A9 A FRAGMENT OF TYPE II PROCOLLAGEN, CHONDROSTATIN, INHIBITS ANGIOGENESIS

Z. Wang[1], J. Bryan[1], B.J. Ell[2], A.C. Rapraeger[2], L. Sandell[1].
[1]Washington University, St. Louis, MO, USA, [2]University of Wisconsin-Madison, Madison, WI, USA

Purpose: A fragment of type II procollagen, chondrostatin, contains the classic integrin binding sequence RGDRGD. This sequence is conserved across species, indicating potential biological function. We have previously shown that the integrin αvβ3 and αvβ5 mediate cell adhesion to chondrostatin. A substantial and persuasive body of data supports the view that integrins plays a critical role in the pathogenesis of arthritic diseases by participating in cell migration, apoptosis and other cellular processes. The goal of this study was to determine effects of chondrostatin on angiogenesis.
Methods: Site-directed mutagenesis was used to mutate the RGD to RAD in chondrostatin. The tube formation assay was performed by seeding HUVEC into a Matrigel-coated plate in the presence of recombinant proteins. The formation of tubes was visualized by light microscopy and quantified by a Q Capture Pro imaging software. The aortic ring formation assay was performed by culturing aorta rings of 5 to 10-week Fischer rat in 3D collagen gels at 37ºC for a week in the presence of FGF and recombinant proteins. The image was captured with an Olympus microscope and total length of the microvessel outgrowth was quantified. Mouse corneal assay was performed by implanting polyHEMA pellets containing FGF into the mouse corneas of 6-week-old Balb/c mice. The mice received osmotic pumps containing recombinant proteins for one week. The mice were sacrificed and the total length of fluorescently labeled vessels was quantified.
Results: Three different assays were performed to determine the ability of chondrostatin to inhibit angiogenesis. In the tube formation assay, recombinant chondrostatin (Fig 1B), but not mutated chondrostatin (data not shown) or GST alone (Fig 1A), inhibited HUVEC cell tube formation in a dose-dependent manner (Fig 1C). In the aortic ring assay, chondrostatin, but not mutated chondrostatin or GST alone, inhibited FGF-stimulated microvessel outgrowth (Fig 2A). The inhibition is also dependent on the concentration of chondrostatin (Fig 2B). In the in vivo mouse corneal assay, chondrostatin inhibited vascularization as seen in the fluorescence-labeled mouse cornea (Fig 3A). The inhibition is dose-dependent, as 5 μM of chondrostatin only inhibited the vessel formation by 40% while 25 μM of chondrostatin suppressed vessel formation by 65% of the control (Fig 3B).

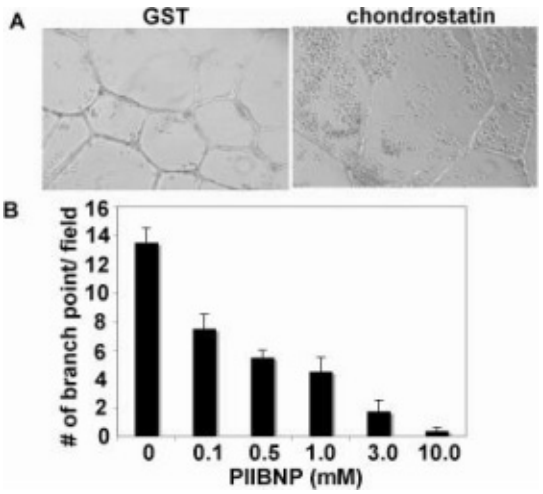

Fig. 1. Chondrostatin inhibits HUVEC tube formation: *in vitro* tube formation assay.

Conclusions: We previously showed that integrins αvβ3 and αvβ5 mediated chondrostatin-cell interaction. As endothelial cells express these integrins, this study was designed to investigate the effect of chondrostatin on angiogenesis. The results from three different angiogenesis assay methods showed that chondrostatin is an effective angiogenesis inhibitor and the inhibition is RGD-dependent. The ability of the chondrostatin to inhibit angiogenesis makes it a potential antitumor agent, since microvascular endothelial cells, which are recruited by tumors, have become an important target in tumor therapy. In addition, chondrostatin is a naturally occurring peptide liberated from the collagen molecule during

