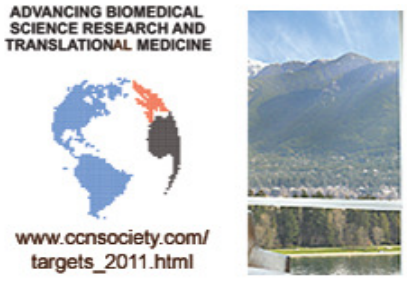

FINAL PROGRAM

Novel targets for cancer and connective tissues diseases

A meeting sponsored by the International CCN Society

Coast Coal Harbour Hotel, Vancouver, BC, Canada

September 24-27, 2011

ORGANIZERS:

- Andrew Leask (London, CANADA)
- Bernard Perbal (Paris, FRANCE)
- Annick Perbal (Paris, FRANCE)
- David Abraham (London, UK)

LOCAL ORGANIZER:

Wun-Chey Sin (Vancouver, CANADA)

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SATURDAY 24 SEPTEMBER 2011

ARRIVAL AND REGISTRATION

4:00-6:00 PM Registration
COAST COAL HARBOUR HOTEL
1180 WEST HASTINGS ST 1-604-697-0202

7:00-? PM Welcome buffet dinner
STEAMWORKS BREWPUB
375 Water Street across from Waterfront Station (at the foot of historical Gastown) 1-604-689-2739
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SUNDAY 25 SEPTEMBER 2011

8:30 AM Plenary talk
Bernard Perbal (Paris, France) The CCN family: Structural and functional issues

9:00 AM Session 1: Tissue remodeling and fibrosis
SPONSORED BY THE CANADIAN INSTITUTES OF HEALTH RESEARCH
(SILVER SPONSOR)
Chairs – Lester Lau and Masaharu Takigawa

9:00-9:30 David Brigstock (Columbus, USA) Targeting CCN2 in liver fibrosis
9:30-10:00 Stephen Twigg (Sydney, Australia) CCN2 is Implicated as a Causal factor in a Model Which Shows Diabetes Is a Progression Factor and Induces Cirrhosis in Non-alcoholic Steatohepatitis
10:00-10:30 coffee/tea
10:30-11:00 Chris Overall (Vancouver, Canada) Quantitative proteomics uncovers new roles for MMP processing of all CCN family members: a new therapeutic approach to fibrosis
11:00-11:30 Karen Lyons (Los Angeles, USA) Role of CCN2 in chondrocytes
11:30-12:00 Enrique Brandan (Santiago, Chile) Essential Role of CCN2 in the Development of Fibrosis and Deleterious Effect on Muscular Strength in Skeletal Muscle Dystrophy

12:00-1:30 Lunch

1:30 PM	Session 2: Young Investigators
Chairs –	Andrew Leask and David Abraham
1:30-1:50	Alyssa Charrier (Columbus, USA) Immune responses in alcohol-induced pancreatic injury are associated with CCN2 expression and action in acinar cells
1:50-2:10	Danilo Janune (Okayama, Japan) Novel effects of CCN3 that may direct the differentiation of chondrocytes
2:10-2:30	Naoki Terada (Baltimore, USA) CCN1 Is Regulated by cAMP-dependent Protein Kinase with Serum Levels Correlating with Prostate Cancer Aggressiveness
2:30-2:50	Takashi Nishida (Okayama, Japan) Effect of CCN2 on FGF2-induced proliferation of and MMP9 and 13 production by chondrocytes
2:50-3:10	Chris Elliott (London, Canada) Periostin modulates myofibroblast differentiation during full thickness cutaneous wound repair.
3:10-3:30	Rohan Samarakoon (Albany, USA) Comparative Analysis of CCN2 and PAI-1 (SERPINE1) Induction in Response to Profibrotic Growth Factors (e.g., TGF-β1, EGF) and Microtubules
3:30-3:45	Alex Lambi (Philadelphia, USA) CCN2 is Required for Normal Intramembranous and Endochondral Ossification
3:45-4:00	Discussion
4:00-5:30	POSTER SESSION/AFTERNOON REFRESHMENTS
5:30 PM	Session 3: Integrins and adhesion
Chairs –	Ruth Lupu and Sushanta Banerjee
5:30-6:00	Brahim Chaqour (Brooklyn, USA) Normalization of Angiogenesis and Vasculogenesis by CCN1: Implications in Occular Neovascular Diseases
6:00-6:30	Lester Lau (Chicago, USA) Cellular Senescence in Wound healing and Fibrosis
6:30-7:00	Hannu Larjava (Vancouver, Canada) The role of integrins in gingival wound healing
7:00-7:30	Shoukat Dedhar (Vancouver, Canada) The role of integrin-linked kinase in cancer
7:30 PM	free evening

MONDAY 26 SEPTEMBER 2011

8:30AM	Session 4: Stem Cells, Cancer and Microenvironment
Chairs –	David Brigstock and Roel Goldschmeding
8:30-9:00	Lynne Postovit (London, Canada) The role of the ECM microenvironment and cancer
9:00-9:30	Andrei Thomas-Tikhonenko (Philadelphia, USA) A microRNA component of the p53-thrombospondin axis
9:30-10:00	Ruth Lupu (Rochester, NY) The role of CCN1 in cancer
10:00-10:30	coffee/tea
10:30-11:00	Sushanta Banerjee (Kansas City, USA) The role of CCN5 in cancer
11:00-11:30	Chiayeng Wang (Chicago, IL) Regulation of CCN3 by PAX3-FKHR oncogenic transcription factor
11:30-1:30	lunch
1:30-4:30	Takaya tour (optional) http://www.takayatours.com/
8:30-	Dinner/Banquet JOE FORTES RESTAURANT 777 Thurlow Street 1-604-669-1940 SPONSORED BY THE INTERNATIONAL CCN SOCIETY (SILVER SPONSOR)

