EVENTS

8th international workshop on the CCN family of genes—Nice November 3–8, 2015

PROGRAM

Tuesday, November 3, 2015

17 h00 Registration begins

19 h00 **Welcome Reception and Buffet Dinner**

Wednesday, November 4, 2015

8 h15–8 h30

Introduction to the Workshop

8 h30–8 h55

Bernard Perbal

CCN Proteins : The path out of the maze

Session I

Structure and function of CCN proteins

Chairpersons—Bernard Perbal and Masaharu Takigawa

9 h00–9 h25

George Bou-Gharios

Novel enhancers in the CCN2 gene that drive cell specific expression

9 h25–9 h50

Takashi Nishida

Induction of CCN2 by low-intensity pulsed ultrasound (lipus) in cultured chondrocytes and its biological significance

9 h50–10 h15

Koichiro Muromachi

BMP-1 regulates CCN2 expression and cell surface glycosylation in human dental pulp

10 h15–10 h45 **Coffee Break**

10 h45–11 h10

Satoshi Kubota

Metabolic impacts of CCN2 in chondrocytes

11 h10–11 h35

Philip Trackman

Lysyl oxidase propeptide stimulates osteoblast and osteoclast differentiation and a possible role for CCN2

11 h35–12 h00

Mitsuhiro Hoshijima

Role of interaction between CCN2 and RAB14 in vesicle trafficking in chondrocytes novel intracellular function of CCN2

12 h00–14 h00 **Lunch**

Session II

Developmental biology

Chairpersons - Lester Lau and Andrew Leask

14 h00–14 h10

Lester Lau

Introduction

14 h10–14 h35

Karen Lyons

CCN1 and CCN4 are essential for bone formation and response to mechanical loads *in vivo*

14 h35–15 h00

Joseph Tarr

CCN2 is essential for secondary palatogenesis

15 h00–15:25

Kunimasa Ohta

Tsukushi, a soluble molecule belonging to the small leucine-rich proteoglycan family, has the diverse roles during the development

15 h25–15 h55 **Coffee break**

15 h55–16 h20

Ying Wayne Wang

Title not available

16 h20–16 h45

Brahim Chaqour

Conditional knockout of the CCN1 gene in mice alters blood vessel growth, specification and remodeling

16 h45–17 h10

Ulf Smith

WISP2 (CCN5) secreted by the adipose tissue is a regulator of mesenchymal tissue growth

17 h10–17 h35

David Brigstock

Exosomes regulate hepatic stellate cell function and experimental liver fibrosis

19 h00 **ICCNS-Sponsored Social Event**

Thursday, November 5, 2015

Section III**CCN proteins in wound healing and tissue regeneration****Chairpersons - Karen Lyons and David Brigstock**

8 h00–8 h10

Karen Lyons

Introduction

8 h10–8 h35

Ole Kaasboell

Novel roles of cardiac CCN1 and CCN2 in healing and tissue remodeling after myocardial infarction

8 h35–9 h00

Andrew Leask

CCN2: A multifunctional regulator of stem cell niches

9 h00–9 h25

Lester Lau

CCN1 in hepatobiliary injury repair

9 h25–9 h50

Masaharu Takigawa

Regenerative effects of CCN2 independent modules and CCN3 on articular chondrocytes/cartilage

9 h50–10 h15

Stephen Twigg

Diabetes and CCN2: a wound healing and a NAFLD perspective

10 h15–10 h30 **Coffee Break****ICCNS - SPRINGER AWARD CONFERENCE**10 h30–10 h45 *Award Presentation – Bernard Perbal*

10 h45–11 h30

Keynote Speaker - Judith Campisi

Cancer and aging: Rival demons and signaling mechanisms

11 h45 Special event

13 h00 **Lunch included in the registration****Free afternoon, dinner on your own****Friday, November 6, 2015****Free morning**14 h15 **SPECIAL CONFERENCE***Moderator: Bernard Perbal*

14 h30–15 h15

Keynote Speaker - Robert Baxter

The role of IGF binding protein-3 in the breast cancer response to DNA damaging chemotherapy

15 h30–16 h00 **Coffee Break****Session IV****Cancer****Chairpersons - Satoshi Kubota and Stephen Twigg**

16 h00–16 h10

Satoshi Kubota

Introduction

16 h10–16 h35

Madhulika Gupta

IGFBP-1 hyperphosphorylation induced by leucine deprivation is mediated by protein kinase CK2 and protein kinase C

16 h35–17 h00

Sushanta Banerjee

CCN5 plays a key role in activation of ER-alpha and promotes tamoxifen action in breast cancer cells

17 h00–17 h25

Celina Kleer

Conditional epithelial cell-specific knockout of CCN6/Wisp3 disrupts normal development of the virgin murine mammary gland

17 h25–17 h50

Andrew Leask

CCN2 expression by tumor stroma is required for melanoma metastasis

17 h50–18 h15

Stephany Barreto

Obesity-related cancers and extracellular matrix with special reference to CCN molecules

18 h15–18 h40

Herman Yeger

A new therapeutic strategy for cancer

20 h00 **ICCNS-Sponsored Social Event**

Saturday, November 7, 2015

Session V

Skin Biology and Fibrosis

Chairpersons - Herman Yeger and Sushanta Banerjee

8 h30–8 h40

Herman Yeger

Introduction

8 h40–9 h05

Gary Fisher

Regulation of CCN1 and CCN2 by transcriptional co-activator YAP in human skin: implications for cancer

9 h05–9 h30

Mary Barbe

Title not available

9 h30–9 h55

Muriel Cario Andre

Is CCN3 a key regulator of epidermal and dermal homeostasis?

9 h55–

Enrique Brandan

Fibrosis associated to skeletal muscle denervation and to amyotrophic lateral sclerosis (ALS) model: role of CTGF/CCN2

10 h20–10 h50 **Coffee break**

10 h50–11 h15

Bethan Monk

WNT3A-mediated rescue of vascular smooth muscle cells (VSMCs) from oxidative stress induced apoptosis by CCN4 and CCN5

11 h15–11 h40

Shahram Ghanaati

Collagen membranes induce different vascularization and cellular inflammatory response in relation to their of origin : *In vivo* and clinical studies

11 h40–12 h05

Choukroun Joseph

A new smart blood concentrate for tissue engineering in regenerative medicine: the i-PRF

12 h05–14 h00 **Lunch**

Session VI

Pathobiology and CCN proteins

Chairpersons - Gary Fisher and Enrique Brandan

14 h00–14 h10

Gary Fisher

Introduction

14 h10–14 h35

Gustavo Matute-Bello

CCN1 expression increases in mice with acute lung injury, and is associated with an enhanced fibroproliferative response

14 h35–15 h00

Shu Wu

The role of CTGF (CCN2)-beta-catenin signaling in the pathogenesis of BPD (broncho pulmonary dysplasia)

15 h00–15 h25

Stephan Klee

WISP1 (CCN4) mediates IL6-dependent proliferation in primary human lung fibroblasts

15 h25–15 h50

Yves Courty

Altered expression of the CCN genes in smoking-related and infectious lung diseases

15 h50–16 h20 **Coffee break**

16 h20–16 h45

Denise Fitzgerald

Title not available

16 h45–17 h00

Jake Bedore

CCN2 deletion from the nucleus pulposus accelerates intervertebral disc degeneration and results in behaviour associated with back pain in mice

17 h10–17 h35

Helen Williams

CCN4 is protective against atherosclerosis and vulnerability to pseudoaneurysm in apoe^{-/-}knockout mice

17 h35–18 h00

Zhiyong Lin

Matricellular protein CCN3 mitigates abdominal aortic aneurysm

16 h40

Bernard Perbal

Concluding remarks

19 h30 **COCKTAIL AND GALA DINNER WITH LIVE MUSIC**

Sunday, November 8, 2015

Departure of Participants

ABSTRACTS

Session I Structure and function of CCN proteins

NOVEL ENHANCERS IN THE CCN2 GENE THAT DRIVE CELL SPECIFIC EXPRESSION

George Bou-Gharios

Institute of Ageing and Chronic disease, University of Liverpool, UK

Connective tissue growth factor (CCN2), a CCN family member, is a secreted protein regulating cellular functions, including fibrosis, apoptosis, adhesion, migration, differentiation, proliferation, angiogenesis, and chondrogenesis. Our group is interested in how CCN2 is regulated at the level of transcription. Over the years, enhancer sequences directing transcription of a particular gene have been identified by a variety of methods, including looking for DNaseI hypersensitivity, evolutionary sequence conservation, histone modifications, and the binding of transcription factors. The ENCODE consortium provides information about tissue-specific histone modifications including H3K4Me1 and H3K27Ac, associated with active enhancer regions. Consequently, for the first time we now have

public access to information which enable us to clearly identify putative enhancer regions *in silico*. We tested limb-specific H3K4Me1 and H3K27Ac histone markers to identify novel regulatory enhancers within the CCN2 gene using transgenic mice. The results of this endeavour revealed new enhancers that are located 5' end of the gene, beyond the BAC sequence (BAC clone RP24-96 J1) that was used in the Gene Expression Nervous System Atlas (GENSAT) Project. Three individual cis-acting elements expressed in different cell types. Work is underway to investigate the factors involved in each of these regulations and their impact on our understanding of CCN2 transcription.

