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SYMPATHICUS EXHIBITS DIFFERENTIAL EFFECTS ON ARTICULAR CARTILAGE, SYNOVIUM AND SUBCHONDRAL BONE DURING OSTEOARTHRITIS DEVELOPMENT

K. El Bagdadi [1], D. Muschter [2], S. Taheri [3], C. Dorn [4], A.F. Schilling [3], S. Grässel [2], A. Meurer [1], F. Zaucke [1], R.H. Straub [5], Z. Jenei-Lanzl [1]. [1] *Dr. Rolf M. Schwiete Res. Unit for Osteoarthritis, Orthopedic Univ. Hosp. Friedrichsheim gGmbH, Frankfurt/Main, Germany;* [2] *Dept. of Orthopaedic Surgery, Experimental Orthopaedics, Ctr. for Med. Biotechnology, Univ. of Regensburg, Regensburg, Germany;* [3] *Clinic for Trauma Surgery, Orthopaedic Surgery and Plastic Surgery, Univ.smedizin Göttingen, Göttingen, Germany;* [4] *Inst. of Pharmacy, Univ. of Regensburg, Regensburg, Germany;* [5] *Lab. of Experimental Rheumatology and Neuroendocrine Immunology, Dept. of Internal Med. I, Univ. Hosp. Regensburg, Regensburg, Germany*

Purpose: Osteoarthritis (OA) is a disease of the whole joint, since its pathogenesis involves articular cartilage, synovial tissue and subchondral bone. OA is characterized by progressive degradation of cartilage, synovial inflammation, osteophyte formation and subchondral bone sclerosis. In contrast to cartilage, its surrounding tissues are innervated by tyrosine hydroxylase(TH)-positive sympathetic nerve fibers and the most important sympathetic neurotransmitter norepinephrine (NE) was detected in the synovial fluid of OA patients. However, the role of the sympathicus in OA progression has not yet been studied. Therefore, we investigated the effects of sympathectomy (Syx) in an experimental OA model.
Methods: Chemical Syx was performed using 6-Hydroxydopamine (6-OHDA) in C57BL/6 mice. OA was induced in wildtype mice (WT) and in Syx mice by destabilization of the medial meniscus (DMM). The efficiency of Syx was evaluated by staining of TH-positive nerve fibers in the synovium and by measurement of (NE) in the spleen. OA progression was assessed by OARSI score, measurement of serum CTX-II, as well as micro- and nano-CT analyses and histochemical detection of tartrate-resistant acid phosphatase (TRAP) in subchondral bone. Additionally, we investigated synovitis by histopathological scoring. Expression of TH, α2A- β2-adrenergic receptors (AR), type II collagen and MMP-13, was detected immunohistochemically in joint tissues.
Results: Syx resulted in significant reduction of synovial TH-positive nerve fibers and significantly less NE in spleen tissue. Interestingly, DMM itself lead to increased NE concentrations compared to sham or untreated mice. DMM surgery also resulted in altered expression of adrenergic receptors and number of TH-positive cells in different tissues of the joint. 8 and 12 weeks after DMM, Syx mice developed significantly lower OA scores compared to WT mice. A less pronounced cartilage degradation and CTX-II release was observed in Syx mice compared to WT mice. Similarly, synovitis score was significantly decreased in Syx mice 8 and 12 weeks after DMM compared to WT mice. Increased synovitis scores were accompanied by higher numbers of TH-positive cells and elevated α2A- and β2-AR expression in the synovium. Additionally, we observed stronger expression of MMP-13 in the synovium in WT mice 2 and 4 weeks after DMM, compared to Syx mice. At the same time, micro- and nanoCT analyses revealed a significant increase in calcified cartilage (CC), subchondral bone plate (SCBP) thickness and bone volume (BV/TV) in Syx mice compared to WT mice 8 weeks after DMM. Both, WT and Syx mice developed osteophytes and meniscal ossicles without any difference between the groups. In contrast to WT mice, osteoclast activity at the site of osteophyte formation and in subchondral bone was reduced in Syx mice 4 weeks after DMM.
Conclusions: Taken together, Syx attenuates cartilage degeneration, synovial inflammation but accelerates OA-specific subchondral bone changes. These findings underline the contribution of the sympathicus in OA pathogenesis. A better understanding of molecular mechanisms will allow the development of novel therapeutic options targeting the sympathicus.