TUESDAY 27 SEPTEMBER 2011

8:30AM	Session 5: Clinical and Translational aspects SPONSORED BY ACTELION (SILVER SPONSOR)
Chairs –	Enrique Brandan and Stephen Twigg
8:30-9:00	Cornelis JF Van Noorden (Amsterdam, Netherlands) Increased CCN2/VEGF ratio drives the angio-fibrotic switch in diabetic retinopathy
9:00-9:30	Michael Underhill (Vancouver, Canada) The role of retinoids in controlling chondrogenesis
9:30-10:00	Chris Denton (London, UK) Novel drug targets in scleroderma
10:00-10:30	Coffee/tea
10:30-11:00	Richard Stratton (London, UK) Epidermal/dermal crosstalk in scleroderma
11:00-11:30	Satoshi Kubota (Okayama, Japan) Role of CCN2 in the remodeling of periodontal tissue upon nicotine exposure
11:30-12:00	Roel Goldschmeding (Utrecht, Netherlands) CCN2 in diabetes

MEETING CLOSES

YOUNG INVESTIGATOR ABSTRACTS

1:30-1:50

Immune responses in alcohol-induced pancreatic injury are associated with CCN2 expression and action in acinar cells

Alyssa Charrier and David Brigstock The Ohio State University and Nationwide Children's Hospital Research Institute, Columbus OH

Introduction Alcoholic chronic pancreatitis (ACP) is characterized by pancreatic necrosis, inflammation, and scarring, the latter of which is due to excessive collagen deposition by activated pancreatic stellate cells (PSC). We have have previously shown that in a novel model of ACP in mice (a species that is usually resistant to the toxic effects of alcohol), PSC are principally responsible for the production of connective tissue growth factor (CCN2) which is correlated spatially and temporally with sites of collagen production (Charrier & Brigstock; Lab Invest, 2010). This is consistent with our earlier in vitro data showing that PSC produce collagen in response to ethanol via the production of CCN2 (Lawrencia et al; Growth Factors, 2009). During the course of the in vivo studies, we observed that, prior to the fibrotic phase (>Day 20), the combination of ethanol and cerulein caused a transient increase in CCN2 production in acinar cells, even though these cells are not collagenic. Thus we adopted in vivo and in vitro approaches to investigate this finding further

Methods C57/Bl 6 mice received ethanol injections for 6-16 days against a background of cerulein-induced acute pancreatitis. Pancreata from mice that were treated with cerulein plus 33% ethanol were examined at Days 6, 9, 13, and 16 for the presence of CCN2 and its relationship to inflammatory responses. Additionally, the rat acinar cell line, AR42J, was grown in culture and treated with either ethanol or CCN2 siRNA prior to determination of interluekin-1B (IL-1β) expression by real-time PCR.

Results In the in vivo model, there was strong immunofluorescent signal for CCN2 in acinar cells by Day 16 of treatment with ethanol + cerulein and this was not co-localized to α-SMA-postive smooth muscle cells of the vasulature. This pattern of CCN2 staining was not apparent in pancreata of mice receiving water + 33% ethanol. F4/80 immunofluorescence showed some macrophage infiltration of pancreatic tissue at days 6 and 9, but this was substantially increased on Day 16 and thus correlated with CCN2 production by acinar cells. Immunofluorescence staining further demonstrated that the increase in acinar CCN2 by ethanol + cerulein was spatially and temporally correlated with than of IL-1β. In vitro studies showed that after treatment of rat AR42J cells with 6.25-100mM ethanol for 24 or 48 hours, the pattern of CCN2 mRNA induction was correlated with that of IL-1β expression. A functional link between CCN2 and IL-1β was shown by the reduction of Ll-1β expression in rat AR42J cells after knockdown of CCN2 mRNA using CCN2 siRNA.

Conclusions Unrelated to its pro-fibrotic role in PSC, CCN2 is robustly expressed in mouse acinar cells during chronic pancreatic injury, the timing corresponds to increased infiltration of activated macrophages as well as of acinar Ll-1β expression. Cultured rat acinar cells demonstrate co-expresison of CCN2 and IL-1β in response to ethanol and an expression of IL-1β that is CCN2-dependent. Taken together, these data support a role for acinar cell-derived CTGF as a modulator of the immune response. We conclude that CCN2 regulates components of the immune response during pancreatic injury prior to exerting its fibrotic role.

1:50-2:10

Novel effects of CCN3 that may direct the differentiation of chondrocytes

Danilo Janune,†‡ Satoshi Kubota,† Takashi Nishida,† Harumi Kawaki,† Bernard Perbal,†† Seiji Iida,‡ and Masaharu Takigawa†
†Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
‡Department of Oral and Maxillofacial Reconstructive Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.
††Laboratoire d'Oncologie Virale et Moléculaire, UFR de Biochimie, Université Paris 7, Paris, France

Background and Hypothesis: CCN3 was suggested to be involved in early chondrogenic differentiation. Later on in the developmental process, chondrocytes follow two distinct pathways: permanent chondrocytes, which will slow down proliferation and produce abundant extracellular matrix and transient chondrocytes, which will enter the organized process of endochondral ossification and give rise to bone. The local molecules responsible for affecting the chondrocyte phenotype are still a field of intense debate. The cells in femoral distal epiphyses of five days old rats are uniform without distinct subpopulations toward ossification and articular cartilage formation. This finding brings a question; what are the effector molecules acting *in loco* telling the cells to enter endochondral ossification pathway and the others to remain as cartilage? This study was conducted to find a clue to answer to this question. CCN3, which seems to be involved in the cartilage development since its beginning, does not have its role in the epiphyseal cartilage unraveled. We posited, then, that CCN3 might play a critical role in the later events of differentiation.