INDUCTION OF CCN2 BY LOW-INTENSITY PULSED ULTRASOUND (LIPUS) IN CULTURED CHONDROCYTES AND ITS BIOLOGICAL SIGNIFICANCE

Takashi Nishida¹, Satoshi Kubota^{1, 2}, Eriko Aoyama², Nobuyasu Yamanaka³, Karen M Lyons⁴, Masaharu Takigawa²

¹Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

²Advanced Research Center for Oral and Craniofacial Sciences, Okayama University Dental School, Okayama, Japan

³Ito Cooperation, Ltd., Tokyo, Japan

⁴Department of Orthopaedic Surgery, UCLA, CA, USA

Previously we reported that CCN family protein 2/connective tissue growth factor (CCN2/CTGF) promoted chondrocyte proliferation and differentiation and that it repaired cartilage in experimental animal osteoarthritis (OA) models. Therefore, we believe that CCN2 is one of the useful molecules in OA therapy. Recently, application of low-intensity pulsed ultrasound (LIPUS) is noted as a low invasive therapy for OA patients. Here, the aim of this study is to investigate whether LIPUS stimulates CCN2 production in chondrocytes, or not and to clarify its biological significance. A human chondrocytic cell line HCS-2/8, a rat chondrocytic cell line RCS, and rat primary articular cartilage cells were treated with LIPUS at a frequency of 3.0 MHz and an intensity of 60 mW/cm² for 20 min using ST-SONIC (Ito Co., Ltd.). After 5 h of treatment, Western blot analysis was performed by using anti-CCN2 antibody. As a result, LIPUS stimulated CCN2 production in cultured chondrocytes. Moreover, p38 MAPK was activated immediately after LIPUS treatment, and then ERK1/2 was activated subsequently. Interestingly, CCN2 was increased in HCS-2/8 cells treated with LIPUS and Y27632, which is inhibitor of Rho-associated kinase (ROCK), more remarkably than those treated with LIPUS alone. On the contrary, LIPUS-induced CCN2 was decreased by pre-treatment with SB203580, PD98059 or NSC23766, which is an inhibitor of p38 MAPK, MEK1 or Rac1, respectively. In addition, Ca²⁺ influx, which is detected by using Fluo-4-AM reagent, was increased in HCS-2/8 cells treated with LIPUS, and stability of mRNAs encoding Ca²⁺ channels, such as TRPV4 and BK_{ca}, was decreased in HCS-2/8 cells treated with siRNA against CCN2. Furthermore, LIPUS-induced structural change of F-actin from stress fibers to cortical fibers, and the gene expression of type II collagen and aggrecan were observed in wild type chondrocytes, but not in *Ccn2*-deficient chondrocytes. These findings suggest that increase in CCN2 production is mediated by activation of RhoA and MAPK signaling caused by LIPUS-stimulated Ca²⁺ influx and that the resultant increase in CCN2 promotes chondrocyte

differentiation. Based on these results, LIPUS may be recommended as an OA therapy on a daily basis.

BMP-1 REGULATES CCN2 EXPRESSION AND CELL SURFACE GLYCOSYLATION IN HUMAN DENTAL PULP

Koichiro Muromachi, Nobuyuki Tani-Ishii

Department of Pulp Biology and Endodontics, Graduate School of Dentistry, Kanagawa Dental University, Yokosuka, Kanagawa, JAPAN

Bone morphogenetic protein-1 (BMP-1) is a metalloprotease belonging to the astacin protease family. This molecule is different from other BMPs due to its protease activity. BMP-1 contributes to tooth development by degradation of dentin-specific substrates such as dentin matrix protein-1 (DMP-1) and dentin sialophosphoprotein (DSPP). CCN2 is a well-known osteogenesis- and chondrogenesis-related protein necessary for tooth development. However, the relationship between BMP-1 and CCN2 in pathological conditioned dentin-pulp complex is still unclear.

In this study, we report that BMP-1 regulates the CCN2 expression and the membrane glycosylation in human dental pulp cells. First, we demonstrated that the expression of BMP-1, CCN2, and DSPP was accelerated in the layer of odontoblast-like cells and reparative dentin subjacent dental caries in human teeth, as shown by immunohistochemistry. BMP-1 induced the expression of CCN2 independently of protease activity in the cells but not that of DSPP or DMP-1. The dynasore, which inhibits dynamin-related endocytosis, suppressed the BMP-1-induced CCN2 expression. To confirm the intracellular dynamics of BMP-1, we observed that fluorescently-labeled BMP-1 was internalized into cytoplasm of human dental pulp cells using confocal microscope. Furthermore, cell membrane glycosylation was altered by BMP-1 stimulation as analyzed by lectin microarray.

These findings indicate that a novel property of BMP-1 which potentially enhances bone-like reparative dentin formation through CCN2 expression and membrane glycosylation in dentin-pulp complex. This novel role of BMP-1 will help to understand the mechanism of dental pulp repair.

METABOLIC IMPACTS OF CCN2 IN CHONDROCYTES

Satoshi Kubota¹, Yurika Murase¹, Aya Maeda-Uematsu¹, Takako Hattori¹, Eriko Aoyama², Takashi Nishida¹, Harumi Kawaki¹, Karen M. Lyons³, Masaharu Takigawa²

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³Department of Orthopaedic Surgery, UCLA School of Medicine, Los Angeles, CA, USA

Until today, a number of studies revealed a novel functionality of CCN2 that promote both differentiation and proliferation of chondrocytes. The positive effects of CCN2 on apparently opposite cytological events suggest the involvement of CCN2 in basic biological events, which is indispensable for any biological activity. Metabolism, especially energy metabolism is the most basic and critical processes to support the life. Therefore, we started our research by evaluating the impact of CCN2

deletion on the metabolism in murine chondrocytes. First, comparative metabolome analysis of metabolites extracted from normal and *Ccn2*-null costal chondrocytes revealed a stable and marked decrease in the cellular ATP levels, suggesting global impairment in endergonic biochemical reactions. Of note, the ATP level in *Ccn2*-null chondrocytes was redeemed by the addition of exogenous recombinant CCN2. This ATP deficiency may be ascribed to the impaired glycolysis, since the gene expression of 3 enzymes catalyzing a series of reactions was found repressed in *Ccn2*-null chondrocytes.

In contrast, mitochondrial membrane potential was maintained in those cells, indicating that aerobic ATP production was not affected by CCN2 deficiency, although pyruvic acid may not be properly supplied by glycolysis. Consistent with this finding, all of the essential amino acid levels were reduced in *Ccn2*-null chondrocytes, indicating catabolic consumption of amino acids for energy production. It should be also noted that cellular protein production was rather accelerated therein, which was accompanied by elevated expression of ribosomal protein genes. These findings collectively indicate a profound role of CCN2 in maintaining the energy supply and harmonized amino acid metabolism in chondrocytes.

LYSYL OXIDASE PROPEPTIDE STIMULATES OSTEOBLAST AND OSTEOCLAST DIFFERENTIATION AND A POSSIBLE ROLE FOR CCN2

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Department of Molecular and Cell Biology,
Boston University Henry M. Goldman, School of Dental Medicine,
Boston, MA, USA

Lysyl oxidase is secreted as a 50 kDa pro-enzyme into the extracellular environment where it is cleaved into the ~30 kDa mature enzyme (LOX) and 18 kDa pro-peptide (LOX-PP). LOX enzyme activity is required for normal collagen and elastin extracellular cross-linking and maturation of the extracellular matrix. LOX-PP acts as a tumor suppressor and inhibits several RAS-related signaling pathways. The underlying hypothesis is that LOX-PP has the potential to promote bone cell differentiation, while inhibiting cancer cell growth in bone. Here we investigate the effect of LOX-PP on bone marrow stromal cell (BMSC) proliferation and differentiation towards osteoblasts or osteoclasts and its modulation of prostate cancer cell conditioned media-induced alterations of proliferation and differentiation of bone marrow stromal cells *in vitro*. In addition, effects of overexpression of rLOX-PP in DU145 and PC3 prostate cancer cell lines on bone structure after intramedullary injections were determined. LOX-PP stimulated osteoblast and osteoclast differentiation in BMSC cultures was assessed by alizarin red staining, TRAP staining and quantitative TRAP enzyme activity assays. Prostate cancer conditioned media inhibited osteoblast differentiation which was reversed by rLOX-PP treatment. Prostate cancer conditioned medium stimulated osteoclast differentiation and was further enhanced by rLOX-PP treatment. rLOX-PP stimulated osteoclast differentiation by inhibiting OPG expression, stimulating CCN2 expression, and increasing osteoclast fusion. *In vivo* studies indicate that rLOX-PP expression by PC3 cells implanted into the tibia of mice further enhanced PC3 cell ability to resorb bone, while rLOX-PP expression in DU145 cells resulted in non-significant increases in net bone formation. LOX-PP enhances both osteoclast and osteoblast differentiation. LOX-PP may serve to enhance coupling interactions between osteoclasts and osteoblasts helping to maintain a normal bone turnover in health, while contributing to osteopenia in disease.

