cartilage degeneration. Knee joints from the mice at 4 and 8 weeks post surgery (n=4 in each group) were collected for morphological analysis. **Results**: 1) We did not find the initiation and acceleration of articular cartilage degeneration by the genetic inactivation of Tgfbr2 in knee joints of mice at the age of 9 months or older. We also did not find hypertrophic chondrocytes in the articular cartilage of the mice. 2) We found that intra-articular injection of neutralizing TGF-β1 antibody into the knee joints of Col11a1+/- mice delayed articular cartilage degeneration approximately 3 months, compared to that in Col11a1+/- mice without the injection of the antibody. It takes about 15 months for Col11a1+/- mice to form OA knee joints. 3) We found that treatment with Losartan delayed articular cartilage degeneration, induced by DMM, compared to that in control littermates. 4) We found that removal of Tgfbr2 in articular cartilage of knee joints delayed articular cartilage degeneration, induced by DMM, at least 6 weeks, compared to that in wild-type littermates. It takes about 16 weeks for mice with DMM to develop OA knee joints.

Conclusion: Inhibition of Tgf-β1 signaling attenuated articular cartilage degeneration in mature knee joints of mouse models of OA. Therefore, inhibition activity of TGF-β1, not application of TGF-β1, may be considered in treatment of OA in mature joints.

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EFFECTS OF OSTEOARTHRITIS ON MENISCUS CELL RESPONSE TO OSTEOGENIC PROTEIN 1

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Purpose: Degenerative meniscus injuries are highly prevalent in aged individuals, increasing the risk and rate of total joint OA onset. Ideally, therapies should incite anabolic matrix production and prevent total joint tissue OA progression. However, anabolic resistance occurs during articular cartilage aging and OA. It is thought to contribute to the risk of OA progression due to insufficient repair of normal tissue microtrauma. Anabolic insensitivity also contributes to net tissue breakdown during OA due to decreased tissue synthesis. Mechanisms of anabolic insensitivity have not been described for meniscus tissue. The objective of this study was to characterize normal and OA meniscus cell response to the anabolic growth factor, Osteogenic Protein 1 (OP1/BMP7), and examine mechanisms of anabolic resistance to OP1 during OA.

Methods: Normal human tissue was obtained through NDRI or Gift of Hope Tissue and Organ Donor Network. Osteoarthritic tissues were obtained as total knee arthroplasty surgical waste under IRB approval. Normal meniscus was grade 2 or less by ICRS scores. Primary meniscus cells were grown as high density monolayer cultures, changed to serum free media upon reaching confluence, and treated with OP1 (100 ng/mL) or comparisons of IL1β (10 ng/mL), TGFβ (10 ng/mL), GDF5 (100 ng/mL), or IGF1 (100 ng/mL). Expression of ECM components and MMPs was determined by RT-qPCR. Total lysates were analyzed by immunoblotting and evaluated using antibodies against activating phosphorylations or total proteins for Smads (1/5/8, 2), MAPKs (ERK, p38), and AKT. Band intensity was measured by ImageJ densitometry, normalized for background and total protein, and expressed relative to unstimulated samples.

Results: By RT-qPCR, OP1 stimulation increased aggrecan and collagen type II (COL2A1) mRNA and decreased MMP-13 mRNA in all samples, however OA meniscus cells showed reduced responses compared to healthy cells. Normal meniscus cells separated from inner and outer meniscus regions displayed similar mRNA levels upon OP1 stimulation, suggesting the decreased response of OA cells to OP1 was due to disease state, not loss of tissue region (data not shown). To identify potential mechanisms of anabolic insensitivity, we evaluated signaling time courses by immunoblot. Normal (n=2) and OA (n=3) meniscus cells, stimulated with OP1, displayed activation of canonical BMP signaling, evidenced by Smad1/5/8 phosphorylation (P-Smad1/5/8), while noncanonical MAPK or AKT signaling was not observed (Figure 1A&B). Maximum P-Smad1/5/8 occured at late time points (≥1.5 hours) with similar kinetics between normal and OA cells. Normal cells responded to OP1 stimulation with enhanced levels of P-Smad1/5/8 compared to OA cells (Figure 1C). Stimulation with IL-1β or IGF1 showed limited Smad 1/5/8 activation, while MAPK or AKT was activated, as expected. Stimulation with TFGβ resulted in both enhanced phospho-Smad 2 and Smad 1/5/8 in normal and OA cells compared to unstimulated controls. TFGβ treatment induced higher phospho-Smad 1/5/8 levels in normal vs. OA meniscus cells, similar to OP1 treated cells.

