Purpose: Recent animal studies suggest that activation of Wnt/β-catenin signaling in articular chondrocytes might be a driving factor in the pathogenesis of osteoarthritis (OA) by stimulating amongst others the expression of matrix metalloproteinases (MMPs). Indeed in animal chondrocytes IL-1β induced MMP expression is mediated by activation of canonical Wnt/β-catenin signaling. This study aimed to investigate the role of Wnt/β-catenin signaling in IL-1β induced MMP expression in human chondrocytes.

Methods: Primary cultures of human articular chondrocytes derived from post mortem healthy donors or patients with end stage osteoarthritis or rheumatoid arthritis were used in all experiments. Cells were stimulated with recombinant growth factors and cytokines. Changes in gene and protein expression were studied using qPCR, Western blot, and enzymatic essays. Multiple strategies for activation and inhibition of signaling pathways in human chondrocytes, such as lentiviral mediated gene overexpression or knockdown were used to study the interaction between β-catenin and NF-kB.

Results: IL-1β potently stimulated MMP1, -3 and -13 expression in human and murine chondrocytes dose-dependently. In marked contrast, co-stimulation with the recombinant Wnt3A, which activates canonical Wnt/β-catenin signaling, potently inhibited IL-1β induced MMP dose-dependently in human chondrocytes but not in murine chondrocytes. This inhibitory effect was found in human chondrocytes irrespective of the disease state of the donor being either healthy, osteoarthritic or rheumatoid arthritis and was also found in human bone marrow derived mesenchymal stem cells. In contrast, Wnt/β-catenin signaling induced MMP expression in chondrocytes of all animal species tested, revealing an unprecedented species difference. Wnt3A’s inhibitory effect was found in both basal conditions and after IL-1β stimulation at the mRNA level, protein level and in enzymatic essays. Western blot indicated that IL-1β indirectly activated β-catenin in human chondrocytes. This was due to IL-1β induced upregulation of the canonical Wnt/β-catenin and down regulation of the Wnt-signaling antagonists DKK1, WIf1 and FRZb in human and murine chondrocytes. This inhibitory effect was found in human chondrocytes irrespective of the disease state of the donor being either healthy, osteoarthritic or rheumatoid arthritis and was also found in human bone marrow derived mesenchymal stem cells. In contrast, Wnt/β-catenin signaling induced MMP expression in chondrocytes of all animal species tested, revealing an unprecedented species difference. Wnt3A’s inhibitory effect was found in both basal conditions and after IL-1β stimulation at the mRNA level, protein level and in enzymatic essays. Western blot indicated that IL-1β indirectly activated β-catenin in human chondrocytes. This was due to IL-1β induced upregulation of the canonical Wnt/β-catenin and down regulation of the Wnt-signaling antagonists DKK1, WIf1 and FRZb as demonstrated by gene knock down and gene overexpression studies. The inhibitory effect of Wnt3A on IL-1β induced MMP expression was independent of TCF/LEF transcription factors, since knock down of TCF4 did not alter Wnt3A’s effect. This was in marked contrast to tumour chondrocytes in which knock down of TCF4 completely blocked IL-1β induced MMP expression. Subsequently, we showed that the inhibitory effect on IL-1β induced MMP expression in human chondrocytes was due to an inhibitory protein-protein interaction between β-catenin and NF-kB/p65RELA blocking the activation of NF-kB promoter reporter constructs as well as inhibiting the expression of established NF-kB target genes like IL-6.

Conclusions: Our study reveals an unexpected and unprecedented species difference between human and animal chondrocytes with respect of the role of Wnt/β-catenin signaling in the regulation of MMP expression. In marked contrast to mouse chondrocytes in which Wnt/β-catenin signaling is part of an IL-1β activated pro-catabolic sequence resulting in increased MMP expression, in human cells β-catenin has an unexpected anti-catabolic role. We provide evidence that Wnt/β-catenin signaling is part of an IL-1β activated negative feedback loop involving upregulation of the canonical Wnt/β-catenin signaling pathways like DKK1. This leads to activation of β-catenin, which in turn inhibits NF-kB activity due to an inhibitory protein-protein interaction with p65RELA. Our results question the relevance of animal models for studying the role of Wnt/β-catenin signaling in osteoarthritis. They furthermore imply that upregulated β-catenin in human osteoarthritic cartilage may be part of an anti-catabolic response counteracting pro-catabolic NF-kB signaling.

Purpose: Recent animal studies suggest that activation of Wnt/β-catenin signaling in articular chondrocytes might be a driving factor in the pathogenesis of osteoarthritis (OA) by stimulating amongst others the expression of matrix metalloproteinases (MMPs). Indeed in animal chondrocytes IL-1β induced MMP expression is mediated by activation of canonical Wnt/β-catenin signaling. This study aimed to investigate the role of Wnt/β-catenin signaling in IL-1β induced MMP expression in human chondrocytes.