ROLE OF INTERACTION BETWEEN CCN2 AND RAB14 IN VESICLE TRAFFICKING IN CHONDROCYTES NOVEL INTRACELLULAR FUNCTION OF CCN2

Mitsuhiro Hoshijima^{1,2}, Takako Hattori³, Eriko Aoyama¹, Takashi Nishida³, Satoshi Kubota³, Hiroshi Kamioka², Masaharu Takigawa¹

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CCN family 2 / connective tissue growth factor (CCN2) is strongly expressed in cartilage and modulates chondrocyte differentiation and proliferation by binding various molecules. The aim of this study is to identify a CCN2-interactive protein and to investigate its role in the physiological activity of CCN2 in chondrocytes. We carried out a yeast two-hybrid screening using CCN2 as a bait and a cDNA library from the human chondrocytic cell line, HCS-2/8, and identified Rab14 GTPase (Rab14) as a CCN2-interactive peptide. Rab14 is known to be involved in vesicle trafficking between the golgi complex and endosomes. For analysis of the CCN2 domain binding to full-length Rab14, we carried out growth tests of yeast transformants in selection media. CCN2 bound to Rab14 through its IGF1R domain among the four domains in CCN2. To detect the interaction between CCN2 and Rab14 in the cells, GFP tagged CCN2 (GFP-CCN2), Halo tagged Rab14 (Rab14 WT), a constitutive active form (Rab14 CA), and a dominant negative form (Rab14 DN) of Rab14 were overexpressed in COS7 cells. Ectopically overexpressed Rab14 showed diffused cytosolic distribution in COS7 cells, however, when Rab14 WT or Rab14 CA overexpressed with GFP-CCN2, Rab14 distribution changed as dotted spot every distributed within cytosol and Rab14 and GFP-CCN2 showed good colocalization. Coexpression of Rab14 DN and GFP-CCN2 also showed dotted codistribution, but more concentrated in perinuclear area. In addition, HCS-2/8 chondrocytic cells were transfected with *rab14* or *ccn2* siRNA, and gene expression of *bip*, *chop* and *aggrecan* mRNA was monitored by real-time PCR. Decrease in *rab14* or *ccn2* mRNA by siRNA enhanced the expression of *chop* and *bip* mRNA significantly. On the other hand, treatment of *ccn2* siRNA decreased *aggrecan* mRNA, but *rab14* siRNA did not change it. Furthermore, to study the effect of interaction between CCN2 and its interactive proteins on proteoglycan synthesis, HCS-2/8 cells were transfected with Rab14 WT or Rab14 CA or Rab14 DN, and the proteoglycan accumulation was monitored by Toluidine Blue or Alcian Blue staining. Rab14 DN overexpressed in HCS-2/8 cells decreased proteoglycan accumulation to less than 75 % as compared to Rab14 WT overexpressed in the cells. Our results suggest that intracellular CCN2 is associated with Rab14 on proteoglycan-containing vesicles during their transport from golgi to endosomes in chondrocytes and that the association play some role in proteoglycan synthesis by chondrocyte

Session II Developmental biology

CCN1 AND CCN4 ARE ESSENTIAL FOR BONE FORMATION AND RESPONSE TO MECHANICAL LOADS *IN VIVO*

Gexin Zhao, Diana Rigueur, Jie Jiang, and Karen M. Lyons

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The Wnt pathway and its inhibitor sclerostin (SOST) play central roles in the maintenance of bone mass. Targeting the Wnt pathway is likely to become the gold standard for osteoporosis therapy, yet the mediators of Wnt actions in bone are largely unknown. CCN1 and CCN4 are among the most highly upregulated targets of Wnts in bone. CCN5 is also elevated by Wnt signaling in osteoblasts. However, the functions of CCN1, CCN4, and CCN5 *in vivo* in bone are unknown. We examined CCN1, CCN4, and CCN5 expression in bone using transgenic reporter mice, and generated mice lacking *Ccn1*, *Ccn4*, or *Ccn5* to address this gap in knowledge. *Ccn1* and *Ccn4* are expressed at high levels in mature osteoblasts and osteocytes. *Ccn5* expression is more restricted. We compared the phenotypes of mice lacking *Ccn1* or *Ccn2* in osteoblasts using floxed alleles. We also examined bone phenotypes of *Ccn4*^{-/-} and *Ccn5*^{-/-} mice. This analysis revealed that mice lacking either *Ccn1* or *Ccn4* have profound low bone mass phenotypes. Production of double mutants is under way. Loss of *Ccn5* did not lead to a detectable bone phenotype. *In vitro* assays demonstrated that commitment of osteoprogenitors to the osteogenic lineage is inhibited in *Ccn1* and *Ccn4* mutants, accompanied by increased adipogenic commitment. In accordance, marrow adiposity is elevated in mutants. Furthermore, bone marrow angiogenesis is significantly impaired in both *Ccn1* and *Ccn4* mutants. Therefore, CCN1 and CCN4 are essential for the osteoblast-angiogenic coupling known to be crucial for maintenance of the osteoprogenitor pool *in vivo*. In addition to defective osteoprogenitor commitment, *Ccn1* mutants exhibited elevated expression of the WNT inhibitor sclerostin (SOST) in osteocytes in cortical bone. In accordance, Wnt signaling output is diminished in *Ccn1* mutant osteoblasts. Mechanical loading on bone, such as that resulting from exercise, is normally protective of bone mass because mechanical signals suppress SOST expression. We find that *Ccn1*-deficient osteoblasts and osteocytes are unable to respond to mechanical loads to suppress SOST. Thus, in addition to its role in osteoprogenitor commitment, *Ccn1* is essential for the ability of osteoblasts and osteocytes to sense and respond to mechanical signals. In summary, we have uncovered essential functions for CCN1 and CCN4 in multiple aspect of osteoblast formation and function.

CCN2 IS ESSENTIAL FOR SECONDARY PALATOGENESIS

Joseph T. Tarr,¹ Timothy G. Visser,¹ James P. Bradley,² Steven N. Popoff^{1*}

¹Anatomy and Cell Biology;

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Non syndromic cleft palate is a common craniofacial birth defect with an incidence of 1 in 700 live births. CCN2 has emerged as an essential player in normal craniofacial skeletal formation. Recent studies suggest that CCN2 acts as a necessary downstream mediator of TGF- β -dependent mesenchymal stem cell proliferation in palatogenesis. Previous work in our laboratory identified numerous craniofacial defects such as failure of formation of the bony palate in CCN2 knockout (KO) mice. In this study, micro-CT and histological analyses showed that in the absence of CCN2, the growth of the palatal shelves is arrested at an early stage in the process of palatogenesis. Additionally, palate organ culture reveals that the absence of CCN2 causes inhibition of palatal shelf fusion. Gene expression analysis by qPCR of palatal tissue revealed increased mRNA expression of TGF- β 1, TGF- β R1 and 2, BMPR2, and FGFR2c. We isolated mesenchyme-derived pre-osteoblasts from crania of WT and CCN2 KO mice for *in vitro* studies. CCN2 KO cells exhibited decreased proliferation with a significant reduction in the number of cells in the S phase and a

concomitant increase in the number of cells accumulating on the G0/G1 phase compared to WT cells. CCN2 KO cells exhibited decreased ability to adhere to extracellular matrices, reduced spreading, altered cytoskeletal organization, and reduced levels of total and activated Rac1 compared to WT cells. The reduction in Rac1 resulted in decreased focal adhesion formation in CCN2 KO cells. Since all of these cell functions are necessary for proper formation of the secondary palate during craniofacial development, we conclude that these defects contribute to the failure of the palatal shelves to form and grow in CCN2 KO mice. Future studies will focus on examining specific proteins (growth factors, receptors, and/or signaling factors) based on data derived from qPCR, isolating mouse embryonic mesenchymal cells directly from the palatal anlage to study cellular function and signaling, and utilizing palate organ culture to further characterize the developmental defects observed in the palates of CCN2 KO mice.

TSUKUSHI, A SOLUBLE MOLECULE BELONGING TO THE SMALL LEUCINE-RICH PROTEOGLYCAN FAMILY, HAS THE DIVERSE ROLES DURING THE DEVELOPMENT

Kunimasa Ohta, M. Asrafuzzaman Riyadh

Department of Developmental Neurobiology, Kumamoto University, Kumamoto, Japan

Small Leucine-Rich Proteoglycan family (SLRP) molecules are involved in several fundamental biological functions (Iozzo and Schaefer, 2015). Previously, we reported that TSK is a unique secreted protein and a member of SLRP family belonging to subclass IV (Ohta et al., 2004). TSK binds nodal/Vg1, BMP4/Chordin, FGF8, Frizzled4, TGF- β 1, and Delta and modulates their downstream intracellular signaling pathways, indicating multiple regulatory functions of TSK (Ohta, 2013). Taken together, we place TSK as signaling mediator at the extracellular region during several biological events.

The mammalian nervous system arises from coordinated proliferation, differentiation and migration of precursor cells during embryonic and early postnatal development. The subventricular zone (SVZ) is one of two regions where neurogenesis persists in the postnatal brain. Four cell types have been described in the SVZ: 1) ependymal ciliated cells (type E), 2) proliferating type A neuroblasts, 3) slowly proliferating type B cells and 4) actively proliferating type C cells. Here we observed aberrant cell proliferation and cell death in TSK knockout mice (TSK^{-/-}). All types of cell (B cell, C cell, A cell) undergo hyper proliferation in TSK^{-/-} mice. The apoptotic cell number increases three fold in TSK^{-/-} mice. Double immunostaining confirmed a large population of A cell experiences apoptosis in knockout mice compared with the wild type. Because of these altered cell proliferation and massive apoptosis, the lateral ventricle is expanded in TSK^{-/-} mice. Using transgenic mice we overexpressed TSK in TSK^{-/-} background and rescued lateral ventricle size. *In vitro* experiments using neurosphere generated from wild type and TSK^{-/-} mice supports *in vivo* findings. Thus using loss of function and gain of function experiment we uncover the role of TSK in neurogenesis.