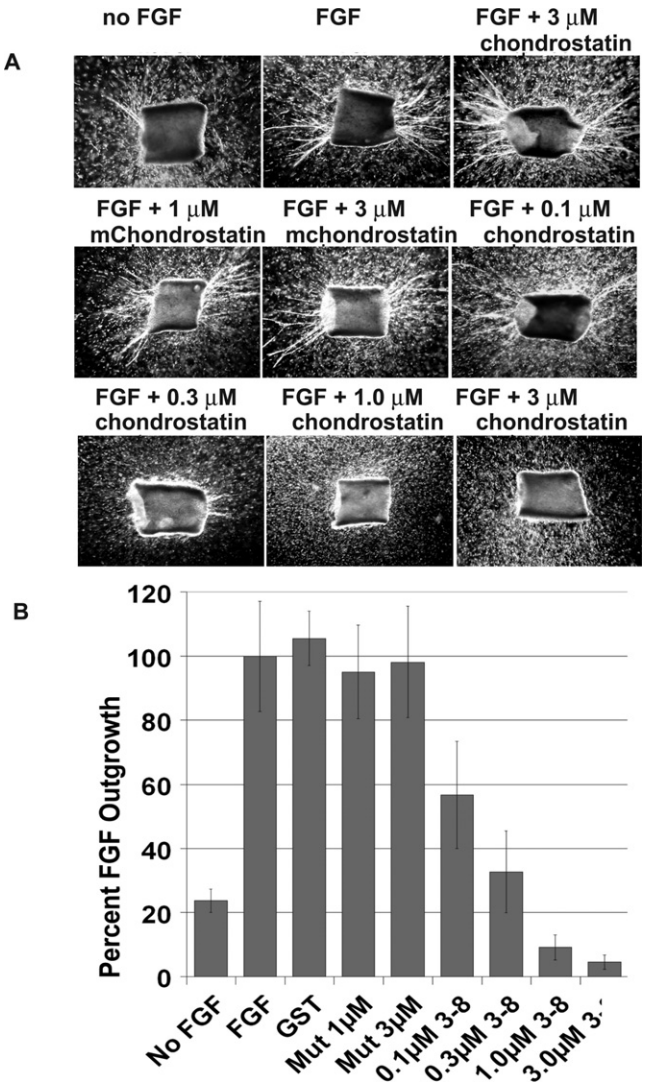

Fig. 2. Chondrostatin inhibits rat aortic ring outgrowth: rat aortic ring assay.

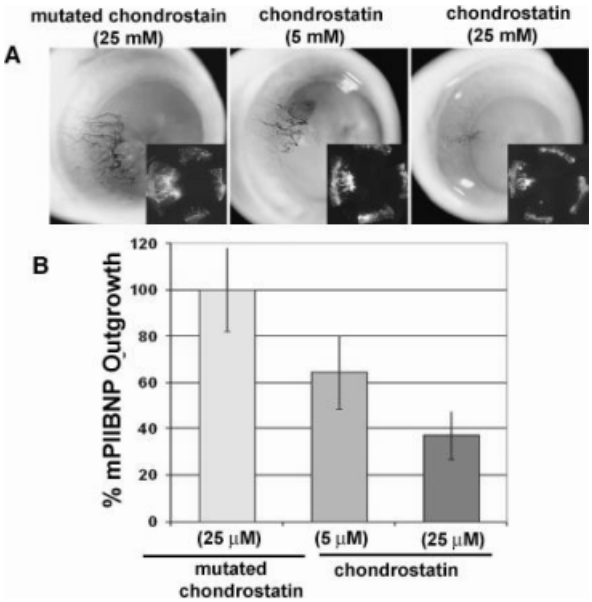

Fig. 3. Chondrostatin inhibits mouse corneal vascularzation: mouse corneal assay.

biosynthesis and contains vicinal RGD motif that bind specifically to αVβ3 and αVβ5 integrins. Since chondrostatin reaches the highest level during cartilage formation and it inhibits angiogenesis, it is an excellent candidate for the molecular mechanism by which cartilage remains avascular.

