades. *Columns*, mean of separate experiments performed in triplicate; *bars*, SE (* = significantly reduced CCN2 mRNA due to inhibitor, $P < 0.05$). Inhibitors used were: 10 μ M bisindolmaleimide I (PKC), 30 μ M SB203580 (SB), 10 μ M PP2, 10 μ M U0126. (a) Expression of CCN2, mRNA (b) Expression of CCN1, CCN3 and CCN4 mRNAs compared to CCN2 mRNA. Basal expression of each mRNA was taken to represent '1'

expressed in B16(F10) cells, although CCN1 mRNA appeared less abundant (Fig. 1).

To investigate the potential mechanism underlying CCN2 expression in B16(F10) murine melanoma cells, we cultured cells until 50% or until 75% confluence and treated them for 24 h with DMSO (as vehicle control) or inhibitors of p38 (30 μ M; SB 203580), MEK/ERK (10 μ M; U0126), FAK/SRC (10 μ M; PP2), and PKC (10 μ M; bisindolmaleimide I). Although inhibition of p38 and PKC did not appreciably affect CCN2 mRNA, inhibition of FAK/SRC and MEK resulted in a potent reduction of CCN2 mRNA expression (Fig. 2a). Results were confirmed using Western blot analysis of whole cell extracts (Fig. 3). Please note that, in our hands, CCN2 migrated as a dimer; this form has been observed before (e.g. Tikellis et al.

2004). Intriguingly, none of the inhibitors tested reduced CCN1, CCN3 or CCN4 expression (Fig. 2b). These results suggest that of the CCN family members examined in our

Fig. 3 Inhibiting MEK and FAK/src potently decreased CCN2 protein expression in B16(F10) murine melanoma cells. Cells were incubated for 24 h with inhibitors of signal transduction cascades. Protein was harvested and subjected to western blot analyses with anti-CCN2 and anti- β -actin antibodies (100 μ g/lane). Experiments were performed thrice, a representative blot is shown

study, only CCN2 is downstream of the highly oncogenic MEK/ERK pathway.

CCN2 is localized to the nucleus in B16(F10) murine melanoma cells

In normal dermal fibroblasts, CCN2 is a secreted protein; cell-associated CCN2 is localized to the Golgi apparatus (Chen et al. 2001). However, recently we showed that CCN2 was localized to the nuclei in human gingival fibroblasts (Thompson et al. 2010). To examine the subcellular localization of CCN2 in B16(F10) murine melanoma cells, we employed indirect immunofluorescence analysis using an anti-CCN2 antibody. Nuclei were counterstained with DAPI. To our surprise, we found that CCN2 was associated with the nucleus in B16(F10) murine melanoma cells at either 50% or >75% confluence (Fig. 4a). Moreover, these data were confirmed when Western blot analysis using an anti-CCN2 antibody was

performed on whole-cell, nuclear and cytoplasmic extracts prepared from B16 (F10) murine melanoma cells (Fig. 4b). Treatment with PP2 or U0126 (24 h, 10 μ M) reduced the appearance of nuclear CCN2. CCN2 was not detected in the conditioned media (Fig. 5). Moreover, treatment with shRNA recognizing CCN2 reduced nuclear localization of CCN2 (Fig. 6). Collectively, these data indicate that CCN2 is localized to the nucleus of B16(F10) murine melanoma cells.

Discussion

There is currently neither an effective biomarker nor a treatment for malignant melanoma; obtaining a greater understanding of the mechanisms underlying melanoma is therefore essential. Previous studies have found that CCN2 is abundant in cancers such as pancreatic cancer, and that this overexpression may be related to cancer progression

Fig. 4 CCN2 is localized to the nucleus of B 16(F10) murine melanoma cells (a) immunofluorescence analysis Cells were cultured in DMEM, 10% FBS and subjected to indirect immunofluorescence with anti-CCN2 antibody and counterstained with DAPI as described in methods. Experiments were performed thrice, a representative photograph is shown. (b) (c) **Western analysis.** Whole cell, nuclear and cytoplasmic extracts of B16(F10) cells were generated as

described in methods, and equal amounts (100 μ g/lane) of protein were subjected to western blot analyses with anti-CCN2 antibody. Conditioned media (1 ml) was concentrated on heparin sepharose as described in methods as was run in parallel. Experiments were performed thrice, a representative photograph is shown. (b) 50% confluency (c) ~75% confluency. Membranes were probed with anti-Sp1 or anti-actin antibodies to verify accuracy of separation procedure

