Purpose: Mitophagy, the process of mitochondrial autophagy involving selective sequestration and subsequent degradation of dysfunctional mitochondria, is implicated in numerous aging-related diseases. Molecularly controlled by the PINK-1/Parkin pathway, the efficient Parkin/PARK2 mediated ubiquitination of mitochondrial proteins is the acknowledged key mechanism. Changes in mitochondrial function have been implicated as an underpinning molecular event that leads to articular cartilage (AC) degeneration and are likely to contribute to osteoarthritis development. We have explored the hypothesis that articular chondrocyte mitophagy is an early event taking place in cartilage that predisposes to OA onset, and have therefore explored whether mitophagic markers are modified at various disease stages in the natural STR/Ort mouse model of osteoarthritis in which AC degradation is initially observed in the medial tibia plateau.
Methods: STR/Ort mouse knee joints from stages before, at onset, and at advancing osteoarthritis stages and age-matched CBA (control) joints were examined by immunohistochemical labelling of autophagy markers (LC3), a protein core to mitochondrial function (VDAC) and one recently described as an inhibitor of the Parkin/PARK2-mediated ubiquitination of proteins (TSPO), both localised on the outer membrane of mitochondria. Hip cartilage explants from 3-4 week-old STR/Ort and CBA mice were also cultured for 24hrs, in absence or presence of 100nM rapamycin, an inducer of macro-autophagy that is likely to also impact the targeted type of autophagy, and mRNA transcripts for a variety cartilage markers assessed by both standard and multiplex PCR analysis. Proteoglycan release from hip cap explants was assessed by a colorimetric assay for aggrecan quantification.
Results: Immunolabelling for TSPO in non-OA prone CBA control mice revealed positive chondrocyte expression in all AC compartments that remained consistent in mice at all ages studied. In contrast, labelling revealed an advancing age-related decline in AC chondrocyte expression of TSPO in chondrocytes of the lateral (unaffected) tibial plateau in STR/Ort mouse joints and importantly minimal TSPO expression in the AC chondrocytes even at ages prior to any overt signs of osteoarthritic pathology in the medial aspect (affected) of the joint. Immunolabelling for LC3 follows the same age-related changes in expression pattern in STR/Ort mice with marked co-localisation with TSPO within identical chondrocytes of the AC in both strains of mice. Addition of 100nM rapamycin to cultured STR/Ort hip explants revealed changes in the chondrocyte mRNA expression involving a decline in levels of transcripts which are normally characteristic of epiphyseal growth chondrocytes, with significant decreases in Col10a1 (0.2 fold) and Enpp1 (0.2 fold) mRNA expression along with concomitant increases in pro-anabolic transcripts, namely Timp1 (1.5 fold) and Col2a1 (2.9 fold) mRNA expression in the presence of this inducer of autophagy. Despite this, no significant differences were observed in proteoglycan release upon rapamycin treatment.
Conclusions: Our data show that STR/Ort mice have an inherent deficit in AC chondrocyte mitochondrial function, exhibited preferentially within the medial tibial plateau compartment known to exhibit greatest vulnerability to OA related AC degeneration. Initial studies indicate that the addition of rapamycin, an inducer of autophagy, stabilises the articular cartilage chondrocyte phenotype and as such, may offer protection against disease pathology.

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FACET JOINT OSTEOARTHRITIS IN LUMBAR SPINAL STENOSIS: HISTOLOGICAL EVALUATION OF CELLULAR PATHOMECHANISMS