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ENHANCING ARTICULAR CARTILAGE REGENERATION BY MODULATING THE CELL CYCLE WITHIN MESENCHYMAL PROGENITORS

A.O. Masson [1], B.A. Besler [1], D. Young [1], A. Dufour [1], W.B. Edwards [1], T.M. Underhill [2], R.J. Krawetz [1]. [1] *Univ. of Calgary, Calgary, AB, Canada;* [2] *Univ. of British Columbia, Vancouver, BC, Canada*

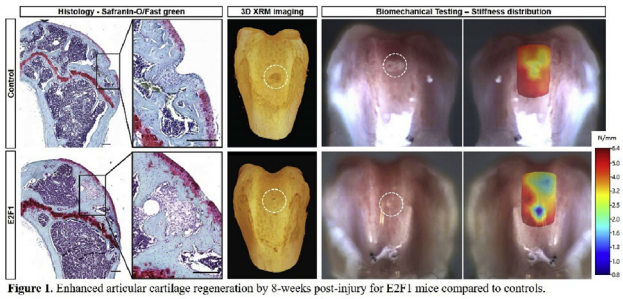

Figure 1. Enhanced articular cartilage regeneration by 8-weeks post-injury for E2F1 mice compared to controls.

Purpose: Most adult tissues contain stem and progenitor cells known to contribute to tissue maintenance and repair. While tissue-resident mesenchymal progenitor cells (MPCs) have been identified in multiple niches in the adult knee joint, articular cartilage shows poor healing capacity following injury. Previously, it has been demonstrated that the constitutive deletion of the p21 gene, a cell cycle regulator, promotes cartilage regeneration. However, the underlying mechanisms of this regenerative phenotype remain largely unknown, such as signaling pathways and cell types involved. We hypothesized that activation of the cell cycle solely in endogenous adult MPCs would enhance cartilage regeneration *in vivo* post-injury.
Methods: To test this, we targeted the p21 downstream effector E2F1 and overexpressed it in quiescent MPCs. This was accomplished by developing a conditionally overexpressing E2F1 mouse that was bred to a *Hic1*^{CreERT2}: *Rosa26*$^{LSL\text{-}TdTomato}$ MPC reporter mouse to generate the *Hic1*^{CreERT2}; *Rosa26*$^{LSL\text{-}TdTomato}$; E2F1^{EGFP} offspring. TdTomato reporter gene expression and E2F1 expression was induced in MPCs following administration of tamoxifen at 8 weeks of age. Subsequently, all mice were subjected to full-thickness cartilage defects in the trochlear groove of their distal femur. Knee joints were harvested at 1-, 2-, 4- and 8-weeks post-injury. Histology (2D structure/composition), high-resolution 3D X-ray microscopy (XRM imaging), immunofluorescence (lineage tracking), quantitative proteomics and biomechanical testing outcome measures were employed to assay for cartilage regeneration.
Results: Overexpression of E2F1 in *Hic1*$^{+ve}$ MPCs resulted in enhanced cartilage regeneration compared to controls. The defect site in control mice lacked proteoglycan staining, surface regularity, and was filled with fibrocartilage-like tissue, while articular cartilage-like tissue was observed in the E2F1 overexpressor by 4-weeks post-injury. Such differences were reflected in the histological score with significant differences between the groups by 4- and 8-weeks post-injury, which was corroborated by the high-resolution 3D imaging of the femurs, indicating an enhanced articular cartilage repair within E2F1 mice. Proteomic analysis demonstrated that extracellular matrix production and secretion were dysregulated between *Hic1*$^{+ve}$ MPCs derived from the synovium and bone marrow in E2F1 mice, with increased production of matrix proteins observed in synovium-derived MPCs. E2F1mice demonstrated altered stiffness distribution within the repaired tissue area, with lower stiffness values compared to uninjured controls (Figure 1). Interestingly, while *Hic1*-lineage cells (TdTomato^{+ve}) were seen within the injury site in both strains, by 4-weeks after injury, few to no cells were present. Furthermore, when the articular cartilage layer was nearly regenerated, new chondrocytes at the injury site were found to be TdTomato^{-ve}.
Conclusions: These findings suggest that activation of cell cycle pathways in MPCs promotes cartilage regeneration. Furthermore, while activated MPCs migrated to the injury site, few to no cells were seen within the newly formed fibro or hyaline-like cartilage tissues in control vs. E2F1, respectively. Therefore, while these MPCs are necessary for articular cartilage regeneration, they are not responsible for new tissue formation. Hence, future studies are required to elucidate the role of E2F1 overexpression and the downstream effects on bone and cartilage tissues after injury.