Fig. 5 Nuclear accumulation of CCN2 in B 16(F10) murine melanoma cells is blocked by U0126 or PP2 Cells were cultured in DMEM, 10% FBS and treated overnight with DMSO, U0126 (10 μ M) or PP2 (10 μ M) and subjected to indirect immunofluorescence with

anti-CCN2 antibody and counterstained with DAPI as described in [methods](#). Experiments were performed thrice, a representative photograph is shown

(Aikawa et al. 2006; Bennewith et al. 2009; Dornhofer et al. 2006). Moreover, CCN expression has also been associated with chondrosarcoma, breast cancer, gastric cancer, ovarian cancer and oral osteolytic mandibular squamous cell carcinoma (Kondo et al. 2006; Eguchi et al. 2007; Mao et al. 2010; Sodek et al. 2009). Given these observation, CCN2 may represent a novel target for drug intervention in cancer (Chen and Lau 2009; Shi-Wen et al. 2008).

Although one prior report used in situ hybridization to show that CCN2 was elevated in tumor stroma of desmoplastic malignant melanoma compared to amelanotic malignant melanoma (Kubo et al. 1998), no prior study has examined basal CCN2 expression in melanoma cancer cells. Our data indicate that CCN2 expression was reduced by inhibition of FAK/src and MEK/ERK. It is interesting to note that CCN2 was previously shown to be downstream of MEK/ERK in PANC-1 cells (Pickles and Leask 2007). Similarly, FAK/src has a critical role in cell adhesion, invasion, proliferation, survival, and angiogenesis during tumor development and cooperates with receptor tyrosine kinases to promote MEK/ERK activation and hence there has been much interest in FAK/src inhibitors as anti-cancer agents (Namkoong et al. 2006; Flaherty et al. 2010). Thus,

it is intriguing that we found CCN2 expression in B6 (F10) cells to be reduced by PP2.

It is interesting to note that, at greater confluency, CCN mRNAs showed elevate expression. It has been suggested that expression of CCN mRNAs are reduced upon confluency (Shimo et al. 2001); it is possible that in the cancer cells used for this study, that are likely to not to show contact-inhibited growth, that in B6 (F10) cells CCN mRNA expression continued to increase as cells became progressively more confluent Intriguingly, mRNA expression of CCN1, CCN3 and CCN4, other members of the CCN family, was not reduced by PP2 or U0126. Thus CCN2 alone is regulated by the MEK/ERK pathway, one of the major pathways previously shown to be activated in human melanoma cells, a pathway highly activated in cancers and the subject of much therapeutic interest (Balmanno and Cook 2009; Kim et al. 2009). Intriguingly, evidence suggests that CCN1 may be a tumor suppressing gene in melanoma (Dobroff et al. 2009), consistent with our observation that CCN1 mRNA expression was less than that of CCN2, CCN3 and CCN4. The situation with CCN3 appears to be complicated and context-dependent (Perbal 2008), nonetheless the preponderance of data suggests that

Fig. 6 Nuclear accumulation of CCN2 in B 16(F10) murine melanoma cells is blocked by shRNA recognizing CCN2. Cells containing control shRNA or shRNA recognizing CCN2 were generated as described in [methods](#). **a** Knockdown of CCN2 was verified using real time PCR. Mela = parental cells. **b** Cells were

cultured in DMEM, 10% FBS and subjected to indirect immunofluorescence with anti-CCN2 antibody and counterstained with DAPI as described in [methods](#). Experiments were performed thrice, a representative photograph is shown

CCN3 is most commonly associated with negative growth regulation and reduced proliferation and invasion including in melanoma/melanocytes (Planque and Perbal 2003; Fukunaga-Kalabis et al. 2008; McCallum et al. 2009). In fact, CCN3 is generally considered to have effects directly

opposing CCN2 (Leask 2009; Riser et al. 2009); down-regulation of CCN3 directly mediates melanoma progression (Fukunaga-Kalabis et al. 2008). Thus, although CCN3 was found to be expressed in B6 (F10) cells but to not be regulated in the same fashion as CCN2 is not surprising.