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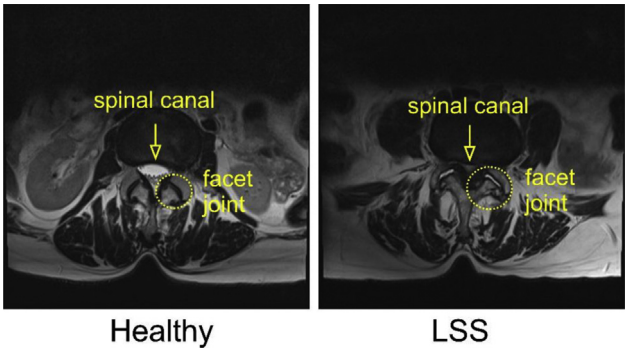
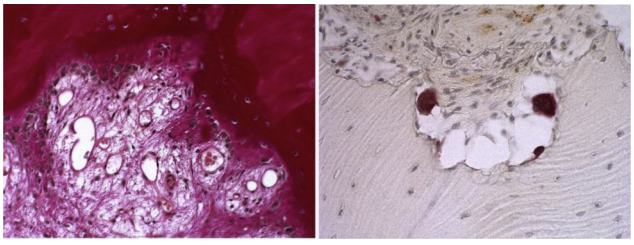
Purpose: Lumbar spinal stenosis (LSS) is a degenerative, age-related narrowing of the lower spinal canal that causes pressure on the nerves, leading to pain and reduced mobility. Hypertrophy of ligament flava and facet joints combined with disc protrusions are a causative for LSS. Osteoarthritic changes to the facet joint, including joint space narrowing, subchondral cysts and osteophyte formation are commonly detected using magnetic resonance imaging (MRI) and computed tomography (CT) scanning. However, the pathomechanisms of facet joint osteoarthritis (OA) at a cellular and molecular level are poorly understood and have been scarcely studied. In this study we sought to investigate the histological features and to uncover cellular pathomechanisms of facet joint OA.
Methods: Fifteen patients undergoing surgical decompression due to degenerative LSS were included in this study (9 female/6 male, median age 66, range 49-85). Routine preoperative X-ray and MRI scanning of the lumbar spine was performed in all subjects. Severity of facet joint OA was assessed in MRI images using the Weishaupt grading system for the lumbar spine. The medial portion of facet joints was collected during surgical decompression of the spinal canal and processed for tissue histology. Tissue morphology was evaluated using haematoxylin and eosin (HE), Safranin-O and van Gieson`s stains. The presence of macrophages, blood vessels and nerve fibers was investigated using immunohistological staining for their respective markers CD68, CD34 and PGP9.5. Functional osteoclasts were visualized using tartrate-resistant acid phosphatase (TRAP) staining. Subchondral bone area fraction (B.Ar/T.Ar) was determined using the ImageJ-plugin BoneJ.
Results: OA was evident in MRI images as evidenced by joint space narrowing, bone edema and cysts and hypertrophy of articular processes (Figure 1).



The severe OA phenotype in facet joints from LSS patients was confirmed at a histological level by complete loss of proteoglycan staining, CD34+ vessel penetration, and fissuring of cartilage tissue. Subchondral B.Ar/T.Ar ranged between 0.6 and 0.8. In all samples, subchondral marrow spaces contained CD34+ blood vessels and CD68+ mononuclear macrophages. CD68+ multinucleated osteoclasts were detected in resorption pits at the bone surface in 80 percent of the patients. Functionality of osteoclasts was confirmed by positive staining of multinucleated bone cells for TRAP in serial sections. Osteoblast activity was demonstrated in 60 percent of the patients and predominantly characterized by large areas of intramembranous bone formation near the osteochondral junction (Figure 2a). Formation of an osteoclast-rich pannus-like tissue was seen in one sample (Figure 2b).
Innervation of subchondral marrow spaces by PGP9.5-positive nerve fibers was scarce and exclusively found in the vicinity of arterioles.



Conclusions: Facet joints in LSS patients display radiological and histological features of OA. Two major OA phenotypes can be distinguished based upon cellular pathomechanisms in subchondral bone tissue: 1) osteoblast-rich intramembranous bone formation and 2) osteoclast/macrophage rich remodeling. Imaging modalities using bone-seeking radiotracers (i.e. SPECT/CT) might enable differential diagnosis of facet joint OA subtypes. Due to abundant osteoclast activity in facet joints, antiresorptive treatments might represent a promising pharmacological intervention for OA-induced LSS.

