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PHARMACOLOGICAL REGULATION OF SENESCENCE-ASSOCIATED SECRETORY PHENOTYPES IN OSTEOARTHRITIS CARTILAGE

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Purpose (the aim of the study): This study aims to investigate the effects of probucol and glyburide on osteoarthritis (OA) progression. We investigated whether treatment with these SASPs-modulating drugs alters senescence-associated inflammation and in vivo senescence during OA progression in mice. This study could provide insights for developing targeted senostatic therapies in OA treatment.
Methods: For *in vivo* studies, mice (C57BL/6J) were used for experimental OA studies. Post-traumatic OA was induced by DMM surgery on 12-week-old male mice; sham-operated male mice were used as controls. For probucol delivery, 10 μl of 200 μM probucol dissolved in DMSO was administered intra-articularly every week for 6 weeks. For glyburide delivery, 10 μl of PBS-suspended hydrogel containing vehicle or 3 mM glyburide was administered intra-articularly every 10 days for 10 weeks. The mice were killed at 6 or 10 weeks after DMM or sham surgery. The degree of cartilage destruction in the knee joints was evaluated by safranin O staining and scored using the OARSI grading system.
Results: Intra-articular injection of probucol accelerated OA progression in DMM-operated mice, evidenced by increased cartilage destruction, enhanced expression of SASPs markers (IL6, MMP13), as well as senescence markers. Treatment with probucol significantly aggravated OA-associated pain in DMM-operated mice. Conversely, intra-articular glyburide treatment significantly delayed OA progression, reduced SASPs expression, and improved pain severity, as evidenced by increased weight-bearing capacity.
Conclusions: Our findings demonstrate that drugs modulating senescence-associated inflammation affect OA progression. This study provides a therapeutic strategy for OA treatment through pharmacological targeting of senescence-associated inflammation, and further studies using clinical data and various mouse models are ongoing.

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EARLY RESPONSE TO JOINT TRAUMA INTERVENTION AFFECTS THE FOLLOWING REPAIR PROCESS AND RISK OF POST-TRAUMATIC OSTEOARTHRITIS

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Purpose (the aim of the study): Osteoarthritis (OA) was recently identified as an epidemic, affecting over 590 million adults worldwide with no cure available. A major impediment in the development of reliable treatments for OA is a lack of understanding of how the disease is initiated and the subsequent underlying mechanisms for it's progression. The onset of OA has been linked to a prolonged pro-inflammatory response induced by a trigger such as trauma. Approximately 40% of patients suffering from joint trauma such as anterior crucial ligament rupture develop post-traumatic OA (PTOA), and the number remains at 37% following reconstruction surgery. This suggest that PTOA is not developed as a function of impaired mechanical stability, but of the biological trauma. We hypothesized that this is caused by an injury-related signal leading to an imbalance in the activation of the immediate pro-inflammatory response upon trauma and the transition from the pro-inflammatory to the pro-regenerative phase.
Methods: To investigate the early response to joint intervention involving different levels of trauma, peripheral blood and synovial fluid was isolated prior, 24h- and 6 weeks post intervention. In addition, synovial membrane was isolated during intervention. Cellular composition, ECM characterization and the presence of biomolecules was investigated employing a combination of flow cytometry, histology, immunohistochemistry and MALDI-imaging. The obtained samples were benchmarked against samples isolated from functional (regenerating) and dysfunction (developing PTOA) joint injury in a pre-clinical animal model. Specifically, pathological evaluation upon injury creation confirmed four distinct degrees of healing after 12 weeks, depending on defect size and immune status.
Results: Analysis of the early mechanism of healing confirmed a corresponding trend between the intervention/trauma level and the immediate response in terms of immune-cell response, skeletal progenitor cell activation and extracellular matrix (ECM) production. This was observed in both the human and preclinical samples. The obtained results confirmed that both the availability of mature T-cells and trauma level significantly affects the healing response at an early phase of healing ($p < 0.5$). Interestingly, scRNASeq from the defect environment of the preclinical model confirmed unique inflammatory and progenitor cell populations present within the defect area in the functional healing model. Next, analysis of cellular communication between specific populations was performed. Elevated COMPLEMENT signaling between chondroprogenitors and immune cell populations was identified in the healing model compared to the non-healing models. Further, elevated growth arrest specific (GAS)-related signaling was found between inflammatory macrophages in the dysfunctional healing models, compared to the healing model. This could be correlated to elevated production of reactive oxygen species (ROS) 96h post injury. To test whether the elevated ROS in the pro-inflammatory environment in the non-healing models was the underlying cause for the failed tissue regeneration, an immunomodulatory cell treatment was developed based on joint-activated and pro-regenerative immune cells that was co-cultured with placenta derived progenitor cells. The treatment was injected intra-articularly one week post injury and evaluation in regards of treatment efficacy for PTOA resolution was investigated after 11 weeks. It was confirmed that the immunomodulatory cell treatment reduced the elevated ROS-related damage. Further, the injectable therapy improved the healing potential in the three models of dysfunctional healing, with a fully regenerated articular unit confirmed based on histology, OARSI scoring and IHC analysis for Collagen type 2 and Lubricin.
Conclusions: These results confirm the integral role of the balanced activation of pro-inflammatory immune cells and pre-regenerative immune- and skeletal progenitor cells for functional tissue regeneration upon trauma or surgical intervention to achieve restored joint homeostasis. Furthermore, the presented findings show articular cartilage regeneration after the intra-articular injection of an immunomodulatory cell treatment upon acute moderate to large joint-injuries and prevention of PTOA development. This suggests a potential early mechanism of molecular, cellular, and ECM-based alterations that lead to either functional (regeneration) or dysfunctional (PTOA-initiation) healing and thus the opportunity identify diagnostic and therapeutic targets for early clinical intervention. In addition, the described immunomodulatory therapy tested in the pre-clinical setting provides an early intervention therapy to avoid the initiation of post-traumatic OA.

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OSTEOCYTE AND CHONDROCYTE RESPONSES TO GRAVITY ARE DIAMETRICALLY OPPOSED

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Purpose (the aim of the study): To evaluate the responses of lunar gravity on osteocyte and chondrocyte in both talocalcaneal and calcaneocuboid joint osteochondral unite (OCU).
Methods: The hind legs of the following two groups of mice provided in collaboration with the Japan Aerospace Exploration Agency (JAXA) were used. Group 1. flight group (1/6G); 24 legs of 12 mice reared in lunar gravity for approximately 4 weeks in an on-orbit cage of JAXA's Kibo launched at 9 weeks of age. 2. ground group (1G); mice of the same age and week as group 1 reared on the ground as control. Except for three mice in the 1/6G group that lost more than 10% body weight. Non-decalcified specimens were prepared and both of osteocyte and chondrocyte morphometry of the OCU in the talocalcaneal and calcaneocuboid joint were measured, then compared between groups. The subchondral bone plate in the OCU was divided into two layers, the non-osteocyte layer following the articular cartilage (AC) and the osteocyte layer underneath it.
Results: In 1/6G group, OCU in calcaneocuboid joint, the number of osteocytes in osteocyte layer in subchondral bone plate (Subcho.BP) was significantly reduced. In contrast, the number of chondrocytes at the