205 EXPRESSION OF CATHEPSIN D AND LIMP-2 CORRELATES WITH CHONDROCYTES DEATH DURING CARTILAGE BREAKDOWN

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Purpose: To evaluate the expression of Cathepsin D and LIMP II in articular cartilage and correlate its expression levels with the chondrocytes death in cartilage explants under oxidative stress and in a surgically-induced rat osteoarthritis model.

Methods: Cultured normal rat articular cartilage explants, were treated with H2O2 at 10 mM and incubated for 0.5, 1, 1.5, 2, and 6 hrs. Explants incubated in absence of H2O2 were used as control. Osteoarthritis (OA) was induced in Wistar rats by unilateral knee partial meniscectomy and post-surgery training for 15 min daily during; 5, 10, 20 and 45 days. Normal rats were used as controls.

Cartilage samples were fixed with 2.5% glutaraldehyde in cacodylate buffer and postfixed in 1% osmium tetroxide. Then, tissues were embedded in Spurr resin to obtain thin sections that were observed in the electron microscope (JEM-2000EX, Jeol). Samples were also fixed with 4% paraformaldehyde in PBS, cryosectioned and processed for Cathepsin D and LIMP II detection by immunofluorescence. Combinations of TUNEL assay with immunofluorescence for Cathepsin D were also performed. Sections were observed in the confocal microscope (LSM-SPC-5MO, Leica Microsystems).

Results: Ultrastructural analysis of chondrocytes from the articular cartilage explants, which were induced to oxidative stress with H2O2 showed changes as: cellular shrinkage, nuclear condensation and large amount of vesicles, suggesting a cell death process. To determine the involvement of lysosomes in the cell death process, we studied the expression of the lysosomal proteins, Cathepsin D and LIMP II. Both proteins were up-regulated in the time course of the stress induction. Furthermore, Cathepsin D and TUNEL labels were found in the same chondrocytes suggesting that lysosomes have an important role in the chondrocytes death. Afterwards, the expression of Cathepsin D during the kinetics of OA pathogenesis in a rat model showed an up-regulation since 5 to 45 days after induction.

Conclusions: Our results suggest an important role of lysosomal pathway in the OA pathogenesis as part of cell death by Chondrioptosis.

206 IMPROVED REGENERATION OF ARTICULAR CARTILAGE BY HUMAN MESENCHYMAL STEM CELLS THROUGH OSTEOSTIGENIC AND BMP2 SIGNALING

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Purpose: Articular cartilage has very limited capacity of regeneration or cartilage repair treatments usually lead to the formation of fibrocartilage. Due to their inferior mechanical properties fibrocartilage only delays the progression of the injury. Therefore, to stop the progression of the injury into osteoarthritis it is capital an effective treatment of chondral lesions. We tested the therapeutic potential of a bi-layered implant containing a porous polymer that sustains chondrogenesis and trabecular bone formation and hyaline-like cartilage formation.

Methods: A critical size osteochondral injury was performed in the femoral condyle of adult sheep. The lesions were filled with bi-layered implants formed by an upper 1 mm thick poly-1-lactic acid layer (PLLA) and a lower 5 mm thick PLLA/hydroxyapatite composite layer (PLLA/HAP). The PLLA layer was cell-free (Scaffold group) or loaded with 5E06 human mesenchimal stem cells (ScaffoldMSC group). The control group consisted of a chondral lesion followed by subchondral bone drilling (Microfracture group).

Results: Twelve weeks post injury we observed increased cartilage formation in the Microfracture and ScaffoldhMSC groups by Safranin O and cartilage markers staining (type II collagen and aggrecan). Strikingly, while in the Microfracture group the new cartilage matrix was mostly fibrocartilage (collagen type I rich), the ScaffoldhMSC group showed hyaline-like neocartilage (collagen type I low). To further investigate the mechanism of neocartilage formation we looked at the injuries after six weeks of treatment. At this point in the Scaffold and ScaffoldhMSC groups the defects were filled with a highly cellular, highly proliferative tissue invading the PLLA/HAP composite, while the Microfracture group showed partially filled defects composed of fibrous tissue with low cellularity. In addition, we observed an increase of osteoid at the edges of the composite layer indicating that the scaffold activates osteogenic signals in the surrounding tissue. Importantly, although in the ScaffoldhMSC group we do not detect hMSCs, it was a statistically significant increased proliferation of multnucleated giant cells when compared to the Scaffold group (p<0.001). The presence of osteoclasts in the reparative tissue was confirmed by TRAP staining. This increase in osteoclasts indicates the presence of hMSCs-induced active trophic factors with actions over the osteoclast-like population.

Purpose: To determine the nature of these osteogenic signals we stained for BMP2 protein and BMP2 mediated signaling (pSmad 1/5/8) and found both signals increased in the ScaffoldhMSC group. Furthermore, we identified that BMP2 secretion and BMP2 signaling were mediated by the giant osteoclast.

Conclusions: Paracrine factors, secreted by hMSCs, act through a population of giant osteoclast that mediated the scaffold resorption, trabecular bone formation and hyaline-like cartilage formation.

207 POTENTIAL OF CARTILAGE REGENERATION BASED ON CHONDROCYTE CLUSTER IN OSTEARTHRITIS

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Purpose: Malalignment of the knee joint increases the risk of osteoarthritis (OA). Cartilage degeneration is often observed site-specific difference in the knee joint and it depends on the degree of mechanical stress based on knee malalignment. Chondrocyte cellularity is altered with OA progression and chondrocyte cluster is recognized as one of the OA pathology with expressing the several catabolic factors. Understanding chondrocyte cellularity based on the effect of mechanical stress may play an important role to elucidate the mechanism of OA pathology, however it is still unclear the effect of mechanical stress based on the knee malalignment for chondrocyte biology.

The purpose of this study is to clarify the chondrocyte cellularity in consequence of mechanical stress and investigate the potential of cartilage regeneration for utilizing the chondrocyte cluster.

Methods: After receiving approval for this study from the Ethics Committee, cartilage explants were obtained from 33 varus OA knees undergoing total knee replacement surgery and harvested from 3 locations at femoral condyle; the center of medial condyle (MC), which was most severe cartilage degeneration, medial adjacent (MA), which was fibrillated, and lateral condyle (LC), which looked normal. Cartilage degeneration was graded by Mankin score and number of chondrocytes were counted in each samples with observing the type of chondrocyte formation; single or cluster. Immunohistochemical analysis was performed with one of the progenitor cell marker STRO-1 and proliferative related marker FGF2 and Ki67. Furthermore, cartilage explants and chondrocytes from MA and LC were cultured by DMEM with 10% calf serum and determined the ability of cartilage regeneration by comparing the area of cartilage erosion before and after tissue organ culture and the product of cartilage like nodules in monolayer culture with toluisin blue staining.

Results: In terms of the cartilage degeneration, MC was most severe (13.3), as compared with MA (7.8) and LC (4.1) according to the Mankin score. Number of chondrocytes was highest in MA more than any other location such as LC and MC (P<0.001). The type of chondrocytes in MA was mainly cluster formation, which expressed STRO-1, FGF2 and Ki67. Furthermore, cartilage explants which involved erosion, was covered with new tissue from MA during two weeks organ culture, moreover chondrocytes from MA proliferated faster and made cartilage like nodules more than LC (Fig. 2).