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OSTEOCYTE CELL DEATH IN SUBCHONDRAL BONE FOLLOWING JOINT INJURY CORRELATES WITH THE SEVERITY OF AGGRECAN LOSS IN OVERLYING CARTILAGE

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Purpose: The destabilisation of the medial meniscus (DMM) mouse osteoarthritis (OA) model is a commonly used model of post-traumatic OA. In this model, the knee joint is destabilised by transecting the medial-meniscotibial ligament which leads to increased loading on the medial tibial compartment, focal articular cartilage damage, subchondral bone accrual and osteophyte formation. In this study, we used the DMM-OA model to determine how tibial subchondral bone structure changes following acute joint injury and how these changes relate to onset and progression of articular cartilage damage.
Methods: 12-week old male C57BL/6 mice underwent DMM or sham surgery on the right knee; left knees served as contra-lateral controls. In vivo micro-CT (Skyscan) was performed prior to surgery, and at 4, 8, and 12 weeks post surgery. A novel approach for data analysis was developed to quantify bone of varying mineralization states. Separate groups of mice were collected for OARSI histologic assessment of bone structure, aggrecan loss and cartilage damage at 1, 4, 8 and 12 weeks post-surgery. Statistics: 2-way ANOVA, Bonferroni post-hoc tests.
Results: Pre-surgery bone volume/tissue volume (BV/TV) and tissue mineral density (TMD) were similar in all limbs. Consistent with increased loading, a focal increase in medial subchondral bone was observed in DMM-OA tibiae: BV/TV and TMD were increased compared to sham from 4 weeks post-surgery ($p<0.001$). There was no systemic effect of DMM-OA on bone structure: BV/TV in the lateral subchondral bone and tibial metaphyseal trabecular bone were similar in all limbs. However, medial subchondral BV/TV of DMM-OA tibiae and contra-lateral tibiae were similar, suggesting an influence of altered gait on the contralateral limb. Histologic analyses showed aggrecan loss and cartilage erosion in the medial compartment of DMM-OA tibiae from 4 weeks post-surgery. Interestingly, the medial subchondral bone in DMM-OA tibiae resembled osteonecrotic bone from 4 weeks post-surgery with numerous empty osteocyte lacunae present, indicating osteocyte death. Furthermore, the number of empty osteocyte lacunae at this site negatively correlated with the width of overlying aggrecan-positive (healthy) cartilage ($p=0.01$, Pearson's $r= -0.92$) suggesting a relationship between aggrecan loss and subchondral bone health.
Conclusions: In summary, focal accrual of medial subchondral bone occurs early in DMM-OA tibiae alongside cartilage damage. Similar to human OA, osteocyte cell death in subchondral bone is a feature of DMM-OA. Mechanical changes or biochemical signals induced by aggrecan loss in articular cartilage may be detrimental to the health of the underlying subchondral bone. Osteocyte cell death could also contribute to local changes in bone integrity, vascularization or cartilage. Finally altered bone structure in the contra-lateral tibiae of DMM-OA mice, suggests that increased subchondral bone per se does not affect overlying cartilage, and serves to highlight the need to include sham-operated mice when using this model.

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ELEVATED LEVELS OF BMP2 COMPENSATE FOR LOSS OF TGF-BETA ON PROTEOGLYCAN LEVEL IN ARTICULAR CARTILAGE DURING EXPERIMENTAL OSTEOARTHRITIS