Methods: Primary cultures of human articular chondrocytes derived from post mortem healthy donors or patients with end stage osteoarthritis or rheumatoid arthritis were used in all experiments. Cells were stimulated with recombinant growth factors and cytokines. Changes in gene and protein expression were studied using qPCR, Western blot, and enzymatic essays. Multiple strategies for activation and inhibition of signaling pathways in human chondrocytes, such as lentiviral mediated gene overexpression or knockdown were used to study the interaction between β-catenin and NF-kB.

Results: IL-1β potently stimulated MMP1, -3 and -13 expression in human and murine chondrocytes dose-dependently. In marked contrast, co-stimulation with the recombinant Wnt3A, which activates canonical Wnt/β-catenin signaling, potently inhibited IL-1β induced MMP dose-dependently in human chondrocytes but not in murine chondrocytes. This inhibitory effect was found in human chondrocytes irrespective of the disease state of the donor being either healthy, osteoarthritic or rheumatoid arthritis and was also found in human bone marrow derived mesenchymal stem cells. In contrast, Wnt/β-catenin signaling induced MMP expression in chondrocytes of all animal species tested, revealing an unprecedented species difference. Wnt3A’s inhibitory effect was found in both basal conditions and after IL-1β stimulation at the mRNA level, protein level and in enzymatic essays. Western blot indicated that IL-1β indirectly activated β-catenin in human chondrocytes. This was due to IL-1β induced upregulation of the canonical Wnt/β-catenin and down regulation of the Wnt-signaling antagonists DKK1, WIf1 and FRZb as demonstrated by gene knock down and gene overexpression studies. The inhibitory effect of Wnt3A on IL-1β induced MMP expression was independent of TCF/LEF transcription factors, since knock down of TCF4 did not alter Wnt3A’s effect. This was in marked contrast to tumour chondrocytes in which knock down of TCF4 completely blocked IL-1β induced MMP expression. Subsequently, we showed that the inhibitory effect on IL-1β induced MMP expression in human chondrocytes was due to an inhibitory protein-protein interaction between β-catenin and NF-kB/p65RELA blocking the activation of NF-kB promoter reporter constructs as well as inhibiting the expression of established NF-kB target genes like IL-6.

Conclusions: Our study reveals an unexpected and unprecedented species difference between human and animal chondrocytes with respect of the role of Wnt/β-catenin signaling in the regulation of MMP expression. In marked contrast to mouse chondrocytes in which Wnt/β-catenin signaling is part of an IL-1β activated pro-catabolic sequence resulting in increased MMP expression, in human cells β-catenin has an unexpected anti-catabolic role. We provide evidence that Wnt/β-catenin signaling is part of an IL-1β activated negative feedback loop involving upregulation of the canonical Wnt/β-catenin signaling pathways like DKK1. This leads to activation of β-catenin, which in turn inhibits NF-kB activity due to an inhibitory protein-protein interaction with p65RELA. Our results question the relevance of animal models for studying the role of Wnt/β-catenin signaling in osteoarthritis. They furthermore imply that upregulated β-catenin in human osteoarthritic cartilage may be part of an anti-catabolic response counteracting pro-catabolic NF-kB signaling.
matrix metalloproteinase (MMP)-13 in cartilage explant culture. Although the intracellular signaling that leads to cartilage destruction is mediated by a cluster of catabolic pathways including mitogen-activated protein kinases (MAPKs), the effect of CB12-II on MAPK pathways remains unclear. Hyaluronan (HA) of high molecular weight is widely used in the treatment of osteoarthritis (OA) by intra-articular injection. An increasing body of evidence indicates that HA suppresses catabolic actions by proinflammatory cytokine like interleukin-1. However, little is known of HA effect on actions of CB12-II through interaction with HA receptor such as intercellular adhesion molecule-1 (ICAM-1).

**Purpose:** This study was aimed to examine activation of p38 MAPK in association with MMP-13 production by CB12-II and its inhibition by HA in chondrocytes.

**Methods:** Cartilage explants harvested from OA knee joints or isolated chondrocytes in monolayer were incubated with CB12-II or its scramble peptide with or without pretreatment with 2700 kDa HA. In another set of experiments, following preincubation with anti-ICAM-1 antibody or non-specific IgG, cartilage explants were incubated with or without HA, followed by coinoculation with CB12-II or the scramble peptide. Enzyme-linked immunosorbent assay for phosphorylated p38 and MMP-13 was performed using total cell lysates and culture supernatants, respectively.