CONDITIONAL KNOCKOUT OF THE CCN1 GENE IN MICE ALTERS BLOOD VESSEL GROWTH, SPECIFICATION AND REMODELING

Bindu Chintala¹, Izabela Krupska¹, Lulu Yan¹, Lester Lau², Maria Grant³, Brahim Chaqour^{1, 4}

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² Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago College of Medicine, Chicago, IL, USA

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Physiological growth of blood vessels depends on the highly coordinated actions of multiple angiogenic regulators. How angiogenic signals originating from the extracellular matrix (ECM) are integrated to yield normal and prevent aberrant tissue vascularization is unknown. CCN1 protein expression is critical for embryonic viability as CCN1 deficiency in mice results in severe cardiovascular defects and placental insufficiency. In adults, the expression of CCN1 is associated with sites of angiogenesis and inflammation and tissue regeneration. However, our understanding of the cellular origins and mode of action of this ECM molecule is incomplete. Using a CCN1 promoter-GFP reporter transgenic mouse, we show that CCN1 is predominantly expressed in angiogenic endothelial cells (ECs) at the leading front of actively growing vessels in the mouse retina. Neither pericytes with a multiprocess morphology on the abluminal surface of the developing vasculature nor the associated astrocytes and microglial cells appear to express the *CCN1:GFP transgene* suggesting that when the angiogenic process is initiated, *CCN1 expression is largely associated with angiogenic ECs*. We further show that endothelial deletion of CCN1 in mice using a Cre/Lox system is associated with EC hyperplasia, loss of pericyte coverage and formation of dense retinal vascular networks lacking the normal hierarchical arrangement of arterioles, capillaries and venules. CCN1 is a product of an immediate-early gene that is transcriptionally induced in ECs in response to stimulation by vascular endothelial growth factor (VEGF). We found that CCN1 activity is integrated with VEGF receptor 2 (VEGF-R2) activation and downstream signaling pathways required for tubular network formation. CCN1 increased the expression of and association between Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) and VEGF-R2 which leads to rapid dephosphorylation of VEGF-R2 tyrosine preventing EC hyperproliferation. Predictably, CCN1 further brings receptors/signaling molecules into proximity that are otherwise spatially separated. In a model of oxygen-induced ischemic retinopathy, CCN1 provided a protective effect against vaso-oblivation and reduced pathological neovascularization subsequent to ischemia-induced VEGF-R2 overactivation. These data highlight novel functions of CCN1 as a naturally optimized molecule fine controlling key processes in physiological angiogenesis and safeguarding against aberrant angiogenic responses.

EXOSOMES REGULATE HEPATIC STELLATE CELL FUNCTION AND EXPERIMENTAL LIVER FIBROSIS

Li Chen¹ and David Brigstock^{1,2}

¹ The Research Institute at Nationwide Children's Hospital, Columbus OH, USA

² Department of Surgery, The Ohio State University, Columbus, OH, USA

Hepatic fibrosis is a debilitating pathology in many chronic liver diseases but lacks effective therapies and can be challenging to clinically assess. The fibrotic response is driven by the actions of hepatic stellate cells (HSC) which are usually quiescent but become activated during liver injury and produce powerful fibrotic mediators such as CCN2 or TGF- β . Our studies of HSC have recently revealed that they are functionally regulated by nanovesicles, or "exosomes", that

are secreted by HSC or hepatocytes. Specifically, HSC activation was stimulated by exosomes from activated HSC or injured hepatocytes, but not by exosomes from quiescent HSC or normal hepatocytes. We have further showed that regulators of CCN2 expression (transcription factors, miRs) as well as CCN2 itself are exosomally shuttled between HSC, allowing their net fibrogenic activity to be fine-tuned based on the molecular information received from neighboring cells. Exosomes have emerged as important regulators of the fibrotic response. In addition, their dynamic molecular payloads have provided new leads for exploring whether exosomes can serve as novel diagnostic or therapeutic tools in liver fibrosis.

Section III CCN proteins in wound healing and tissue regeneration

CCN2: A MULTIFUNCTIONAL REGULATOR OF STEM CELL NICHES

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Normally, CCN2 expression is restricted to progenitor cells in the skin, notably in Sox2-expressing cells such as the dermal papillae of the hair follicle. In response to injury, CCN2 is transiently induced in (myo)fibroblasts. In fibrotic conditions, myofibroblasts persist in fibrotic tissue, and CCN2 is overexpressed in these cells. In injured skin tissue, only a small number of myofibroblasts are positive for progenitor cell markers. CCN2, although required for the recruitment of myofibroblasts derived from Sox2-expressing cells, is not required for tissue repair. Conversely, in fibrotic tissue, essentially all myofibroblasts appear to have a progenitor cell origin; CCN2 is required for their recruitment/differentiation and hence fibrosis. Thus the role of CCN2 *in vivo* appears to be for the recruitment and differentiation of dermal progenitor into smooth-muscle-like myofibroblasts. Paralleling these results, *in vitro*, progenitor cells differentiate into myofibroblasts in response to serum in a fashion dependent on the FAK/MRTFA-dependent induction of CCN2. In the skin, crosstalk between dermal and epithelial stem cells results in hair follicle cycling due to modulation of wnt/beta catenin activity. Consistent with a role of CCN2 in skin progenitor cell function, loss of CCN2 from the dermal papillae results in an increase in wnt/beta catenin-dependent promoter activity and an increase in hair follicle cycling due to the ability of CCN2 to block binding of wnts to the receptor LRP6. Overall, these data point to a key central role of CCN2 in modulating progenitor cell function in the skin.

CCN1 IN HEPATOBILIARY INJURY REPAIR

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Liver fibrosis occurs as a wound-healing response to chronic hepatic injuries irrespective of the underlying etiology and may progress to life-threatening cirrhosis. We show that the matricellular protein CCN1 (CYR61) plays key roles in multiple aspects of hepatobiliary injury repair through distinct mechanisms. First, CCN1 mediates bile duct regeneration in response to cholestatic injury by activating NF- κ B through integrins α v β 5/ α v β 3, leading to *Jag1* expression, Jag1/Notch signaling, and

cholangiocyte proliferation. In addition, CCN1 promotes the differentiation of hepatic progenitor cells into cholangiocytes by triggering Jag1/Notch signaling in progenitor cells. Knock-in mice expressing a CCN1 mutant that is unable to bind $\alpha v\beta 5/\alpha v\beta 3$ were impaired in bile duct regeneration, leading to massive hepatic necrosis and mortality after bile duct ligation. Second, CCN1 dampens and limits liver fibrogenesis induced by either hepatotoxin intoxication (carbon tetrachloride) or cholestasis (bile duct ligation). Mechanistically, CCN1 acts by triggering cellular senescence in activated hepatic stellate cells and portal fibroblasts by engaging integrin $\alpha 6\beta 1$ to induce reactive oxygen species accumulation through the RAC1-NADPH oxidase 1 enzyme complex, whereupon the senescent cells express an anti-fibrotic genetic program. Moreover, tail-vein injection of CCN1 accelerates resolution of established liver fibrosis. Third, CCN1 suppresses the formation of hepatocellular carcinoma by inhibiting hepatocarcinogen-induced compensatory hepatocyte proliferation, thus limiting the expansion of damaged and potentially oncogenic hepatocytes. CCN1 effectively inhibits epidermal growth factor receptor-dependent hepatocyte proliferation through integrin $\alpha 6$ -mediated accumulation of reactive oxygen species, thereby triggering p53 activation and a cell cycle block. Consequently, *Ccn1*-deficient mice exhibit diminished p53 activation and elevated compensatory hepatocyte proliferation, resulting in increased hepatocarcinogen-induced tumors. Taken together, these results show that CCN1 plays critical roles in hepatobiliary injury repair to promote parenchymal regeneration, enhance resolution of fibrosis, and suppress carcinogen-induced hepatocellular carcinomas.

REGENERATIVE EFFECTS OF CCN2 INDEPENDENT MODULES AND CCN3 ON ARTICULAR CHONDROCYTES/CARTILAGE

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We previously reported that CCN2 promotes the regeneration of articular cartilage. For future clinical application, it is important to discover a more robust and effective CCN2 derivative to induce regeneration because it is difficult to prepare large amount of stably active full length CCN2. Also, we previously reported that CCN3 directed epiphyseal chondrocytes toward the articular chondrocyte phenotype, suggesting that CCN3 is an additional useful CCN protein for regeneration of articular cartilage. Therefore, in this study, we investigated (1) the effects of the 4 modules independently and their combinations on chondrocytic cells in vitro and damaged cartilage *in vivo*, especially in relation to full length CCN2 and (2) the effects of CCN3 on articular chondrocytes in vitro and damaged cartilage *in vivo*.

In vitro evaluation using human chondrocytic cells showed a remarkable enhancing effect of several single modules on the gene expression of cartilaginous extracellular matrix components, whereas combinations of 2 or 3 modules rather diminished such effects. Interestingly, combination of all 4 modules redeemed the effect of intact CCN2 *in vivo*. Next, the thrombospondin 1 type 1 repeat module (TSP1), which was found most promising in the experiments in vitro, and the combination of 4 modules were forwarded further to *in vivo* confirmation using 2 rat osteoarthritis (OA) models. As a result, TSP1 displayed more prominent regenerative effects than intact CCN2 on damaged cartilage. Unexpectedly, the

combination of 4 modules showed limited effects *in vivo*. These results indicate the utility of TSP1 in the regenerative therapeutics of OA.

Strong immunostaining for CCN3 was observed in rat articular cartilage but not in articular cartilage of monoiodoacetate (MA)-induced osteoarthritis rat. However, even in OA cartilage, chondrocyte clusters in which chondrocytes are trying to repair cartilage showed positive staining. Forced overexpression of CCN3 in cultured chondrocytes promoted aggrecan accumulation and gene expression of type II collagen and lubricin a specific marker of articular cartilage. Administration of CCN3 with gelatin hydrogel into the knee joints protected cartilage degeneration caused by MA injection. These findings indicate that CCN3 is also useful for regeneration of articular cartilage.

DIABETES AND CCN2 : A WOUND HEALING AND A NAFLD PERSPECTIVE

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Diabetes complications are characterised by excessive inflammation in some tissues and organs such as in foot ulcers and wounds and by excessive fibrosis in others, such as in the diabetic kidney, heart and liver in non-alcoholic fatty liver disease (NAFLD), especially the non-alcoholic steatohepatitis (NASH) sub-type. Over the years, we have related these organ changes to functional loss and dysregulation of the CCN family of genes and proteins, especially CCN2. This presentation will focus on the contrasting dysregulation of CCN2 and other CCN family members in wounds and in NASH in diabetes, implicating CCN2 as therapy in one complication and CCN2 inhibition strategies in others.

ICCNS - SPRINGER AWARD CONFERENCE

CANCER AND AGING: RIVAL DEMONS AND SIGNALING MECHANISMS

Judith Campisi

Aging is the single largest risk factor for developing a panoply of diseases, including diseases as diverse as neurodegeneration and cancer.