A10 TRANSFORMING GROWTH FACTOR-BETA INDUCES PERSISTENT SYNOVIAL FIBROSIS WHILE CONNECTIVE TISSUE GROWTH FACTOR-INDUCED FIBROSIS IS REVERSIBLE. ROLE FOR LYSYS HYDROXYLASE

E.N. Blaney Davidson[1], E.L. Vitters[1], N. Oliver[2], W.B. van den Berg[1], P.M. van der Kraan[1]. *[1]University Medical Centre St. Radboud, Nijmegen, NETHERLANDS, [2]FibroGen Inc., South San Francisco, CA, USA*

Purpose: The main characteristics of osteoarthritis are cartilage damage, osteophyte formation and synovial fibrosis. Synovial fibrosis can contribute to the loss of the function of an OA joint. TGF-β is considered one of the main players in fibrotic diseases. However, CTGF, which can be induced by TGF-β, has been reported to be an important player as well, stated to contribute to maintenance of fibrotic tissue. We investigated the underlying mechanism in that leads to the observed difference in fibrosis persistence between TGF-β- and CTGF.
Methods: We injected C57Bl/6 mice intra-articularly with Ad-TGF-β or Ad-CTGF. After 3, 7 and 21 days knee joint synovial tissue was isolated for RNA isolation or whole knee joints for histology. With Q-RT-PCR relative mRNA levels were determined of matrix molecules, proteases, protease inhibitors, and growth factors. To analyze the enzymes involved in collagen cross link formation, mRNA expression of lysyl hydroxylase (LH) was determined.
CTGF-levels induced by TGF-β were measured by ELISA in 24 hour patella-synovial wash-outs of C57Bl/6 mice intra-articularly injected with Ad-TGF-β (3, 7 and 14 days).
Results: TGF-β and CTGF induced synovial fibrosis in murine knee joints as shown by histology (as observed by an increase in synovial width). However, TGF-β-induced fibrosis was very persistent, whereas CTGF-induced fibrosis resorbed by day 28.
TGF-β induced elevated collagen type I and very high levels of aggrecan expression and hardly any changes in collagen type II and III. CTGF induced no clear changes in collagen type I, II and III. CTGF even decreased aggrecan expression (day 21 only).
CTGF induced no changes in MMP3, -9, -13, ADAMTS-4 or -5, whereas TGF-β increased MMP-3, -13 and ADAMTS4. Thus elevated levels of matrix degrading enzyme expression cannot explain the less persistent CTGF-induced fibrosis.
TIMP1 (associated with matrix accumulation in fibrotic disorders), was highly up regulated by TGF-β (all days) and only slightly up regulated by CTGF (day 7). TGF-β also induced elevated mRNA levels of TGF-β1 and CTGF.
Only TGF-β induced high levels of LH expression, especially LH2b, which has been implicated in hard-to-degrade collagen linkage and was found up regulated in fibrotic lesions.
As TGF-β can induce CTGF and up regulated CTGF mRNA in our experiments, we measured CTGF levels in 24 hour patella-synovial wash outs and found that TGF-β induced 64 ng CTGF/ml after 3 days and that CTGF levels were still elevated by day 14.
Conclusions: TGF-β induced increased collagen type I and aggrecan mRNA levels in synovial tissue, but also high levels of degrading enzymes. The latter does not explain the persistent nature of TGF-β versus CTGF-induced synovial fibrosis. Lack of collagen type I mRNA induced by CTGF corresponds to findings of other groups. Despite this fact, fibrosis is found. Only TGF-β induced high levels of TIMP1 expression, which was previously found associated with strain dependent fibrosis sensitivity in mice and implicated in accumulation of ECM in fibrotic disorders. TGF-β, not CTGF, induced high levels of LH which are crucial for ECM cross linking. Moreover, LH2b was reported by van der Slot et al. to induce harder-to-degrade crosslinks compared to LH2a. The 2b splicing variant was highly expressed upon stimulation with TGF-β. Since TGF-β clearly up regulated MMP expression a dominant role for TIMP appears unlikely. The strong induction of LH2b by TGF-β compared to CTGF, and consequently harder to degrade cross links, appears to be the most likely cause of the induction of irreversible fibrosis by TGF-β.