**Results:** When cartilage explants or chondrocytes in monolayer were incubated with CB12-II, the peptide activated p38 and MMP-13 in association with enhanced MMP-13 production. Inhibition studies with the specific inhibitor (SB203580) indicated the requirement of p38 for CB12-II-induced MMP-13 production. Pretreatment with HA resulted in significant suppression of CB12-II-stimulated MMP-13 production in cartilage as well as in chondrocyte monolayer cultures. HA suppressed p38 activation by CB12-II, leading to a decrease in MMP-13 production. Anti-ICAM-1 antibody reversed HA effect on CB12-II action.

**Conclusions:** The present study clearly demonstrated that HA suppressed CB12-II stimulated p38 via ICAM-1. Hence, OA articular chondrocytes HA could down-regulate the catabolic action of type II collagen fragments in osteoarthritic joints through the mechanism demonstrated in this study.

**258 IDENTIFICATION OF NEW ALTERNATIVE PATHWAYS AND THERAPEUTIC TARGETS IN TNF-α-MEDIATED INFLAMMATION AND OSTEOLYSIS INDUCED BY ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE**

Z. He 1, D.J. Leong 1, R.J. Majeska 2, J.A. Hardin 1, N.J. Cobelli 1, H.B. Sun 1, 3 Albert Einstein Coll. of Med., Bronx, NY; 2 The City Coll. of New York, New York, NY, USA

**Purpose:** Osteoarthritis is the common cause of failure for joint replacement surgeries. Wear debris of prosthetic implants provoke inflammatory responses, leading to osteoclast differentiation, osteolysis and ultimately, implant loosening. Debris from implants, including ultra-high molecular weight polyethylene (UHMWPE), one of the most common materials used in arthroplasty, induce a variety of inflammatory responses, including increased secretion of pro-inflammatory cytokine TNF-α (Tumor necrosis factor alpha). A large body of literature has established the critical role of TNF-α in UHMWPE-induced inflammation and osteolysis, predominantly mediated via the RANKL/NF-κB pathway. However, extensive efforts to prevent and treat prosthetic wear-induced osteolysis by interfering with the TNF-α-associated RANKL/NF-κB pathway has seen limited success. This suggests the existence of alternative pathways for TNF-α-mediated inflammation and osteolysis. Accordingly, in the current studies, we explored alternative molecular pathways by which TNF-α might mediate inflammation and osteolysis induced by UHMWPE.

**Methods:** Osteoclast differentiation induction, UHMWPE treatment and TNF-α inhibition: Bone marrow was harvested from TNF-α knockout and wild-type C57/BL6 mice (Jackson Laboratories) according to our IACUC-approved protocol and cultured. Marrow cells were induced for osteoclast differentiation by M-CSF and RANKL. After 2 days of differentiation induction, the cells were treated with UHMWPE particles (53-75μm, Sigma) alone or with TNF-α inhibitor Infliximab (Centocor Biotech). For some experiments, cultures were treated with NF-κB inhibitor for 72 hrs (Santa Cruz). Cellular and molecular assays: The cells were harvested for qPCR and Western blot to measure targeted gene expression, or subject to TRAP staining (kit from Clontech) to determine osteoclast differentiation. Cells without UHMWPE treatment were used as controls.

**Results:** Exposure to UHMWPE promotes differentiation of wild-type marrow cells to osteoclasts (increased number of TRAP-positive osteoclast-like cells), increases expression of TNF-α, TNF-α receptor 1 (TNF-αr1), and multiple TNF-α targets including Snail1 and survivin in these cells, over control (non-UHMWPE-treated) cultures. UHMWPE exposure fails to enhance osteoclast differentiation, or increase expression of TNF receptor or its downstream targets in TNF-α knockout cells, confirming the critical mediating role of TNF-α in UHMWPE-induced inflammation and osteolysis. Interestingly, TNF inhibitor α Infliximab suppresses the osteoclast differentiation and the upregulated expression of factors induced by UHMWPE treatment in wild type cell culture; however, the UHMWPE upregulated expression of Snail1 and survivin appeared not affected by NF-κB inhibition.

**Conclusions:** Our results, in addition to substantiating the critical role of TNF-α in UHMWPE-induced inflammation and osteolysis, identified several downstream targets of TNF-α that are activated in response to UHMWPE but are beyond the NF-κB pathway typically implicated in osteoclastogenesis. These TNF-α targets have been shown to serve important and relevant functions. Snail1 is critical for inflammatory-induced cell migration, and survivin is an apoptosis inhibitor. Our results, although preliminary, provide evidence of alternative pathway/factors in addition to NF-κB through which TNF-α mediates UHMWPE-induced inflammation and osteolysis. Our results are supported by recent reports of TNF-α involvement in regulation of some signaling molecules, whose expressions are not directly regulated by NF-κB. Further elucidation of these alternative pathways will facilitate our understanding of the osteolytic process induced by UHMWPE. The identification of new TNF-α targets may also promote prevention and treatment of osteolysis and the consequent implant loosening induced by UHMWPE.