I will discuss recent progress in the common signaling mechanisms and cell fate responses that drive disparate age-related diseases. At the heart of this convergence is the cell fate decision termed cellular senescence. The pleiotropic senescence response entails a complex signaling cascade that ultimately determines important physiological responses ranging from tumor suppression to wound healing.

SPECIAL CONFERENCE

THE ROLE OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 (IGFBP-3) IN THE BREAST CANCER RESPONSE TO DNA DAMAGING CHEMOTHERAPY

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About 15 % of breast cancers are triple-negative for estrogen and progesterone receptors, and HER2 overexpression. These typically aggressive cancers are generally treated with DNA-damaging chemotherapy drugs that induce DNA double strand breaks (DSB). The ability to repair DSB damage allows breast cancer cells to develop treatment resistance. We previously reported that IGFBP-3 interacts in the nucleus of basal-like triple-negative breast cancer cells with the epidermal growth factor receptor (EGFR) and DNA-dependent protein kinase (DNA-PKcs) to modulate DSB repair by non-homologous end-joining (Lin MZ et al., *Oncogene* 33:85-96, 2014). Nuclear localization of EGFR and IGFBP-3, and of their complex measured by coimmunoprecipitation or proximity ligation assay, was enhanced by etoposide or doxorubicin, and inhibited by EGFR kinase inhibition. Nuclear DNA-PKcs-IGFBP-3 interaction peaked 4 h after treatment. IGFBP-3 downregulation by siRNA attenuated the stimulation of nuclear DNA-PKcs-EGFR complexes and of DNA repair activity in a NHEJ assay. An unbiased proteomic analysis of IGFBP-3-interacting proteins in basal-like breast cancer cell lysates has revealed potential new binding partners, with known DNA- and RNA-binding activity, that may be involved, together with IGFBP-3, in the DNA damage response in basal-like breast cancer. Interaction of IGFBP-3 with these proteins in response to chemotherapy is prevented by poly ADP-ribose polymerase (PARP) inhibition, suggesting a role for PARP in IGFBP-3-dependent DSB repair. We propose that targeting the DNA repair function of IGFBP-3 may sensitize basal-like triple-negative breast cancers to chemo- or radiotherapy. Supported by the Australian Research Council and Cancer Institute NSW.

Session IV Cancer

IGFBP-1 HYPERPHOSPHORYLATION INDUCED BY LEUCINE DEPRIVATION IS MEDIATED BY PROTEIN KINASE CK2 AND PROTEIN KINASE C

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Fetal growth restriction (FGR) predisposes infants to childhood and adult metabolic and cardiovascular disease. FGR is associated with impaired maternal-fetal amino acid transfer and diminished fetal availability of essential amino acids, such as leucine. Insulin-like growth factor binding protein-1 (IGFBP-1) secreted by the fetal liver is a key regulator of IGF-I bioavailability and fetal growth. Increased IGFBP-1 phosphorylation increases IGF-I affinity and is associated with FGR. We recently showed that IGFBP-1 hyperphosphorylation in response to leucine deprivation is regulated via activation of the amino acid response (AAR) signaling pathway. We hypothesized that amino acid (leucine) deprivation-induced hyperphosphorylation of IGFBP-1 is mediated by protein kinase CK2, PKC and PKA.

HepG2 cells (model for fetal hepatocytes) cultured with/without leucine (450/0 μ M) were treated with kinase inhibitors (CK2:TBB (1 μ M); pan-PKC: Bisindolylmaleimide (BIS) (7.5 μ M); PKA: PKI (5–24) (100 nM)). Alternatively, cells were transfected with siRNA targeting CK2 holoenzyme (α + α' + β subunits), or pan-PKC (α / β / β II/ γ / δ / ϵ / η / θ / ζ / ν isoforms) and cultured with/without leucine.

Using cell media, quantitative Multiple Reaction Monitoring Mass Spectrometry (MRM-MS) and immunoblotting (phospho-site-specific antibodies) were performed for IGFBP-1 phosphorylation and IGF-I bioactivity (IGF-1R autophosphorylation). Kinase activity was determined using enzymatic assays, ANOVA/Dunnett's Multiple Comparison Post-Test used ($n=3$) for analyses.

MRM MS analysis showed that purified CK2 directly phosphorylated Ser101/119/169. Leucine deprivation in cells stimulated CK2 activity (+300 %) and induced IGFBP-1 phosphorylation (Ser101+800 %, Ser119+300 %, Ser169+600 %). Inhibition of CK2 or PKC, but not inhibition of PKA, prevented IGFBP-1 phosphorylation in response to leucine deprivation. MRM MS analysis validated these findings. Functionally, IGF-I-induced-IGF-1R autophosphorylation was decreased (-85 %) due to leucine deprivation-induced IGFBP-1 phosphorylation, but remained stimulated when CK2 or PKC were inhibited. Stimulation of CK2 by leucine deprivation was effectively prevented by BIS (which specifically inhibited PKC in presence of normal leucine). Unlike CK2, which contains precise or close consensus sequence motifs at Ser101/119/169, PKC lacks these consensus sequences. We suggest that PKC promotes IGFBP-1 phosphorylation at Ser101/119/169 in response to leucine deprivation indirectly via activation of CK2. This study provides a mechanistic link between decreased amino acid availability and reduced fetal growth in FGR.

CONDITIONAL EPITHELIAL CELL-SPECIFIC KNOCKOUT OF CCN6/WISP3 DISRUPTS NORMAL DEVELOPMENT OF THE VIRGIN MURINE MAMMARY GLAND

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Background: CCN6 is a secreted extracellular matrix-associated protein which belongs to the CCN family (named after CTGF, Cyr61 and Nov). CCN6 knockdown in benign human breast cells leads to an epithelial-to-mesenchymal transition and increased resistance to detachment-induced cell death. Further, CCN6 overexpression in breast cancer cells reduces invasion and growth *in vivo* and *in vitro*. Although CCN6 knockout mice were reported to have no evident phenotype, the consequences of mammary epithelial cell-specific CCN6 knockout have never been explored. We hypothesized that the conditional mammary-specific deletion of CCN6 in mice may lead to defects in mammary gland development and may induce mammary tumors.

Methods: We created a novel transgenic mouse model in which CCN6 is conditionally knocked out in mammary epithelial cells using the Cre-Lox recombination system. Conditional knockout was confirmed via PCR, and mice were maintained on an FVB background. We collected groups of CCN6 knockout mice and littermate controls at 8 weeks, 4 months, and 12 months of age to observe the effect of CCN6 knockout on the development of the virgin mammary gland and on *de novo* tumor formation in aged mice. We also collected groups of CCN6 knockout and control mice at early pregnancy, late pregnancy, lactation and involution. Whole mounts of mammary glands were prepared for each time point in order to characterize the phenotype, a portion of the inguinal gland was collected for immunohistochemical analysis, and whole mammary gland lysates were collected for protein and RNA analysis.

Results: Conditional CCN6 knockout in the murine mammary gland results in defective mammary gland development in pubertal (8-week-old) and post-pubertal (4-month-old) virgin female mice compared to controls. At 8 weeks of age, virgin CCN6 knockout mice exhibited

significantly fewer terminal end buds and fewer bifurcated terminal end buds. At 4 months of age, virgin CCN6 knockout mice had fewer lobuloalveolar units and a hypobranching phenotype compared to controls. We observed no phenotypic differences in the pregnancy and lactation timepoints between CCN6 knockout mice and controls. Conditional CCN6 knockout mice spontaneously formed late malignant mammary tumors with spindle cell morphology and led to metastasis.

Conclusion: We provide new evidence that CCN6 is an important signaling molecule in the mammary gland *in vivo*, as CCN6 is necessary for normal mammary development in virginal animals. CCN6 knockout resulted in development of late mammary carcinomas with metastasis, validating our previous work in cell lines and human tissues. We are currently investigating the mechanisms by which CCN6 promotes tumorigenesis.

CCN2 EXPRESSION BY TUMOR STROMA IS REQUIRED FOR MELANOMA METASTASIS

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Metastatic melanoma is characterized by an extremely poor prognosis with few durable remissions. The secreted matricellular protein connective tissue growth factor (CTGF, CCN2) is overexpressed in cancers including melanoma and may represent a viable therapeutic target. However, the mechanism underlying the contribution of CCN2 to melanoma progression is unclear. In this report, we use the highly metastatic murine melanoma cell line B16 (F10) and syngeneic mice in which CCN2 expression is knocked out in fibroblasts, to demonstrate that loss of CCN2, either in melanoma cells or in the niche, impedes the ability of melanoma cells to invade. Specifically, loss of CCN2 in melanoma cells diminished their ability to invade through collagen *in vitro* and loss of fibroblast-derived CCN2 decreased spontaneous metastases of melanoma cells from the skin to the lungs, *in vivo*. Proliferation and tumor growth were not affected by loss of CCN2. CCN2-deficient B16 (F10) cells showed reduced expression of the matricellular protein periostin; addition of recombinant periostin rescued the *in vitro* invasion defect of these cells. Immunohistochemical analysis of CCN2-deficient mice confirmed loss of periostin expression in the absence of CCN2. CCN2 and periostin mRNA levels are positively correlated with each other and with the stromal composition of human melanoma lesions but not BRAF mutations. Thus CCN2 promotes invasion and metastasis via periostin and should be further evaluated as a possible therapeutic target for melanoma.