Design: Localization of CCN3 in distal femoral epiphysis before secondary ossification was analyzed by immuhistochemistry and semi-quantified by immunoblotting. Effect of exogenous CCN3 on epiphyseal cells collected at this developmental stage was analyzed *in vitro* regarding changes in chondrocytic marker gene expression, proteoglycan synthesis and cell proliferation. To further confirm the effects of CCN3 loss-of-function in a CCN3 downregulation scenario was evaluated by knocking CCN3 down with an shRNA in the prechondrocytic cell line ATDC5.

Results: CCN3 is expressed throughout the epiphyseal head of rats before secondary ossification. Exogenous CCN3 increased the mRNA expression of tenascin-C and aggrecan, while repressing type X collagen mRNA expression in primary cells from epiphyseal heads. In parallel to the effects on matrix metabolism, exogenous CCN3 repressed cell proliferation. The knock-down experiment showed an expected loss-of-function regarding matrix metabolism in the ATDC5 cells, with increased type X collagen mRNA expression and decreased tenascin-C mRNA expression.

Conclusion: Our results indicate a pivotal role of CCN3 in directing the pathway of differentiation of epiphyseal chondrocytes.

2:10-2:30

CCN1 Is Regulated by cAMP-dependent Protein Kinase with Serum Levels Correlating with Prostate Cancer Aggressiveness

Naoki Terada¶, Takumi Shiraishi¶, Yu Zeng¶, Steven M. Mooney¶, David B. Yeater¶, Leslie A. Mangold¶, Alan W. Partin¶, Prakash Kulkarni¶* & Robert H. Getzenberg¶*±

¶Departments of Urology, *Oncology, ±Pharmacology and Molecular Sciences, and the James Buchanan Brady Urological Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21287

Purpose:

Cysteine-rich angiogenic inducer 61 (Cyr61, CCN1) is an extracellular matrix protein involved in the transduction of growth factor and hormone signaling. Previously we demonstrated that Cyr61 was highly expressed in prostate cancer (PCa) but that the expression levels were associated with a lower risk of PCa recurrence. In the present study, we demonstrate that serum Cyr61 is a potential biomarker that correlates with PCa aggressiveness. Furthermore, we also explore the potential mechanism underlying the changes in Cyr61 expression during PCa progression.

Materials and Methods:

Cyr61 concentrations in the medium from PCa cell lines and in serum samples obtained from PCa patients were measured by sandwich ELISA. Serum Cyr61 levels were correlated with disease characteristics and the association between Cyr61 expression changes by several types of stimulation or stress and cAMP/cAMP-dependent protein kinase (PKA) pathway were examined.

Results:

There was a positive correlation between Cyr61 levels in cell supernatants and mRNA expression in these cell lines. Serum Cyr61 levels were significantly higher in non-organ-confined PCa patients (116.3±140.2ng/ml) than in organ-confined PCa patients (79.7±56.1ng/ml) (p=0.031). Cyr61 expression was up-regulated in response to both lysophosphatidic acid and androgen treatments which promoted PCa cell invasion. Serum starvation and phosphoinositide-3-kinase inhibition also resulted in Cyr61 up-regulation however they suppressed cell proliferation. Cyr61 up-regulation was correlated with an increase in cAMP and suppressed by PKA inhibition.

Conclusions:

These findings suggest that Cyr61 expression in PCa is regulated by the cAMP/PKA pathway and that circulating Cyr61 levels are a potential serum-based biomarker for characterizing PCa.

2:30-2:50

Effect of CCN2 on FGF2-induced proliferation of and MMP9 and 13 productions by chondrocytes

Takashi Nishida,[1] Satoshi Kubota,[1] Eriko Aoyama,[2] Danilo Janune,[1] Azusa Maeda,[1] and Masaharu Takigawa[1, 2]
[1]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan;
[2]Biodental Research Center, Okayama University Dental School, Okayama, Japan

CCN2 (also known as connective tissue growth factor: CTGF) interacts with several growth factors involved in endochondral ossification via its characteristic 4 modules and modifies the effect of such growth factors. Presently we investigated whether or not CCN2 interacts with fibroblast growth factor 2 (FGF2). Solid-phase binding assay, immunoprecipitation-Western blot analysis, and surface plasmon resonance (SPR) spectroscopy revealed that the C-terminal module of CCN2 (CT) directly bound to FGF2 with a dissociation constant (Kd) of 5.5 nM. Next, we examined the combinational effects of CCN2 and FGF2 on the proliferation of and matrix metalloproteinase (MMP) 9 and 13 productions by cultured chondrocytes. FGF2 promoted not only the proliferation but also the production of MMP9 and 13, however, combined of FGF2 with CT module nullified the enhancement of both MMPs productions and proliferation. To clarify the mechanism, we investigated the binding of CCN2 or its CT module to FGF receptor 1 (FGFR1). As a result, we found that CCN2 bound to FGFR1 with a Kd of 362 nM, whereas the CT module did not. In addition, when we tested FGF signaling in HCS-2/8 cells stimulated by the combination of FGF2 with CT module, the level of ERK1/2, p38 MAPK, and JNK phosphorylation was decreased compared with that found with FGF2 alone. These findings suggest that CCN2 may regulate the proliferation and matrix degradation of chondrocytes by forming a complex with FGF2 as a novel modulator of FGF2 functions.