Conclusions: OA meniscus cells are resistant to OP1 stimulated extracellular matrix transcription compared to normal meniscus cells. MMP expression is not induced by either normal or OA meniscus cells, indicating that OP1 is not harmful and has potential therapeutic benefit for OA meniscus. Both normal and OA meniscus cells respond to OP1 stimulation by activating canonical BMP signaling (Smad1/5/8), but not non-canonical signaling. Our current signaling experiments using OP1 and including TFGβ suggest that meniscus anabolic resistance does not affect kinetics of Smad activation, but influences total amount of Smad phosphorylation. Stimulation with TFGβ also resulted in differential Smad 1/5/8 activation, suggesting that Smad signaling is a target pathway for overcoming anabolic resistance during meniscus cell OA. Discovering mechanisms of OA cell anabolic resistance will enhance therapeutic targeting to overcome resistance and contribute to restoration of tissue homeostasis beneficial to the whole knee joint.
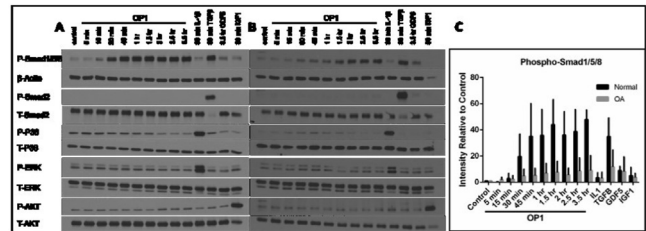

Figure 1. OP1 activates canonical (P-Smad 1/5/8 signaling in A) healthy and B) OA meniscus cells. C) P-Smad 1/5/8 densitometry, normalized to β-actin, relative to unstimulated controls (mean +/- SD).

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EFFICACY OF CULTURE-EXPANDED MESENCHYMAL STROMAL CELLS VERSUS CONCENTRATED BONE MARROW IN AN EXPERIMENTAL OSTEOARTHRITIS SHEEP MODEL

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Purpose: Despite the advantages in the field of regenerative medicine for cartilage repair, the research of available and effective treatments for osteoarthritis (OA) represents a major challenge for clinicians. To this regard, the use of bone marrow concentrate (BMC), containing different cell populations including Mesenchymal Stromal Cells (MSCs) surrounded by their microenvironment with a cocktail of multiple growth factors, could represent a novel therapeutic approach for OA management. This methodology allows to perform a one -step procedure through a density gradient centrifugation of autologous bone marrow avoiding all the problems related to cell manipulation. Most recent studies demonstrated benefits of the use of BMC for osteochondral reconstruction in critical-sized defects.The purpose of this study was, therefore, to assess the efficacy of the use of BMC seeded onto a hyaluronan scaffold compared to the use of expanded isolated MSCs in a sheep model of OA.

Methods: Skeletally mature female Merino sheep were used for this study.The induction of OA was done surgically through unilateral partial medial meniscectomy (MMX) to achieve a mild grade of OA at 12 weeks. Treatment approaches consisted of autologous BMC and/or MSC seeded onto Hyaff-11 from bone marrow and transplanted into the knee joint; no treatment (OA group) and empty Hyaff-11 were used as controls. Cartilage from femoral condyle and tibial plateau, synovial membranes and medial menisci were assessed by macroscopic and histological analysis supplemented by specific scoring systems. Protein expression of various fibrous, cartilaginous, catabolic, hypertrophic and inflammatory markers were examined by immunohistochemistry. All the assessments were carried out 3 months after implantation. Data were reported using 95% confidence of interval of the mean and/or mean with standard deviation.