Consistent with this notion, TGF β elevates CCN2 but suppresses CCN3 (Riser et al. 2010; Leask et al. 2003; van Beek et al. 2006). No prior study has examined CCN4 expression in melanoma; however CCN4 appears to reduce metastasis of lung cancer cells (Soon et al. 2003).

CCN proteins are normally thought of as secreted matricellular proteins (Bleau et al. 2005; Leask and Abraham 2006). However, evidence has gradually accumulated that CCN family members can also be localized to the nucleus. For example, truncated CCN3 variants are in the nucleus in several tumor cells (Perbal 1999; Planque et al. 2006; Subramaniam et al. 2008). CCN3 has also been found in the nucleus in normal skin (Rittie et al. 2011). Similarly, CCN5 can also be present in the nucleus (Wiesman et al. 2010). (CCN1) is in the nucleus of mechanically stretched bladder smooth muscle cells, but absent in the unstretched cells (Tamura et al. 2001). CCN2 can be in the nucleus of human mesangial cells (Wahab et al. 2001) and in gingival fibroblasts (Thompson et al. 2010). In the case of mesangial cells, CCN2 designated for the nucleus migrated at approximately 46 kDa as this form was highly phosphorylated (Wahab et al. 2001). In this report, we observed an approximately 80 kDa which could represent a dimer, or it could represent a highly phosphorylated or modified version of CCN2. Future efforts will be expended in differentiating between these possibilities. In any event, these data suggest that modification of CCN2 to other than its secreted 36–38 kDa form is associated with nuclear localization. Localization of all of these proteins occurs in spite of an obvious, functional ‘standard’ nuclear localization signal. Although the significance of nuclear CCNs is unclear, all these studies associate nuclear CCN proteins with potential transcriptional activity.

In summation, CCN2 is expressed in B 16 (F10) murine melanoma cells downstream of oncogenic FAK/src and MEK. CCN2 may therefore operate downstream of FAK/src and MEK to contribute to the phenotype of melanoma cells. However, the precise effect of endogenous CCN2 in melanoma is unclear, and awaits further studies.

Acknowledgements Our work is supported by grants from the Canadian Institutes of Health Research to the Canadian Foundation for Innovation. A.L. is a New Investigator of the Arthritis Society (Scleroderma Society of Ontario) and a recipient of an Early Researcher Award. WS is the recipient of a postdoctoral fellowship from the Canadian Scleroderma Research Group.

References

Aikawa T, Gunn J, Spong SM, Klaus SJ, Korc M (2006) Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. *Mol Cancer Ther* 5:1108–1116