2:50-3:10

Periostin modulates myofibroblast differentiation during full thickness cutaneous wound repair.

Christopher G. Elliott[1], Jian Wang[2], Shi-wen Xu[3], Mark Eastwood[4], Jianjun Guan[5], Andrew Leask[6], Simon J. Conway[2] and Douglas W. Hamilton[1,6]

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[2]Riley Heart Research Center, Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 West Walnut, Indianapolis, IN.
[3]Centre for Rheumatology, Royal Free and University College Medical School, London, United Kingdom
[4]School of Life Sciences, University of Westminster, London, United Kingdom
[5]Department of Materials Science and Engineering, The Ohio State University, 2041 College Road, Columbus, Ohio.

The various influences of matricellular proteins on wound repair provide a multitude of avenues for exploration of potential targets for fibrosis and chronic wounds. Despite mounting evidence for the importance of matricellular proteins in wound contraction and re-epithelialization, no clinical trials are currently employing these proteins for the resolution of chronic wounds. A more thorough examination of the role of matricellular proteins in dermal wound repair is needed in order to determine their suitability as therapeutics. The matricellular protein periostin is expressed in the skin. Although periostin has been hypothesized to contribute to dermal homeostasis and repair, this hypothesis has not been directly tested. To assess the contribution of periostin to dermal healing, 6 mm full thickness excisional wounds were created in the skin of periostin knockout and wild-type/sex-matched control mice. In wild-type mice, periostin was potently induced 5-7 days post-wounding. In the absence of periostin, day 7 wounds showed reduced numbers of myofibroblasts, as visualized by α-smooth muscle actin (α-SMA) expression within the granulation tissue. Isolated wild-type and knockout dermal fibroblasts did not differ in in vitro assays of adhesion or migration; however, in 3D culture, periostin knockout fibroblasts showed significantly reduced ability to contract a collagen matrix, and adopted a dendritic phenotype. Recombinant periostin, in a fashion which was sensitive to a neutralizing anti-β1-integrin and to the FAK/src inhibitor PP2, restored the defects in cell morphology and matrix contraction displayed by periostin-deficient fibroblasts. We propose that periostin promotes wound contraction by facilitating myofibroblast differentiation and contraction.

3:10-3:30

Comparative Analysis of CCN2 and PAI-1 (SERPINE1) Induction in Response to Profibrotic Growth Factors (e.g., TGF-β1, EGF) and Microtubule Alterations

Rohan Samarakoon and Paul J. Higgins
Albany Medical Center 43 New Scotland Avenue, Albany, NY 12208

Profibrotic matricellular proteins CTGF (CCN2) and PAI-1 are highly-induced during the progression of renal fibrosis and cardiovascular disorders. Since PAI-1 and CTGF are prominent downstream targets of TGF-β1, a major profibrogenic cytokine growth factor, a comparative analysis of signaling cascades (e.g., SMAD and non-SMAD) involved in PAI-1 and CTGF induction in vascular smooth muscle cultures and fibroblasts was undertaken. TGF-β1 stimulation of CTGF and PAI-1 expression required SMAD3 activation as RNA interference and pharmacological inhibition of SMAD3 virtually eliminated these responses. TGF-β1 mediated activation of Src-FAK-EGFR signaling cascades were critical for activation of both CTGF and PAI-1 genes as induction was largely absent in EGFR, Src and FAK-deficient mouse embryo fibroblasts. Moreover, pharmacological blockade of EGFR signaling by AG1478 as well as DN-EGFR expression effectively suppressed PAI-1 and CTGF induction by TGF-β1 in VSMCs. Surprisingly, EGF-stimulated PAI-1 and CCN2 expression also required SMAD3 signaling further highlighting cross talk between receptor tyrosine kinase (e.g, EGFR) and serine threonine kinase (e.g., TGF-β1 receptor) cascades in gene induction. Interestingly, cell shape alterations by disruption of microtubule cytoskeleton also activated EGFR and Rho-A and SMAD signaling and EGFR and Rho-A activation is critical for both CCN2 and PAI-1 expression while SMAD activation is dispensable for CCN2 induction but not PAI-1. These findings highlight a complex network of both common and unique signaling control elements that can be of potential translational value in the simultaneous targeting of CTGF and PAI-1, major causative factors of fibrotic and vascular pathologies (Supported by NIH Grant GM057242).

3:30-3:45

CCN2 is Required for Normal Intramembranous and Endochondral Ossification

[1]Lambi, A G; [3]Pankratz, T L; [1]Hendesi, H; [1]Razmpour, R;[1]Pixley R A; [1]Barbe, M F; [3]Richtsmeier, J T; [1,2]Safadi F S [1,2,*]Popoff, S N.[1]Department of Anatomy and Cell Biology, [2]Department of Orthopaedic Surgery, Temple University School of Medicine, Philadelphia, PA, [3]Department of Anthropology, Pennsylvania State University, University Park, PA