OBESITY-RELATED CANCERS AND EXTRACELLULAR MATRIX WITH SPECIAL REFERENCE TO CCN MOLECULES

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The extracellular matrix (ECM) is no longer considered an inert substance; while obesity is associated with an increase risk of certain cancers including breast cancer in postmenopausal women, special attention must be given to the microenvironment. The cellular components fibroblasts and macrophages, and the cytokine transforming growth factor- β , have implications in inflammation, cancer, regulation of ECM molecules, adipogenesis and obesity. Obesity along with type 2 diabetes and other metabolic dysfunctions in insulin resistant states, dysregulates the ECM by altering protease levels, which can provide biomarkers of disease states and can correlate with aggressive cancer phenotypes. Moreover, obesity and its associated diseases have been linked to alterations in the expression of the matricellular proteins like CTGF-Cyr61-Nov (CCN), thrombospondins and the secreted protein acidic and rich in cysteine (SPARC). An important growth factor is insulin-like growth factor (IGF), which is concomitant with the matricellular protein family CCN; intriguingly, obesity is also accompanied by aberrant expression of these proteins. Matricellular proteins are non-structural components of the ECM that modulate cell-ECM interactions, have important roles in tumorigenesis including obesity-associated cancers, and possibly contribute to diabetic complications. Of interest were the interactions of matricellular proteins and pro-inflammatory cytokines/adipokines. Obesity promotes an imbalance towards pro-inflammatory adipokines, results in impaired regulation of the sex-steroid hormone estrogen, and necessitates extensive ECM remodeling. ECM components including CCN have a pivotal role in the relation of obesity with subsequent inflammation and possible neoplastic transformation and progression; the precise understanding of which can provide potential therapeutic targets to combat obesity-related pathologies.

A NEW THERAPEUTIC STRATEGY FOR CANCER

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There is growing evidence that all cancers harbor a self-renewing cancer stem cell (CSC) subpopulation responsible for their maintenance and ultimate progression. CSCs, identified by their expression of embryonic relevant stemness markers, have the properties of an unwavering self-renewal capacity and an intrinsic resistance to apoptosis and drugs that constitute the basis of their resilience. The oncogenic drivers and passengers for many cancers have already been defined leading to development of novel therapeutic approaches based on selective targeting. Knowledge about the contrasting metabolome of cancers has also generated new drug targets, and dramatic recent successes with immunotherapy have raised the hopes of many with cancer. Despite these encouraging advances there is still a long way to go. The actual statistics on cancer frequency (appreciable decreases for some types, e.g. lung) but 2nd for deaths overall (1 of every 4, USA), with often limited, durable responses, and even more limited proven cures, temper the enthusiasm. Altering lifestyle habits could play a big part in reducing the cancer burden and here effective chemoprevention might be advantageous. Ultimately, drug resistance and the ever-changing cellular and genetic landscape during malignant progression bring an obvious degree of reality to the effort and one that continues to spur renewed intensive attempts to solve the cancer mystery. In considering all these aspects we took the view that cancers, like normal cells, must rely on a set of homeostatic controls that prevent collapse of the system. How then can one really pull the plug on cancer and especially on the CSCs of cancers?

We have obtained evidence that particular enzymes governing pH control (homeoregulation), the carbonic anhydrases (CA), play a critical role in

tumor cell survival, clonogenicity *in vitro* and tumorigenicity in xenograft models. We have shown that small molecules with robust cancer chemopreventive or epigenetic modulating properties, and overt anti-tumor capability via interference in tumor cell signaling pathways, cooperate with pharmacological inhibitors of CA to potently suppress tumor growth and survival and the ability for self-renewal. Observed loss of stemness marker expression *in vivo* (pre-clinical xenograft models), and diminished capacity for serial heterotransplantation, after treatment with the combination of such molecules, supports the idea of abrogation of the cancer stem cell subpopulation. We will present evidence on both pediatric (neuroblastoma) and adult cancer (carcinoids, bladder) models that support our working hypothesis. Importantly, clinical amenability and negligible intrinsic toxicity favor their clinical implementation of such therapeutic combinations.

Session V Skin Biology and Fibrosis

REGULATION OF CCN1 AND CCN2 BY TRANSCRIPTIONAL CO-ACTIVATOR YAP IN HUMAN SKIN: IMPLICATIONS FOR CANCER

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Yes-associated protein (YAP) is a transcriptional co-activator that is the downstream effector of the hippo signaling pathway, which plays an important role in control of organ size and tumorigenesis. YAP activity is also controlled by the cellular mechanical environment, and thereby mediates responses of cells to substrate adhesion. Interestingly, both CCN1 and CCN2 are directly regulated by YAP. In human skin basal cell carcinoma (BCC) tumor islands, YAP and its downstream transcriptional targets CCN1 and CCN2 are markedly elevated in keratinocytes. In human keratinocytes, knockdown of YAP significantly reduces expression of CCN1 and CCN2 and represses proliferation and survival. This inhibition of proliferation and survival is rescued by restoration of CCN1 expression, but not by CCN2 expression. In BCC stroma, CCN2-regulated genes type I collagen, fibronectin, and α -smooth muscle actin are highly expressed. Furthermore, atomic force microscopy (AFM) reveals increased tissue stiffness in BCC stroma, compared to normal dermis. These data provide evidence that up-regulation of YAP in BCC impacts both aberrant keratinocyte proliferation, via CCN1, and tumor stroma cell activation and stroma remodeling, via CCN2. Targeting YAP and/or CCN1 and CCN2 may provide clinical benefit in BCC.

IS CCN3 A KEY REGULATOR OF EPIDERMAL AND DERMAL HOMEOSTASIS ?

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We have previously observed that CCN3 is modulated in vitiligo, a hypopigmentary disorder and in systemic sclerosis, a dermal disease associated with pigmentary troubles. *In vivo*, in a mouse skin xenograft model, we have induced a caucasian-negroid switch which was associated with a differential expression of CCN3. However, the correlation between the level of CCN3 and the degree of pigmentation differed according to physiological or pathological the skin status. To clarify the link CCN3/pigmentation, we revealed CCN3 on skin sections with various phototypes by immunohistochemistry and quantified fluorescence in epidermal and dermal cells. CCN3 was significantly higher in epidermis and dermis of phototypes III-VI as compared to phototypes I-II. This result was in accordance with those observed in xenografted skin. Thus, in basal condition CCN3 level seemed associated with the level of eumelanin. CCN3 is regulated by molecules implicated in pigmentation such as estrogen, endothelin 1, FGF-2, vitamin D, UV. Thus, to determine if CCN3 is directly implicated in regulation of pigmentation, we produced a shRNA lentivirus inhibiting CCN3 and expressing GFP. We then transduced melanocytes, keratinocytes and fibroblasts the main cells implicated in pigmentation. Melanocytes did not survive when CCN3 was inhibited whereas keratinocytes and fibroblasts proliferated. To understand this discrepancy we analyzed expression of CCN3 in these cells, over the time after transduction by western blot. To our surprise we noticed that CCN3 in melanocytes was clearly inhibited and this inhibition induced in turn an inhibition of DDR1 which explained the detachment of shCCN3-transduced melanocytes in culture. On the contrary for keratinocytes and fibroblasts we observed a transient decrease in CCN3. Indeed, around 4-6 days post-transduction CCN3 was inhibited whereas 10-15 days post-transduction, even if GFP was already expressed in cells, CCN3 was no longer inhibited. Since it was difficult to analyze CCN3 effect on pigmentation using this transduced cells in our reconstructed models due to variations of CCN3 level, we cultured fibroblasts from scleroderma patients with hyperpigmentary troubles. On one hand we analyzed CCN3 expression in these cells and in the other hand we incubated normal reconstructed epidermis with medium conditioned by scleroderma fibroblasts. Preliminary data revealed that scleroderma fibroblasts expressed different isoforms of CCN3 and that conditioned media modified melanocytes homeostasis. In conclusion, these results point out the critical role of CCN3 in skin cells, revealed that CCN3 is tightly regulated in fibroblasts and keratinocytes and that pathways induced by CCN3 inhibition were skin cell-dependent

FIBROSIS ASSOCIATED TO SKELETAL MUSCLE DENERVATION AND TO AMYOTROPHIC LATERAL SCLEROSIS (ALS) MODEL: ROLE OF CTGF/CCN2

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Background: Muscular fibrosis corresponds to an excessive accumulation of extracellular matrix (ECM) replacing functional tissue, a characteristic found in several myopathies and neuropathies. Therefore, the knowledge of pro-fibrotic factors biology and its regulation is critical. Chronic muscular damage reduces vascularization and adequate blood flow, challenging the maintenance of adequate oxygen availability. This triggers ischemic foci and activate Hypoxia-inducible pathways. Connective Tissue Growth Factor (CTGF/CCN2), a key factor promoting fibrosis is up-regulated by Hypoxia-inducible Factor α (HIF-1 α) in different models of renal fibrosis; whether this modulation also occurs in skeletal muscle remains unknown.

We evaluated skeletal muscle fibrotic markers and the role of CTGF and HIF-1 α associated to mouse sciatic denervation and to an Amyotrophic Lateral Sclerosis (ALS) model (hSOD1^{G93A} transgenic mouse), a fatal neurodegenerative disease characterized by degeneration of upper and lower motor neurons, causing muscle denervation, atrophy and progressive paralysis.

Design/Methods: Fibrotic markers, CTGF and HIF-1 α pathway were evaluated by IFI and WB in our models of sciatic denervation and ALS. Decreased CTGF in our models was used to evaluate whether fibrotic characteristics on muscle of both models are CTGF dependent. Pharmacological stabilization of HIF-1 α was used to evaluate whether HIF-1 α by itself is able to induce CTGF and fibrosis.

Results: In our models of sciatic denervation and symptomatic ALS we found an increase of TGF- β signaling and CTGF together with ECM molecules. In both models, the increment in fibrotic markers parallel with an augmented expression and nuclear localization of HIF-1 α , together with increased target genes of this pathway. When the same parameters were evaluated in CTGF deficient mice, we observed that the extent of fibronectin and collagen deposition was reduced compared to control mice. Pharmacological stabilization of HIF-1 α increases CTGF and ECM deposition in both healthy and damaged (denervated) skeletal muscle.