- Balmano K, Cook SJ (2009) Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ* 16(3):368–377, Epub 2008 Oct 10
- Bennewith KL, Huang X, Ham CM, Graves EE, Erler JT, Kambham N, Feazell J, Yang GP, Koong A, Giaccia AJ (2009) The role of tumor cell-derived connective tissue growth factor (CTGF/CCN2) in pancreatic tumor growth. *Cancer Res* 69(3):775–784
- Bleau AM, Planque N, Perbal B (2005) CCN proteins and cancer: two to tango. *Front Biosci* 10:998–1009
- Chen CC, Lau LF (2009) Functions and mechanisms of action of CCN matricellular proteins. *Int J Biochem Cell Biol* 41(4):771–783
- Chen Y, Segarini P, Raoufi F, Bradham D, Leask A (2001) Connective tissue growth factor is secreted through the Golgi and is degraded in the endosome. *Exp Cell Res* 271(1):109–117
- Chin L, Garraway LA, Fisher DE (2006) Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* 20:2149–2182
- Dobroff AS, Wang H, Melnikova VO, Villares GJ, Zigler M, Huang L, Bar-Eli M (2009) Silencing cAMP-response element-binding protein (CREB) identifies CYR61 as a tumor suppressor gene in melanoma. *J Biol Chem* 284(38):26194–26206
- Dornhofer N, Spong S, Bennewith K, Salim A, Klaus S, Kambham N, Wong C, Kaper F, Sutphin P, Nacamuli R, Hockel M, Le Q, Longaker M, Yang G, Koong A, Giaccia A (2006) Connective tissue growth factor-specific monoclonal antibody therapy inhibits pancreatic tumor growth and metastasis. *Cancer Res* 66:5816–5827
- Eguchi T, Kubota S, Kawata K, Mukudai Y, Ohgawara T, Miyazono K, Nakao K, Kondo S, Takigawa M (2007) Different transcriptional strategies for ccn2/ctgf gene induction between human chondrocytic and breast cancer cell lines. *Biochimie* 89(3):278–288, Epub 2007 Jan 3
- Flaherty KT, Hodi FS, Bastian BC (2010) Mutation-driven drug development in melanoma. *Curr Opin Oncol* 22(3):178–183
- Fukunaga-Kalabis M, Martinez G, Telson SM, Liu ZJ, Balint K, Juhasz I, Elder DE, Perbal B, Herlyn M (2008) Downregulation of CCN3 expression as a potential mechanism for melanoma progression. *Oncogene* 27(18):2552–2560
- Hartel M, Di Mola FF, Gardini A, Zimmermann A, Di Sebastiano P, Guweidhi A, Innocenti P, Giese T, Giese N, Büchler MW, Friess H (2004) Desmoplastic reaction influences pancreatic cancer growth behavior. *World J Surg* 28(8):818–825, Epub 2004 Aug 3
- Kim LC, Song L, Haura EB (2009) Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol* 6(10):587–595
- Kondo S, Kubota S, Mukudai Y, Moritani N, Nishida T, Matsushita H, Matsumoto S, Sugahara T, Takigawa M (2006) Hypoxic regulation of stability of connective tissue growth factor/CCN2 mRNA by 3'-untranslated region interacting with a cellular protein in human chondrosarcoma cells. *Oncogene* 25(7):1099–1110
- Kubo M, Kikuchi K, Nashiro K, Kakinuma T, Hayashi N, Nanko H, Tamaki K (1998) Expression of fibrogenic cytokines in desmoplastic malignant melanoma. *Br J Dermatol* 139(2):192–197
- Leask A (2009) Yin and Yang: CCN3 inhibits the pro-fibrotic effects of CCN2. *J Cell Commun Signal* 3(2):161–162, Epub 2009 May 29
- Leask A, Abraham DJ (2006) All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci* 119:4803–4810
- Leask A, Holmes A, Black CM, Abraham DJ (2003) Connective tissue growth factor gene regulation. Requirements for its induction by transforming growth factor-beta 2 in fibroblasts. *J Biol Chem* 278:13008–13015
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-(Delta Delta C_T)} Method. *Methods* 25(4):402–408