Results: A mild grade of OA was developed after MMX at 12 weeks. A series of OA changes, including cartilage loss, cell clones, hypertrophic processes, subchondral bone thickness, osteophytes, hyperplasia of

synovial lining and fibrous and inflammatory processes, were noticed in OA group. Hyaluronan scaffold displayed only a minor role in favoring the wound-healing processes in the cartilage, synovial membrane and menisci. There were instead a general beneficial effect on these tissues, when this scaffold was implanted with BMC and/or MSC. Macroscopic, histological and immunohistochemical assessments clearly showed in detail in BMC group an enhancement of cartilage repair in terms of a down-regulation of some fibrous, catabolic, hypertrophic and inflammatory markers particularly in medial femoral condyle respect OA group (P<0.05). Moreover, the use of BMC determined an improvement of various histological aspects in synovial membrane and menisci reducing the fibrous and inflammatory states respect OA group. MSC group displayed a positive contribution particularly on medial femoral condyle and low effect in tibial plateau due to the biomechanics of the articular joint in sheep.

Conclusions: The sheep was chosen for this work because of its similarities with humans, which render it an useful model. The findings obtained in this study would support the use of BMC in clinical practice for OA management, as useful therapeutic tool, leading to a reduction of the degenerative and inflammatory processes in cartilage, synovial membrane and menisci during OA setting. Moreover, the clinical application of BMC in OA management is attractive because it would allow a one- step procedure, avoiding all the complications related to in vitro cell manipulation, thus complying with US Food and Drug Association (FDA) restrictions.

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ALTERATIONS OF THE SUBCHONDRAL BONE INDUCED BY PTH [1-34] PROVOKE EARLY OSTEOARTHRITIS *IN VIVO*

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Purpose: Systemic application of the 1-34 amino acid segment of the parathyroid hormone (PTH [1-34]) stimulates bone formation, enhances diaphyseal fracture healing, and is approved for the clinical treatment of postmenopausal osteoporosis. However, the effect of PTH [1-34] on the subchondral bone is poorly understood. Here, we tested the hypothesis that subcutaneous application of PTH [1-34] over 6 weeks induces changes in the microarchitecture of the subchondral bone that reciprocally affect the integrity of the articular cartilage within a naïve osteochondral unit *in vivo*.

Methods: Daily subcutaneous injections of 10 μg PTH [1-34] per kg body weight were given to the treatment group (n = 4 adult female Chinchilla bastard rabbits) for 6 weeks, controls received saline. The animals were allowed full weight bearing and were fed a standard diet. Blood samples were continuously collected to monitor renal function. After sacrifice, the subchondral bone plate and subarticular spongiosa of the femoral heads (n = 16) were separately assessed by micro-computed tomography. Osteoarthritic changes of the articular cartilage were evaluated by macroscopic assessment and histological grading of safranin orange/fast green stained sections, by polarized light microscopy, and by computerized assessment of the mean immunostaining intensities for type-I, type-II, and type-X collagen (Acris, Hiddenhausen, Germany). Absolute and relative extents of hyaline and calcified articular cartilage layers were determined histomorphometrically in a standardized fashion on Goldner's trichrome stained sections. The effect of PTH [1-34] on apoptosis and PTH receptor (PTH1R) expression of osteocytes and chondrocytes was determined by immunostaining, applying a polyclonal caspase-3 (Cell Signaling Technology, Frankfurt, Germany) and a monoclonal mouse PTH1R antibody (Abcam, Cambridge, UK), respectively. Statistical comparison was conducted using a linear model with generalized estimating equations. The Pearson correlation coefficient was applied to determine the strength of association between PTH-induced key changes in subchondral bone and articular cartilage. Data are given as mean ± standard deviation. Two-tailed values of $p < 0.05$ were considered significant.