Connective tissue growth factor (CTGF, CCN2) has emerged as an important growth factor for skeletogenesis. Its importance in skeletogenesis is seen in global CTGF knockout (KO) mice, which demonstrate defects in growth plate chondrogenesis and skeletal defects including kinked ribs, tibiae, radii and ulnae, and craniofacial abnormalities. Ultimately, global ablation of CTGF results in neonatal lethality from respiratory failure. This study addressed the effects of global CTGF ablation on skeletogenesis using quantitative techniques, such as micro-CT. Furthermore, to discern any differences in the separate processes of ossification affected by CTGF ablation, specific bone sites and cells were studied. To address our goals, we used: (a) micro-CT analysis of long bones and skulls to assess phenotypic differences, (b) histomorphometric analyses to assess parameters of endochondral bone formation, and (c) ex vivo studies to assess bone cell function. Analysis of distal femoral and proximal tibial metaphyses demonstrated a decrease in trabecular bone volume in CTGF KO mice compared to WT littermates. In CTGF-null tibial diaphyses (the region of the structural kink), total tissue volume, bone volume and bone perimeter were markedly increased, compared to WT tibiae. CTGF-null skulls demonstrated decreases in cranial bone mineralization (being absent in some sites), increases in suture and fontanel size, and a clear failure of midline convergence of the maxillary and palatine processes to form the palate. This was also accompanied by asymmetry of the vomer, a consistent feature found in CTGF-null skulls. Histomorphometric analyses of WT and CTGF-null tibiae showed differences in various parameters of endochondral bone formation, including decreased osteoblast cell numbers immediately below the growth plate, decreased bone matrix formation, and increased hypertrophic zone thickness. We also demonstrated a unique dichotomy of bone formation in the kinked tibiae, where ossification at the kink appears to form by a mechanism distinct from the region under the growth plate. Lastly, ex vivo studies from isolated primary osteoblasts revealed a decreased ability of CTGF-null osteoblasts to adhere to bone matricellular proteins, such as fibronectin, and decreased ability to proliferate. This study is crucial in elucidating the role of CTGF in bone development, because it has for the first time addressed the effects of CTGF ablation on bone development using quantitative techniques, such as micro-CT and histomorphometric analyses. Using such techniques, we have demonstrated quantitative defects in both endochondral and intramembranous ossification processes by studying long bone trabeculae and skull flat bones, respectively. Our *ex vivo* studies using CTGF-null primary osteoblasts showed defects involving differentiation and function. These studies provide new information on the bone phenotype observed in CTGF KO mice, and thereby help define the importance of CTGF for normal bone development.

POSTER SESSION ABSTRACTS

1. CCN2 is a key mediator of Idiopathic pulmonary fibrosis (IPF)

Shi-Wen X[1], Renzoni EA[2], Eastwood M[3], Stratton R[1], Denton CP[1], Wells AU[2], Leask A[4] and Abraham DJ[1]
[1]Centre for Rheumatology, UCL Medical School (Royal Free Campus), London, UK.
[2]Interstitial Lung Disease Unit, NHLI, Royal Brompton Hospital/Imperial College, London, UK;
[3]School of Biosciences, University of Westminster, London, UK.
[4]School of Dentistry, University of Western Ontario, London, Ontario, Canada.

Background: Idiopathic pulmonary fibrosis (IPF) is a devastating disease, with progressive scarring of the lungs, poorly responsive to current treatment options. Lung cancer is frequently associated with IPF. Connective tissue growth factor (CTGF/CCN2) is a cysteine-rich secreted matricellular protein involved in wound healing and tissue repair. Enhanced and prolonged expression of CCN2 has been associated with tissue fibrosis. Several lines of evidence implicate the angiotensin system in the pathogenesis of lung fibrosis. This study was undertaken to investigate whether CCN2 effects are mediated through activation of angiotensin system signaling transduction pathways.

Methods: Lung fibroblasts isolated from IPF and from control lungs (6 each) were used between passage 2-5. IPF and control fibroblast treated with or without Angiotensin II (Ang II; 100 nM) expression of CCN2, type I collagen (Col I), PTEN, angiotensin receptors, and alpha-smooth muscle actin (alpha-SMA) were examined using immunofluorescence staining and Western blot. The ability of Ang II to enhance fibroblast contraction in a collagen gel contraction and migratory ability were also examined. Ang II and CCN2 levels in fibroblast supernatants were examed by Elisa. Signalling pathways were evaluated using selective pharmacological inhibitors, including PI3-kinase/AKT inhibitors wortmanin (0.1 μM) and LY294002 (20 μM).

Results: IPF fibroblast and control cells treated with AngII were expressed significantly higher CCN2 ($p<0.05$) and other matrix proteins and lower PTEN ($p<0.05$) than in control cells. IPF fibroblasts secrete increased CCN2 and Ang II levels compared to control fibroblasts. AngII significantly enhanced the ability of control lung fibroblasts to contract a collagen gel matrix by inducing the expression of CCN2, alpha-SMA and by increasing lung fibroblast migratory capability; these features were significantly inhibited by AT1 specific antagonist Losartan ($p<0.05$). IPF fibroblasts displayed higher levels of CCN2, alpha-SMA and increased collagen gel contractility compared to controls; these characteristics were significantly reversed by treatment with Losartan, as well as by PI3kinase/AKT inhibitors, compared to controls,

Conclusions: CCN2 is key mediator in IPF. Treatment of IPF lung fibroblasts with either Losartan or PI3 kinase/AKT inhibitors reduces IPF fibroblasts contractility and matrix to levels seen in control lung fibroblasts, with potential clinical implications for the treatment of fibrotic lung diseases.

2. Characterization of the effect of CCN2 modules independently and in different combinations on chondrocytic cells

Tarek Abd El Kader[1,2], Satoshi Kubota[1], Takashi Nishida[1], Takako Hattori[1], Eriko Aoyama[3], Danilo Janune[1], Takuo Kuboki[2], Masaharu Takigawa[1,3]
[1]Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, [2]Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,[3]Biodental Research Center, Okayama University Dental School, Okayama Japan.