- Mao Z, Ma X, Rong Y, Cui L, Wang X, Wu W, Zhang J, Jin D (2010) Connective tissue growth factor enhances the migration of gastric cancer through downregulation of E-cadherin via the NF- κ B pathway. *Cancer Sci*. doi:10.1111/j.1349-7006.2010.01746.x
- McCallum L, Lu W, Price S, Lazar N, Perbal B, Irvine AE (2009) CCN3: a key growth regulator in chronic myeloid leukaemia. *J Cell Commun Signal* 3(2):115–124
- Namkoong J, Martino JJ, Chen S (2006) From existing therapies to novel targets: a current view on melanoma. *Front Biosci* 11:2081–2092
- Perbal B (1999) Nuclear localisation of NOVH protein: a potential role for NOV in the regulation of gene expression. *Mol Pathol* 52(2):84–91
- Perbal B (2001) NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. *Mol Pathol* 54:57–79
- Perbal B (2008) CCN3: doctor Jekyll and Mister Hyde. *J Cell Commun Signal* 2(1–2):3–7
- Pickles M, Leask A (2007) Analysis of CCN2 promoter activity in PANC-1 cells: regulation by ras/MEK/ERK. *J Cell Commun Signal* 1(2):85–90
- Planque N, Perbal B (2003) A structural approach to the role of CCN (CYR61/CTGF/NOV) proteins in tumourigenesis. *Cancer Cell Int* 3(1):15
- Planque N, Long Li C, Saule S, Bleau AM, Perbal B (2006) Nuclear addressing provides a clue for the transforming activity of amino-truncated CCN3 proteins. *J Cell Biochem* 99(1):105–116
- Riser BL, Najmabadi F, Perbal B, Peterson DR, Rambow JA, Riser ML, Sukowski E, Yeger H, Riser SC (2009) CCN3 (NOV) is a negative regulator of CCN2 (CTGF) and a novel endogenous inhibitor of the fibrotic pathway in an in vitro model of renal disease. *Am J Pathol* 174(5):1725–1734
- Riser BL, Najmabadi F, Perbal B, Rambow JA, Riser ML, Sukowski E, Yeger H, Riser SC, Peterson DR (2010) CCN3/CCN2 regulation and the fibrosis of diabetic renal disease. *J Cell Commun Signal* 4(1):39–50
- Rittie L, Perbal B, Castellot JJ, Orringer JS, Voorhees JJ, Fisher GJ (2011) Spatial-temporal modulation of CCN proteins during wound healing in human skin in vivo. *J Cell Commun Signal* 5(1):69–80
- Shimo T, Nakanishi T, Nishida T, Asano M, Sasaki A, Kanyama M, Kuboki T, Matsumura T, Takigawa M (2001) Involvement of CTGF, a hypertrophic chondrocyte-specific gene product, in tumor angiogenesis. *Oncology* 61(4):315–322
- Shi-Wen X, Leask A, Abraham D (2008) Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. *Cytokine Growth Factor Rev* 19(2):133–144
- Sodek KL, Ringuette MJ, Brown TJ (2009) Compact spheroid formation by ovarian cancer cells is associated with contractile behavior and an invasive phenotype. *Int J Cancer* 124(9):2060–2070
- Soon LL, Yie TA, Shvarts A, Levine AJ, Su F, Tchou-Wong KM (2003) Overexpression of WISP-1 down-regulated motility and invasion of lung cancer cells through inhibition of Rac activation. *J Biol Chem* 278(13):11465–11470
- Subramaniam MM, Lazar N, Navarro S, Perbal B, Llombart-Bosch A (2008) Expression of CCN3 protein in human Wilms' tumors: immunohistochemical detection of CCN3 variants using domain-specific antibodies. *Virchows Arch* 452(1):33–39
- Tamura I, Rosenbloom J, Macarak E, Chaqour B (2001) Regulation of Cyr61 gene expression by mechanical stretch through multiple signaling pathways. *Am J Physiol Cell Physiol* 281(5):C1524–C1532
- Thompson K, Hamilton DW, Leask A (2010) ALK5 inhibition blocks TGF β -induced CCN2 expression in gingival fibroblasts. *J Dent Res* Oct 5. [Epub ahead of print]
- Tikellis C, Cooper ME, Twigg SM, Burns WC, Tolcos M (2004) Connective tissue growth factor is up-regulated in the diabetic retina: amelioration by angiotensin-converting enzyme inhibition. *Endocrinology* 145(2):860–866, Epub 2003 Oct 30
- Van Beek JP, Kennedy L, Rockel JS, Bernier SM, Leask A (2006) The induction of CCN2 by TGF β 1 involves Ets-1. *Arthritis Res Ther* 8:R36
- Wahab NA, Brinkman H, Mason RM (2001) Uptake and intracellular transport of the connective tissue growth factor: a potential mode of action. *Biochem J* 359(Pt 1):89–97
- Wenger C, Ellenrieder V, Alber B, Lacher U, Menke A, Hameister H, Wilda M, Iwamura T, Beger HG, Adler G, Gress TM (1999) Expression and differential regulation of connective tissue growth factor in pancreatic cancer cells. *Oncogene* 18(4):1073–1080
- Wiesman KC, Wei L, Baughman C, Russo J, Gray MR, Castellot JJ (2010) CCN5, a secreted protein, localizes to the nucleus. *J Cell Commun Signal* 4(2):91–98, Epub 2010 Mar 18