Conclusions: Our observations suggest that ALS skeletal muscle fibrosis might be related to muscle denervation and not necessarily to transgene expression. On the other hand, while HIF-1 α activation could be a compensatory muscle response leading to improve oxygen availability upon damage and under pathological conditions, it might also be participating in the development of muscular fibrosis. Our results suggest that HIF-1 α pathway needs CTGF to fully exert its fibrotic role. We emphasize the importance of understanding the balance between these two effects in order to develop therapeutic alternatives.

(*Both authors contributed equally. Supported by FONDECYT 115016 to EB, 3140357 to DR, predoctoral fellowship from CONICYT to DG and CARE-UC PFB12/2007)

WNT3A-MEDIATED RESCUE OF VASCULAR SMOOTH MUSCLE CELLS (VSMCs) FROM OXIDATIVE STRESS INDUCED APOPTOSIS BY CCN5

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Cardiovascular diseases remain the leading cause of global mortality. Atherosclerosis is the underlying cause of cardiovascular disease and is characterised by lipid-filled inflammatory plaques within the blood vessel wall. Viability of vascular smooth muscle cells (VSMCs) within the fibrous cap of atherosclerotic plaques is pivotal to preventing plaque rupture, subsequent thrombosis and myocardial infarction. We previously demonstrated that oxidative stress-induced VSMC apoptosis is reduced by Wnt5a via induction of CCN4 via a β -catenin/CREB dependent, TCF independent, signalling pathway. In this study, we reveal that another Wnt member, Wnt3a, can also rescue VSMCs from oxidative stress-induced apoptosis but contrastingly this is via up-regulation of CCN5 via β -catenin/TCF signalling.

We detected Wnt3a protein expression within VSMC-rich regions of atherosclerotic plaques from apolipoprotein-E deficient mice. We showed 400 ng/ml recombinant Wnt3a protein significantly suppressed H₂O₂-induced apoptosis by 60.7 $\pm$ 2.6 % ($n=4$, $p<0.05$) in primary mouse VSMCs. Furthermore, we found Wnt3a, in the presence of H₂O₂, resulted in significantly increased β -catenin nuclear translocation (2.0 $\pm$ 0.2 fold vs. control, $n=4$, $p<0.05$). Unlike Wnt5a, Wnt3a did not induce CREB activity as shown by Western blotting for phospho-ser133-CREB. Interestingly, we

found that unlike Wnt5a, Wnt3a in the presence of H₂O₂, activated TCF as shown by TOPFLASH luciferase assay (40.9 $\pm$ 11.6 fold vs. control, $n=6$, $p<0.05$) and induction of the TCF-responsive genes Axin-2 and Tcf-7 (66.0 $\pm$ 13.2 and 3.9 $\pm$ 0.5 fold vs. control, respectively, $n=3$, $p<0.05$). Furthermore, we showed that TCF was necessary for the anti-apoptotic effect of Wnt3a as inhibition of β -catenin/TCF signalling using CCT031374 hydrobromide significantly reduced Wnt3a-mediated rescue by 58.9 $\pm$ 19.4 % ($n=4$, $p<0.05$). We showed that Wnt3a induced expression of both CCN4 (1.9 $\pm$ 0.2 fold vs. control, $n=6$, $p<0.05$) and CCN5 mRNA (2.2 $\pm$ 0.3 fold vs. control, $n=9$, $p<0.05$). Importantly, CCN4 siRNA did not impair the anti-apoptotic effects of Wnt3a whereas neutralisation of CCN5 significantly inhibited Wnt3a-mediated rescue of apoptosis by 40.9 $\pm$ 14 % ($n=5$, $p<0.05$), suggesting that CCN5 but not CCN4 is necessary for Wnt3a-mediated survival.

Our data suggests that Wnt3a protein is expressed in atherosclerotic plaques within VSMC-rich regions. We have shown that Wnt3a can rescue primary mouse VSMCs from oxidative stress-induced apoptosis. Unlike Wnt5a, the anti-apoptotic effect of Wnt3a involves β -catenin/TCF signalling and CCN5 expression. Further understanding of the effects of Wnt3a and CCN5 in VSMCs may enable identification of therapeutic targets to promote VSMC survival in atherosclerosis.

A NEW SMART BLOOD CONCENTRATE FOR TISSUE ENGINEERING IN REGENERATIVE MEDICINE: THE I-PRF

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Platelet concentrates, as PRP and PRF, are used routinely in medicine and reconstructive therapies. However the use of platelets is not enough to enhance the regeneration of a tissue.

We started to work on a new concept of blood concentrate which will provide platelets, fibrin, plasma protein, inflammatory cells and mesenchymal stem cells. And we named it a "smart blood concentrate".

The most difficult is to obtain stem cells simply and with low invasive approach. Until now, I was impossible to collect the MSCs from the blood. Obviously because the centrifugation concept was wrong.

We introduce with Dr Ghanaati the "low speed concept" which is a centrifugation of the whole blood at a very low speed. In order to collect more inflammatory cells and mesenchymal stem cells.

We present in this lecture our results of the data, our clinical results in cartilage regeneration and how we can produce easily, in the medical office, many thousands of MSCs in few minutes and with a low cost.

Session VI Pathobiology and CCN proteins

CCN1 EXPRESSION INCREASES IN MICE WITH ACUTE LUNG INJURY, AND IS ASSOCIATED WITH AN ENHANCED FIBROPROLIFERATIVE RESPONSE

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We have previously shown that CCN1 overexpression is associated with the development of acute lung injury (ALI) in mice. In humans, ALI is characterized by an acute inflammatory response followed by a fibroproliferative repair phase. In this study, we seek to, first, confirm that CCN1 is upregulated in different models of ALI, and second, determine whether CCN1 modulates the fibroproliferative response that follows ALI. Specifically, we investigate CCN1 expression in the following three models of ALI: Fas activation, mechanical ventilation (VILI) and bleomycin. We found that CCN1 expression increases in the alveolar walls of mice exposed to the Fas-activating antibody Jo2. Similarly, CCN1 expression also increases in the lungs of mice exposed to tidal volumes of 20 cc/kg for 5 h. Exposure of the lung alveolar epithelial cell line MLE12 to 1 h of stretch resulted in increased expression of CCN1. Finally, CCN1 expression increased 10 days after bleomycin instillation, and returned to normal on days 15 and 24. The next step was to determine whether CCN1 modulates the fibroproliferative response that follows bleomycin exposure. Interestingly, we found that the administration of recombinant CCN1 to mice enhanced collagen expression in bleomycin-treated mice. Similarly, adenoviral expression of CCN1 for 8 days resulted in increased collagen expression. We conclude that CCN1 expression increases in the lungs of mice in the setting of lung injury, and is associated with an increased fibroproliferative response.

THE ROLE OF CTGF-BETA-CATENIN SIGNALING IN THE PATHOGENESIS OF BPD

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Bronchopulmonary dysplasia (BPD) is the most common and serious chronic lung disease of premature infants that is characterized by impaired alveolarization and interstitial fibrosis. While oxygen toxicity plays a key role in the pathogenesis of this disease, the cellular and molecular mechanisms are poorly understood and there is no effective therapy. Recent studies have found that CTGF expression is increased in clinical BPD and in hyperoxia-induced rodent models of BPD. In order to determine the mechanistic role of CTGF in the pathogenesis of BPD, we generated a novel inducible transgenic mouse model with overexpression of CTGF in type II alveolar epithelial cells (AEC). Induction of CTGF expression in the neonatal lung resulted in hypoalveolarization and interstitial fibrosis, the pathological hallmarks of BPD. In vitro overexpression of CTGF in AEC induced epithelial to mesenchymal transition (EMT). These histopathological changes were associated with activation of β -catenin signaling. Furthermore, inhibition of β -catenin signaling improved alveolarization and decreased interstitial fibrosis in CTGF transgenic lungs. Utilizing neonatal rat model of BPD, we tested the effects of CTGF neutralizing antibodies and β -catenin inhibitors in the prevention of hyperoxia-induced lung injury. We found that treatment with CTGF antibodies increased alveolarization, decreased interstitial fibrosis, reduced pulmonary vascular remodeling and pulmonary hypertension in hyperoxia exposed rats. In addition, CTGF antibody blocked β -catenin signaling activation during hyperoxia. Similarly, treatment with β -catenin inhibitors improved alveolarization, and decreased pulmonary vascular remodeling and pulmonary hypertension in hyperoxic rats. These finds indicate that CTGF- β -catenin signaling pathway plays a critical role in the pathogenesis of BPD, and targeting this pathway may provide novel therapy for this disease.