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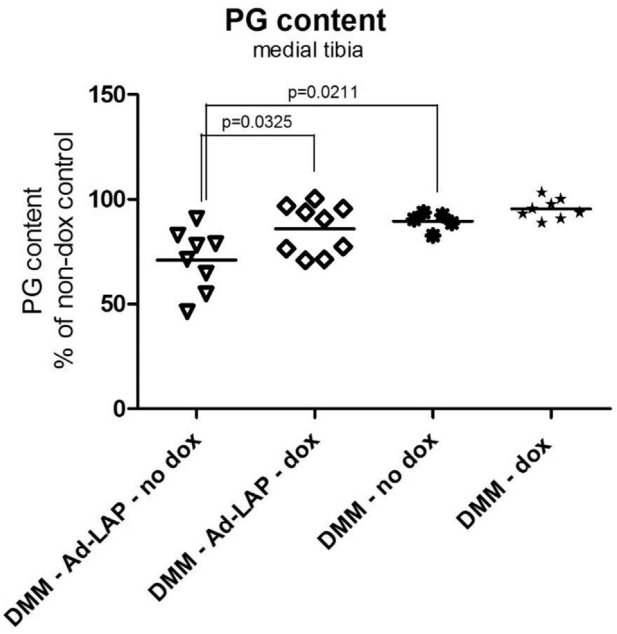
Purpose: We have demonstrated that in aging murine articular cartilage TGF-beta signaling via Smad2/3 is drastically reduced and that loss of Smad2/3-related TGF-beta signaling predisposed cartilage for OA development. In addition, we have previously shown that inhibition of TGF-beta reduces the proteoglycan content in articular cartilage. In contrast, during OA elevated levels of BMP2 are found in chondrocytes surrounding cartilage lesions. However, it is unclear what is the effect of this BMP2 presence on the articular cartilage. Therefore, we have investigated whether elevated BMP-2 expression can counteract the loss of TGF-beta signaling during OA.
Methods: We made a unique transgenic mouse which expresses human BMP2 under control of the Col2a1 promoter but only when exposed to doxycycline (Col2a1-rtTA-BMP2). This results in a chondrocyte-specific overexpression of human BMP2 which is inducible by doxycycline. Functionality of this transgenic mouse was tested by isolating mRNA from articular cartilage, spleen and liver 72 hours after exposure to doxycycline food or standard diet. With Q-PCR we analyzed the expression of human BMP2 mRNA. In Col2a1-trTA-BMP2 mice we induced OA by destabilization of the medial meniscus (DMM-model) while treating them with doxycycline in food versus standard diet. To study the effect of loss of TGF-beta activity during OA in these young mice, we additionally intra-articularly injected an adenovirus over-expressing the TGF-beta inhibitor LAP (Ad-LAP). Four weeks after induction of DMM knee joints were isolated for histology. OA was scored based on cartilage damage (adapted OARSI score, scale of 0-30) In addition, we measured proteoglycan (PG) content with digital image analysis in Safranin O stained articular cartilage of the medial tibia, which is most affected during DMM.
Results: Treatment of the Col2a1-rtTA-BMP2 transgenic mice with doxycycline clearly elevated the expression of hBMP2 mRNA in articular cartilage, but not in spleen and liver thereby confirming functionality of the transgenic animals. Doxycycline exposure in Col2a1-rtTA-BMP2 up to 8 weeks did not result in any detectible alterations in healthy articular cartilage. When OA was induced there was a clear increase in OA score (average of all DMM groups of 16.9 versus 2.5 in non-DMM groups), but this was not significantly affected by the presence of elevated chondrocyte-specific BMP2. TGF-beta inhibition with LAP did not affect the OA-score either . However, TGF-beta inhibition during DMM significantly reduced the proteoglycan content by 18% compared to DMM alone. BMP2 did not have an effect on the proteoglycan content during DMM (see figure). Nevertheless, the proteoglycan depletion that occurred by the inhibition of TGF-beta during DMM could significantly and nearly completely be counteracted by elevated chondrocyte-specific BMP2.



Conclusions: Our data show that in healthy articular cartilage and in cartilage affected by osteoarthritis in young animals elevated levels of BMP2 did not have any detectible effects. However, when TGF-beta signaling was lost, a phenomenon occurring in aged individuals, this resulted in decreased levels of PG content in articular cartilage during OA. In this setting, elevated levels of BMP2 could compensate this loss of PG. Therefore the elevated levels of BMP2 near OA lesions could be a reparative response of the articular cartilage. Especially with ageing, when TGF-beta signaling is drastically reduced this compensatory mechanism could be of great importance as an attempt to restore damaged articular cartilage. Overall our data show that with respect to proteoglycan content elevated levels of BMP2 can compensate for the loss of TGF-beta signaling, indicating that the presence of elevated BMP2 during OA could be a potential compensatory mechanism of the articular cartilage for the fact that TGF-beta signaling is lost. This potential attempt to repair the lost proteoglycans could be a mechanism of great importance especially in the elderly. Therefore our study provides novel insight into the mechanisms involved in OA.