Objective
CCN family proteins play important roles in differentiation and regeneration of cartilaginous tissues. CCN2/CTGF is known to promote the regeneration of articular cartilage by promoting cell growth and extracellular matrix production. This protein comprises 4 highly interactive independent modules, each of which was suggested to have its own biological activity. Based on these findings, it is suspected that different combinations of these modules may exert unexpected biological effects through mutual molecular interaction. This study aims to assess the effects (resulting from these different combinations) on chondrocytic cells especially in relation to full length CCN2, as well as the direct interaction between these modules.
Methods
Four independent modular proteins of human CCN2 were produced and purified through recombinant protein production system with *Brevibacillus choshinensis*. Physical interaction of 2 modules was kinetically examined by a surface plasmon resonance (SPR) methodology. Effect of a single module, or a combination of independent modules was evaluated with human chondrocytic HCS-2/8 cells. Chondrocytic phenotype was estimated by the gene expression of aggrecan, type II collagen, and matrix metalloproteinase 13 via real time RT-PCR analysis. Proteoglycan synthesis and proliferation of chondrocytes were evaluated by [^{35}S]-sulfate incorporation assay and [^{3}H]-thymidine incorporation assay, respectively.
Results
Functional analysis with HCS-2/8 cells revealed an activity comparable to the full length CCN2 in a particular module, which was rather diminished by the combination with other modules. Interestingly, mixed application of all of the 4 modules almost reconstructed the bioactivity to the level of the full length CCN2 as evaluated by gene expression and biochemical analyses. SPR analysis uncovered significant interaction via particular modules of CCN2, whereas the other modules showed no interaction even with full-length CCN2.
Conclusion
These results favor the theory of independent bioactivity of each of the modules and raises questions about what mediates the interaction between the modules to construct a possible I-V-T-C complex. It also indicates the complexity of inter-modular interaction in regulating the bioactivity of CCN2

3. **Mechanical tension increases CCN2 expression and proliferation in gingival fibroblasts via a TGFβ–dependent mechanism**

Fen Guo, David E. Carter and Andrew Leask
Department of Dentistry, University of Western Ontario, London, ON, Canada

Introduction: Unlike skin, oral gingival do not scar in response to tissue injury. Fibroblasts, the cell type responsible for connective tissue repair and scarring, are exposed to mechanical tension during normal and pathological conditions including wound healing and fibrogenesis. Understanding how human gingival fibroblasts respond to mechanical tension is likely to yield valuable insights not only into gingival function but also into the molecular basis of scarless repair.

Objective: To study how human gingival fibroblasts respond to mechanical stress and understanding the molecular mechanism underlying the ability of strain to modulate gene expression in gingival fibroblasts.

Methods: Gingival fibroblasts were subjected to cyclical strain (up to 72 hours) using the Flexercell system and the response of mechanical strain on gene expression in gingival fibroblasts was investigated by monitoring the alterations in (a) CCN2 expression using real-time polymerase chain reaction (PCR) and Western blot analyses; (b) total/active TGFbeta expression and Endothelin1 expression using real-time PCR and ELISA; (C) genome-wide mRNA expression using micro-array profiling.

Results: Strain caused the rapid activation of latent TGFβ, in a fashion that was reduced by blebbistatin and FAK/src inhibition, and the induction of endothelin (ET-1) mRNA and protein expression. Strain did not cause induction of α-smooth muscle actin or collagen type I mRNAs (proteins promoting scarring); but induced a cohort of pro-proliferative mRNAs and cell proliferation. Compared to dermal fibroblasts, gingival fibroblasts showed reduced ability to respond to TGFβ by inducing fibrogenic mRNAs; addition of ET-1 rescued this phenotype. Pharmacological inhibition of the TGFβ type I (ALK5) receptor, the endothelin A/B receptors and FAK/src significantly reduced the induction of CCN2 and pro-proliferative mRNAs and cell proliferation.

Conclusion: Controlling TGFβ, ET-1 and FAK/src activity may be useful in controlling responses to mechanical strain in the gingiva and may be of value in controlling fibroproliferative conditions such as gingival hyperplasia; controlling ET-1 may be of benefit in controlling scarring in response to injury in the skin.

4. CCN2 expression and localization in B 16 (F10) murine melanoma cells

Wei Sha and Andrew Leask
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Background: 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.

Methods: 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 , and 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.

Results: 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.

Conclusion: 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.

5. A Model of Arthrofibrosis Using Intra-Articular Gene Delivery of Transforming Growth Factor-β1

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Idiopathic adhesive capsulitis (IAC) of the shoulder is a disease of unknown etiology characterized by painful, chronic fibrotic expansion of the synovium and joint capsule, gradually leading to loss of joint motion. Although IAC affects approximately 3-5% of the population, and 20% of diabetics, little is known about its pathogenesis. While the underlying causes are diverse, it is likely that many of the harmful aspects of fibrosis are mediated by transforming growth factor-β1 (TGF-β1), a pleiotropic cytokine. Previously, we used an adenovirus to deliver and chronically overexpress TGF-□1 at high levels in the knee joints of immunocompromised nude rats, which led to very severe arthrofibrosis (Watson et al, 2010). The amount of TGF-β1 produced by the virus delivered in these experiments represented an extreme response to stimulation with this transgene. While relevant to various diseases of the joint, including synovial chondromatosis, chondrometaplasia, osteosarcoma and chondrosarcoma, it showed no signs of resolving.