WISP1 (CCN4) MEDIATES IL6-DEPENDENT PROLIFERATION IN PRIMARY HUMAN LUNG FIBROBLASTS

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Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease, which is characterized by progressive scarring of the lung, alveolar epithelial type II (ATII) cell hyperplasia and an impaired epithelial-mesenchymal crosstalk. We recently showed that Wnt1-inducible signaling protein 1 (WISP1) is involved in this crosstalk. WISP1 increases ATII cell proliferation in vitro and upregulates collagen production and myofibroblast marker in lung fibroblasts. The regulation of WISP1 as well as how WISP1 exerts its downstream function in primary human lung fibroblasts (phLFs), however, is only incompletely understood. We here show that WISP1 is a common downstream target of the pro-fibrotic cytokines Tissue growth factor β 1 (TGF β 1) and Tumor necrosis factor α (TNF α) in phLF using RT-qPCR and ELISA. TGF β 1 and TNF α significantly increased WISP1 mRNA expression (TGF β 1: 1.92 ± 0.23 fold; TNF α : 4.61 ± 0.68) and secretion (control vs. TGF β 1: 24.02 ± 5.88 pg/ml vs. 75.52 ± 1.98 pg/ml; control vs. TNF α : 20.47 ± 3.66 pg/ml vs. 35.02 ± 3.63 pg/ml). Moreover, the presence of WISP1 was required for TGF β 1- and TNF α -dependent induction of IL6 in phLFs. Upon siRNA-mediated knockdown of WISP1, we observed a significant reduction of TGF β 1- and TNF α -induced IL6 expression (siCtrl+TGF β 1 vs. siWISP1+TGF β 1: IL6 mRNA 1.81 ± 0.36 fold over control vs. 0.81 ± 0.1 fold over control; siCtrl+TNF α vs. siWISP1+TNF α : IL6 mRNA 5.04 ± 1.29 fold over control vs. 2.78 ± 0.81 fold over control) and IL6 secretion (siCtrl+TGF β 1 vs. siWISP1+TGF β 1: 2.55 ± 0.16 pg/ml vs. 0.96 ± 0.11 pg/ml; siCtrl+TNF α vs. siWISP1+TNF α : 22.35 ± 0.55 pg/ml vs. 12.21 ± 2.4 pg/ml) compared to control siRNA transfected phLFs. Furthermore, loss of WISP1 led to a significant reduction in phLF proliferation by 22.9 ± 2.8 %. This loss in proliferation was in part mediated by the reduction in IL6. Taken together, our results show that WISP1 is a target gene of TGF β 1 and TNF α . Moreover, the presence of WISP1 is required for TGF β 1- and TNF α -dependent induction of IL6 in phLFs. Importantly, WISP1 mediates proliferation of phLFs via IL6. These results further strengthen the pro-fibrotic functions of WISP1 in lung fibrosis and thus corroborate the potential benefit of targeting WISP1 in IPF therapy.

ALTERED EXPRESSION OF THE CCN GENES IN SMOKING-RELATED AND INFECTIOUS LUNG DISEASES

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Inflammation and tissue repair are involved in smoking-related and infectious lung diseases. However, despite the role played by the matricellular CCN proteins in these processes in several organs, only few reports have documented their expression in acute or chronic pulmonary diseases. We therefore examined whether several triggers of lung inflammation alter the expression of the CCN genes *in vivo*. Using mouse models, we investigated the effect of cigarette smoke (CS) exposure and viral and bacterial infections on the lung expression of CCN1 to CCN5 (*Cyr61*, *CTGF*, *Nov*, *WISP-1* and *WISP-2*, respectively). CCN1 and CCN2 were down-regulated by acute smoke exposure (4 days) whereas a prolonged exposure (2 months) to CS decreased mRNA expression of CCN1, CCN3 and CCN4. Influenza A virus infection triggered an induction of CCN1, 2, 4 and 5 expression while expression of CCN3 was significantly decreased. Lung infection with nontypeable *Haemophilus influenzae*, and LPS or LTA administration revealed that both Gram-positive and Gram-negative bacteria may rapidly modulate expression of the CCN genes. We also analyzed the CCN expression in human lung tissues in association with smoke exposure, chronic obstructive pulmonary disease (COPD) and non-small cell lung cancer (NSCLC). Consistent with our observations on the effects of a prolonged CS exposure in mice, expression of CCN3 and CCN4 was significantly decreased in tissue of smokers compared with lung tissue from nonsmokers ($p < 0.05$). In patients suffering from COPD, the mRNA levels of CCN1 significantly increased in relation to the degree of severity of this disease ($p < 0.01$). Finally, CCN1, CCN2, CCN3 and CCN5 were found down-regulated and CCN4 upregulated in NSCLC ($p < 0.001$). Collectively, our data support the hypothesis that CCN proteins may play distinctive roles in several lung diseases including bacterial and viral pneumonias, COPD and NSCLC.

CCN2 DELETION FROM THE NUCLEUS PULPOSUS ACCELERATES INTERVERTEBRAL DISC DEGENERATION AND RESULTS IN BEHAVIOUR ASSOCIATED WITH BACK PAIN IN MICE

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Introduction: Currently, our ability to treat intervertebral disc (IVD) degeneration is hampered by an incomplete understanding of the pathways regulating disc aging and the proteins contributing to symptomatic vs. asymptomatic disc degeneration. In particular, the specific function of matricellular proteins including CCN2, in modulating these pathways remains enigmatic. Recently, we reported that a mouse strain with a targeted deletion of CCN2 from the notochord and nucleus pulposus (NP) (referred to as CCN2-deficient) demonstrated not only aberrant matrix content during development but also accelerated age-related degeneration. Herein, we further characterize the mechanism underlying IVD degeneration observed in CCN2-deficient mice by examining markers of inflammation and matrix breakdown. Moreover, we determine whether CCN2-deficient mice show behaviours associated with discogenic back pain.

Methods: Inflammation and matrix breakdown were assessed in IVD tissues from notochord-specific CCN2-deficient mice and littermate controls at 6, 9, 13 and 18 months of age using gene expression, histological and immunohistochemical analyses. Behaviours associated with axial pain were evaluated using established grip force, tail suspension and locomotor assays to measure stretch-induced axial discomfort.

Results: Compared to control mice, CCN2-deficient mice showed histological hallmarks of disc degeneration by 9 months of age. Analysis of gene expression demonstrated increased expression of the proinflammatory cytokines *Tnf- α* , *Il-1 β* and *Mmp9* in CCN2-deficient mice at 13 months of age, and increased expression of brain-derived neurotrophic factor (*Bdnf*), *Mmp3* and *Mmp7* at 18 months of age. Immunolocalization showed increased accumulation of MMP-mediated aggrecan cleavage products in the NP of CCN2-deficient mice at 18 months of age. CCN2-deficient mice showed reduced grip force at 9, 13 and 18 months of age. 9 and 13-month-old CCN2-deficient mice also show decreased time spent in full extension and an increased time spent self-supported in tail suspension, indicative of increased discomfort induced by axial stretching. Lastly, the locomotor activity of CCN2-deficient mice at 13 months of age was significantly reduced immediately following tail suspension, indicative of increased movement-evoked discomfort.

Significance: CCN2 expression by nucleus pulposus cells regulates IVD tissue health, as accelerated degeneration in CCN2-deficient mice was associated with inflammatory cytokine expression and MMP-mediated matrix degradation. Furthermore, our findings suggest that CCN2-deficient mice demonstrate symptomatic disc degeneration. The ability of CCN2 to regulate the composition of the intervertebral disc suggests that it may represent an intriguing target for the treatment of disc degeneration. Further, our CCN2-deficient mouse model may be useful to probe the connection between disc degeneration and associated back pain.

CCN4 IS PROTECTIVE AGAINST ATHROSCLEROSIS AND VULNERABILITY TO PSEUDO-ANEURYSM IN APOE^{-/-} KNOCKOUT MICE

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We have previously demonstrated that CCN4 promotes smooth muscle cell migration following vascular injury, and suppresses smooth muscle cell apoptosis. In this study we examined its effect on progression of atherosclerosis and plaque composition.

Apolipoprotein E null (ApoE^{-/-}) mice were crossed with CCN4^{-/-} mice to produce ApoE^{-/-}CCN4^{-/-} and ApoE^{-/-}CCN4^{+/-} littermate controls. Eight week-old male mice were fed a high-fat diet for 10 weeks to induce atherosclerotic lesion formation. After which the mice were perfusion fixed and the brachiocephalic arteries were removed for histological analysis.

Deletion of CCN4 resulted in a 3.7 fold increase in brachiocephalic artery lesion size, leading to an increase in the occlusion of the lumen from 20.3 $\pm$ 6.3 % to 57.7 $\pm$ 10.5 % ($P < 0.05$; t-test). The brachiocephalic arteries from ApoE^{-/-}CCN4^{-/-} mice also exhibited an increased incidence in breaks within the medial elastic lamellae (pseudo-aneurysms) from 62 % to 100 % ($P < 0.05$; Fisher's exact test), implying a higher vulnerability for aneurysm formation. The total number of apoptotic cells increased from 0.17 $\pm$ 0.13 % in wild-type to 1.53 $\pm$ 0.81 % in plaques from ApoE^{-/-}CCN4^{-/-} mice ($P < 0.05$; Mann Whitney test). Enhanced apoptosis may therefore underlie the transition to a more vulnerable plaque phenotype and pseudo-aneurysm formation observed in ApoE^{-/-}CCN4^{-/-} mice.

This study indicates that CCN4 may be protective against both atherosclerosis and aneurysm formation. Consequently, strategies to increase CCN4 expression may prove useful for the treatment of cardiovascular diseases.

MATRICELLULAR PROTEIN CCN3 MITIGATES ABDOMINAL AORTIC ANEURYSM

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Abdominal aortic aneurysm (AAA) is a major cause of morbidity and mortality and our understanding of mechanisms involved in disease initiation and progression is incomplete. Extracellular matrix proteins play an integral role in modulating vascular homeostasis in health and disease. In this study, we find that matricellular protein CCN3 expression is strongly reduced in rodent models of AAA (Angiotensin II (AngII) infusion onto an *Apoe*-null background), findings recapitulated in human AAA aortas. Using both AngII infusion and elastase-induced rodent models of AAA, we provide evidence that germline deletion of CCN3 in mice results in severe AAA characterized by elastin fragmentation, vessel dilation, vascular inflammation/dissection, heightened reactive oxygen species generation and smooth muscle cell loss. Conversely, lentiviral mediated overexpression of CCN3 mitigates elastase-mediated AAA progression in mice. Bone marrow transplantation experiments indicate the AAA phenotype of CCN3-deficient mice is intrinsic to the vasculature. Mechanistic studies utilizing genetic and pharmacological approaches suggest the ERK1/2 pathway is a critical regulator of CCN3-dependent AAA development. These results demonstrate CCN3 as a nodal regulator in AAA biology and identify CCN3 as a potential therapeutic target for vascular disease.