A11 EFFICACY OF A SINGLE ULTRASOUND GUIDED INJECTION IN HIP OSTEOARTHRITIS

I. Atchia[1], M. Reed[1], D. Kane[2], J. Isaacs[2], F. Birrell[1]. *[1]Northumbria Healthcare NHS Foundation Trust, Northumberland, UNITED KINGDOM, [2]Musculoskeletal Research Group, Newcastle, UNITED KINGDOM*

Purpose: There is increasing evidence for intra-articular hyaluronic acid and especially corticosteroids in the treatment of osteoarthritis (OA) from studies using fluroscopy guidance or triple ultrasound guided injections. Having previously demonstrated the accuracy of ultrasound guided injection and how competency can be achieved we aimed to demonstrate the duration of objective improvement in pain, function and global health assessment in patients with moderate/severe hip OA with a single intra-articular injection.
Methods: 77 patients with hip OA were recruited to a prospective, randomised controlled trial and randomised to one of four groups: standard care (non-injection group), normal saline (3 mls), non-animal stabilized hyaluronic acid (durolane, 3 mls/60 mg) and methylprednisolone acetate (depomedrone, 3 mls/120 mg). All patients met the ACR criteria for hip OA. Those in the injection groups received a single ultrasound guided injection. All baseline data were obtained with both patients and investigators blinded to the group allocation, and injection patients remained blinded throughout the study. The primary outcome measure was 'worst pain' using a numerical rating scale (NRS 0−10), and other outcome measures included the aggregate pain and function scales of the WOMAC. These evaluations were performed at baseline and weeks 1, 4, 8 and 16.
Results: Mean age was 69 (SD 8) years. Mean BMI was 28.5 (SD 5.2) $kg \times m^{-2}$. The 4 groups were matched with respect to age, BMI, and scores of the outcome measures (ANOVA). At week 1 and week 4 there was a significant improvement in NRS pain, as well as WOMAC pain and function for the steroid arm with the differences being statistically significant compared to all the other groups; the effect sizes were striking: 1.5 for NRS pain, 1.9 for WOMAC pain and 1.3 for WOMAC function at week 1. There were 22 responders as defined by the OMERACT-OARSI responder criteria in an intention to treat analysis at week 1: 14 in the steroid group (74%), 4 in saline group (21%), 2 in durolane group (11%) and 2 in non-injection group (10%); Chi-squared test for comparison between the groups, $p < 0.001$. At 8 weeks the differences were still significant between the steroid group and the saline group (NRS pain: t test, p 0.018).
Conclusions: This is the first single ultrasound guided injection study in hip OA comparing a moderately high dose of corticosteroid, hyaluronic acid, a control injection and standard care. Our data demonstrate the efficacy of intra-articular corticosteroid, with a statistically significant and clinically important improvement in pain and function sustained for at least 8 weeks post injection, suggesting this is an important and practical treatment option for those with moderate/severe hip OA.

A12 DOSE DEPENDENT CHONDROPROTECTIVE EFFECTS OF ALLOGENIC STRO-3+ MESENCHYMAL PRECURSOR STEM CELLS FOLLOWING DIRECT INTRA-ARTICULAR INJECTION INTO JOINTS OF AN OVINE MODEL OF EARLY OSTEOARTHRITIS

R. Read[1], M. Cake[1], T. Smith[1], C.B. Little[2], M.M. Smith[3], R.C. Appleyard[3], S. Itescu[4], **P. Ghosh[5]**. *[1]Murdoch University, Perth, AUSTRALIA, [2]University of Sydney, Sydney, AUSTRALIA, [3]Royal North Shore Hospital, Sydney, AUSTRALIA, [4]Mesoblast Ltd, Melbourne, AUSTRALIA, [5]Mesoblast Ltd, Sydney, AUSTRALIA*

Purpose: Purpose: Our previous studies had shown that bilateral total meniscectomy (BTM) in merino sheep resulted in pathological changes in articular cartilage (AC), subchondral bone and synovial tissues that were progressive and simulated the development of early human osteoarthritis (OA). We previously used this animal model to evaluate potential disease-modifying OA drugs. Mesenchymal stem cells (MSC) + bio-scaffolds have been widely investigated for their ability to enhance repair of chondral/osteochondral defects, but their chondroprotective effects when injected directly into OA joints has received limited attention. The objective of this study was to address this deficiency using immunoselected Stro-3+ mesenchymal precursor stem cells (MPC) in the BTM model.
Methods: BTM was undertaken in 36 adult Merino wethers. Two weeks post BTM, joints were randomly injected with either 2 mL high MW Hyaluronan (HA) or 2 mL allogenic Stro-3+ MPC suspended in HA. Four doses of MPC were studied: Group A = 10 million (mil) MPC [n = 6]; Group B = 25 mil MPC [n = 6]; Group C = 100 mil MPC [n = 18] and Group D = 150 mil MPC [n = 6]. Groups A, B and D were sacrificed