Therefore, in an effort to understand the biological processes that contribute to the development and resolution of arthrofibrosis and frozen shoulder, we wanted to establish an animal model that more closely reflected the level of disease in humans. As cells expressing nonhomologous transgenic proteins are immunologically cleared from the joints of immunocompetent Wistar rats within 21 days (Gouze et al, 2007), we used this system to examine the effects of short term production of TGF-β1 intra-articularly, with the goal of inducing a fibrotic event that will more closely mirror the stages of IAC. To enable a comprehensive description of the biology of IAC at the molecular level, we performed human genome array analyses to determine the differences in global transcription patterns between normal capsular tissues and those from patients with IAC.

By observing animals over the course of 120 days and delivering a low dose of Ad.TGF-β1, we hypothesized that the animals would undergo a remodeling process similar to stage 4 IAC. To follow the changes in gene expression over the course of disease in the capsular tissues, we used real-time PCR technology and histologic analysis. We found that TGF-β1 gene transfer very rapidly induced a fibrotic condition that completely encased and immobilized the injected joint, induced a chondrogenic response, and over the course of the 120 day experiment, gradually resolved into a less aggressive, less fibrotic, and overall less cellular tissue. Using PCR arrays, we were able to establish the signaling patterns occurring throughout this process. These patterns were comparable to expression data obtained from human patients with IAC, with similar extracellular matrix, MMP, adhesion and collagen proteins upregulated in both human and rat fibrosis. Histologic examination, as well as focused expression arrays of the joint tissues over time, suggested the developing fibrotic tissue adopted a phenotype similar to that of IAC and gradually remodeled with time.

6. Gap junction protein Cx43 as a prognostic marker for brain injury

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Glia scar consisting mostly of reactive astrocytes with upregulated glial fibrillary acidic protein (GFAP) is often present in lesion sites of brain injuries such as a stab wound or ischemia. It is similar but distinct from the inflammatory response observed in fibrosis that involves hardening of connective tissue. The formation of glia scar is believed to prevent harmful substances released by the damaged cells from affecting the surrounding healthy brain tissue. However, the same scar tissue also hinders regeneration of neurons necessary for full recovery of brain function. Until now, the intermediate filament GFAP is the most reliable marker for a glia scar. This study therefore seeks to explore alternative proteins that can be accessible for targeted therapeutic intervention. The gap junction protein connexin43 (Cx43) is widely expressed in adult astrocytes and its expression is upregulated in the peri-lesional region. As a channel protein, Cx43 is predicted to play a key role in intercellular communication critical for proper wound repair and neuroprotection. By correlating the temporal and spatial expression of Cx43 by immunohistochemistry to the activation of astrocytes and microglia (the immune cells in the brain) in response to an acute needle stab wound, we showed that the kinetics of Cx43 expression primarily followed the temporal and spatial distribution of reactive astrocytes. We also examined the presence of nestin-expressing progenitors that are known to be recruited to lesion sites. Our results revealed that the initial response of the host brain involves proliferation of reactive microglia that were devoid of Cx43 within the injury site from 3 hours to 3 days post injury (dpi), with a concurrent migration of Cx43- and GFAP-positive reactive astrocytes towards the lesion. Increased Cx43 and GFAP protein expression were detected within the lesion from 6 dpi onwards. Although there was also a transient increase of nestin-positive cells in the needle wound, only Cx43 and GFAP proteins were detected at 2 weeks post injury. Taken together, our findings indicate Cx43 is a good alternative biomarker for brain injury.

Steamworks Dinner Buffet

Passed Canapés on Crostini

Mascarpone Cheese & Fresh Basil Pesto
with roma tomato on crostini
Prosciutto & Roasted Roma Tomato
aged asiago cheese
Warm Brie Cheese
strawberry & balsamic glaze
Asian Wonton Wrapped Prawns
spicy dipping sauce
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Freshly Baked Artisan Breads
Mesclun Green Salad
dried blueberries & cranberries, roasted almonds
balsamic vinaigrette
Greek Salad
bell pepper, red onion, cucumber, vine ripened tomato,
oregano & feta cheese

Halved Roasted Roma Tomatoes
fresh herbs & parmesan cheese
Roasted Herb Baby White Potatoes
Medley of Seasonal Vegetables

Asparagus Lemon Risotto
grilled asparagus spears, lemon zest & parmesan
Dijon Marinated Chicken Breast
creamy mushroom sauce
Grilled Wild Pacific Red Spring Salmon
sweet soy marinated
roasted red pepper & scallion jus
Grilled Roast of Striploin
rock salt & cracked pepper crust
brandy black peppercorn sauce
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Creamy Espresso Layered Tiramisu Cake
Coffee or Tea
3 Drink tickets (Beer/wine) included

JOE FORTES ENGLISH BAY DINNER

Fresh local oysters

Appetizer

West Coast Clam Chowder
cream, bacon, thyme
OR
ClassicCaesarSalad
crisp romaine hearts, freshly grated parmesan

Main

Wild sockeye Salmon
maple brown butter
OR
miso marinated sablefish
jasmine rice, sweet soy
OR
Arctic Char
carmelized lemon, extra virgin olive oil
OR
Grilled pesto chicken Linguine artichoke, sun dried tomato, pine nuts, parmesan
OR
New york striploin7oz
Canadian Angus reserve
garlic mashed potatoes, seasonal vegetables
OR
Grilled portobello mushroom

Dessert

tiramisu
mascarpone, espresso
OR
Vanilla gelato

2 glasses of white wine included

TOURISTIC SUGGESTIONS

Within the Vancouver/Victoria area, the following are suggested:

Bill Reid Gallery of Northwest Coast Art
639 Hornby
http://www.billreidgallery.ca/
Walking distance

Vancouver Art Gallery
750 Hornby
http://vanartgallery.bc.ca/
Walking distance

Dr Sun Yat Sen Classical Chinese Garden
578 Carrall Street
http://www.vancouverchinesegarden.com/
Walking distance

Stanley Park including Aquarium
North foot of Georgia Street
http://vancouver.ca/parks/parks/stanley/
Walking distance

van Dusen Gardens
5251 Oak St
http://www.vandusengarden.org/
Public transit: From downtown Vancouver, take **#17 Oak** bus. You catch it on the east side of Seymour Street or the transit stop on the west side of Howe Street just south of Robson across from Chapters books. Ask the bus driver to let you off at West 37th Avenue. Once at West 37th Avenue, cross the street and you're right there.