Results: PTH [1-34] enhanced bone volume (BV/TV; 45.58 ± 3.69 versus 40.12 ± 3.35%; $p = 0.010$), mineral density (BMD; 1,062.48 ± 104.16 versus 931.59 ± 60.94 mg calcium hydroxyapatite/cm³; $p = 0.017$), and trabecular thickness (Tb.Th; 0.14 ± 0.01 versus 0.13 ± 0.01 mm; $p =$

0.042) within the subarticular spongiosa, without affecting the subchondral bone plate. Besides, PTH [1-34] significantly increased the thickness of the calcified cartilage layer (285.32 ± 46.17 versus 163.45 ± 31.30 μm; $p = 0.001$). Moreover, PTH [1-34] also increased degeneration of the articular cartilage (4.28 ±1.47 versus 1.97 ± 0.46 overall score points; $p = 0.040$), reflected by cartilage surface irregularities (1.11 ± 0.96 versus 0.25 ± 0.59; $p < 0.001$) and reduced matrix staining (2.75 ± 1.65 versus 1.68 ± 0.55; $p = 0.029$). Importantly, statistical analysis revealed that such early osteoarthritic changes of the articular cartilage correlate with (r = 0.56) and are ascribed to the increased thickness of the calcified cartilage layer ($p = 0.026$) and the enhanced mineral density of the subarticular spongiosa ($p = 0.001$). Systemic administration of PTH [1-34] neither affected the rate of apoptotic osteocytes and chondrocytes within the osteochondral unit nor induced renal failure, as serum levels of creatinine, phosphate, and urea nitrogen remained constant over the observation period and were similar between treatment and control group ($p > 0.05$ for all time points). The macroscopic aspect of the articular cartilage, type-I, type-II, type-X collagen content and distribution, and PTH1R expression remained unaffected (all $p > 0.05$).

Conclusions: These findings identify a mechanism by which systemic PTH [1-34] may provoke early osteoarthritis of the hyaline articular cartilage by inducing microarchitectural modifications of the subarticular spongiosa and broadening of the calcified cartilage layer.

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SENSITIZATION AND PAIN OVER TWO YEARS: THE MULTICENTER OSTEOARTHRITIS (MOST) STUDY

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Purpose: Peripheral and central sensitization is associated with knee pain severity in knee osteoarthritis (OA). Whether sensitization occurs prior to or only concurrently with development of OA pain is not known as prior studies have only been cross-sectional. Such insights would have implications for understanding whether sensitization may be an inherent trait vs. an induced state. We report here the first longitudinal evaluation of the effect of sensitization and its change over two years to the incidence of knee pain and clinically meaningful change in pain severity.

Methods: The Multicenter Osteoarthritis (MOST) Study is a NIH-funded longitudinal cohort of persons with or at risk of knee OA. Subjects had x-rays and WOMAC pain severity obtained at each study visit, and a standardized somatosensory evaluation of mechanical temporal summation and pressure pain thresholds (PPT) at the wrist and patella at 60- and 84-months. Temporal summation was defined by increased pain during repeated mechanical stimulation (1 Hz x 30-sec) with a 60g monofilament. PPT was assessed with an algometer (1cm2 tip, 0.5 Kg/ sec) as the point at which the subject felt the pressure change to slight pain. The average of 3 PPT trials was categorized into sex-specific tertiles. Lower PPT indicates more sensitivity. Incident consistent frequent knee pain (CFKP, pain on most days of the past 30 days on both a telephone screen and clinic visit) at the 84-month visit was determined from among knees that did not have CFKP at the 60-month visit. Incident symptomatic knee OA (SxOA) at the 84-mo visit was defined as radiographic knee OA (ROA, KL grade ≥2) plus CFKP, assessed among knees free of pain at the 60-mo visit with or without ROA. We used the minimal clinically important difference (MCID) in WOMAC pain to examine sensitization effects on clinically meaningful changes in pain severity. We examined the relation of the baseline and 2-year changes in somatosensory tests to each of these pain outcomes using logistic regression with GEE. All analyses were adjusted for potential confounders (see Table for list).

Results: A total of 2308 subjects met eligibility criteria for these analyses (mean age 67.6, mean BMI 30.9, 61% female). Neither temporal summation nor PPT were associated with incident CFKP or incident SxOA (ORs 0.7-1.2, all p>0.05). Baseline temporal summation at the wrist was associated with a trend towards increased risk of MCID in WOMAC pain worsening at follow-up, but baseline PPT was not associated (Table). However, a decrease in PPT (¬sensitivity) over 2 years