Queen Elizabeth Park and Blodel Conservatory
Cambie Street at West 33rd Avenue
http://vancouver.ca/parks/parks/queenelizabeth/
Public transit: King Edward Station Canada Line

Museum of Anthropology
UBC Campus
6393 N.W. Marine Drive Vancouver, B.C. V6T 1Z2
Tel: 604.822.5087
http://www.moa.ubc.ca/
Public transit: From downtown take either the 4, 9, 17, 25, 41, 43, 44, 49, 99, 258, 480, N17 to "UBC," or contact Translink. The UBC bus loop is located at the centre of campus and is a 7 minute walk from the museum.

Capilano Suspension Bridge
3735 Capilano Road, N Vancouver
http://www.capbridge.com/
Free shuttle: every 15 minutes from either Canada Place or Marriott hotel at 1128 West Hastings Street.

Rocky Mountaineer Whistler Sea to Sky Climb train
Reservations are only available by Calling Toll Free 1.888.403.4727
Whistler Sea to Sky Climb Schedule:
Daily Departures, except Tuesdays & Wednesdays
May 20 - September 26, 2011
http://www.whistlerblackcomb.com/getting_here/whistler_sea_to_climb/index.htm

Pacific Coast Lines Bus to Victoria or Whistler
Reservations & Information 1 800 661 1725
Local: 604 662 7575
Pickup: Waterfront Skytrain Station (beside Fairmont Waterfront Hotel) OR Burrard Skytrain Station (Melville and Burrard St.) OR Pacific Station Bus terminal (Main Street-Science World Skytrain Station).
http://www.pacificcoach.com/

Royal BC Museum
675 Belleville Street
Victoria, BC
http://www.royalbcmuseum.bc.ca/MainSite/default.aspx

RESTAURANTS (all within walking distance)

Alibi Room
Gastropub
157 Alexander St
Vancouver, BC
www.alibi.ca

Boathouse
Seafood
1795 Beach Avenue
Vancouver, BC
http://www.boathouserestaurants.ca/vancouver-restaurant-locations.php

Cardero's
American Fish/Chop house
1583 Coal Harbour Quay
Vancouver, BC
http://www.vancouverdine.com/carderos0experience.aspx

C
Seafood
1600 Howe Street
http://www.crestaurant.com/

Blue Water Cafe + Raw Bar
Seafood
1095 Hamilton Street
Vancouver, BC
http://www.bluewatercafe.net/

Cactus Club - Robson Street
Casual fine dining (local chain)
1136 Robson Street
Vancouver, BC
http://www.cactusclubcafe.com/locations/

Five Sails Restaurant
In Pan Pacific Hotel
West coast cuisine
410 - 999 Canada Place Way
Vancouver, BC
http://www.fivesails.ca/

Il Giardino di Umberto
Italian
1380 and 1382 Hornby Street
http://www.hotelvilladelia.com/restaurants.cfm

Imperial Chinese Seafood Restaurant
Chinese
355 Burrard Street
Vancouver, BC
http://www.imperialrest.com/

Irish Heather
Gastropub
217 Carrall Street
http://irishheather.com/

Keg Steakhouse & Bar
595 Hornby Street
Vancouver, BC
Information & Reservations
604-687-4044
www.kegsteakhouse.com

Kirin Mandarin
Chinese
1172 Alberni Street
http://www.kirinrestaurants.com/

Miyako Sushi
Japanese
829 West Pender St.
Vancouver, BC
miyakosushi.ca

Old Spaghetti Factory
Family dining (local chain)
53 Water Street
http://www.oldspaghettifactory.ca/

Phnom Penh
Cambodian
244 E Georgia St
http://www.dineouthere.com/restaurants/phnom-penh-cambodian-vietnamese-restaurant-in-vancouver-chinatown

Raincity Grill
Local, westcoast
1193 Denman
http://raincitygrill.com/

Salt Tasting Room
Wine/cheese/charcouterie
in Gastown's historic Blood Alley, which runs south of Water St. between Carrall St. and Abbott St.
http://salttastingroom.com/

Shizen Ya
Japanese
985 Hornby St
Vancouver, BC
shizenya.ca

White Spot
Burgers/family dining (local chain)
1616 West Georgia Street
718 Drake Street
580 West Georgia Street
http://www.whitespot.ca/

ALSO HIGHLY RECOMMENDED (but further away)

Bishop's
Fresh local French style
2183 West 4th Avenue
http://www.bishopsonline.com/
Transit: #4 Bus
Over the past 20 years, consistently considered best in Vancouver
Reservations required

Seasons in the Park
Queen Elizabeth Park
West 33rd Avenue & Cambie Street
Transit: King Edward (Canada Line), or #15 Bus
http://www.vancouverdine.com/seasons0experience.aspx
Best views in Vancouver, good local food.