Changes in Knee Joint Following Non-Invasive Tibial Compression in Genetic Mouse Strains

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Introduction: Joint trauma can result in a spectrum of acute lesions and potentially lead to post-traumatic osteoarthritis (PTOA). We showed that early molecular events include apoptosis and rearrangement of aggrecan distribution in C57Bl/6 mice (1). In this study, we asked whether mice that could heal their cartilage showed resistance to these early molecular changes. Two recombinant inbred mouse strains, (LGXSM-6 and LGXSM-33) generated from a LG/J by SM/J intercross, have been shown to differ in various phenotypes: LGXSM-6 has the capacity to heal full-thickness articular cartilage lesions and ear wounds and is relatively protected from developing PTOA compared to LGXSM-33 (2, 3). Here, we hypothesized that LGXSM-6 and LGXSM-33 will respond differently to mechanical loading for various cytological and histological parameters including events in the cartilage and in the synovium.

Methods: To identify cells responsive to injury, a double nucleoside analog cell-labeling scheme was used: iododeoxyuridine (IdU) for slow cycling cells and chlorodeoxyuridine (CldU) for rapid cycling. For labeling of slow-cycling cells, 3-week old male mice received IdU (1 mg/mL) in the drinking water for 30 days, followed by a 40-day washout period. After washout period, right knees of all mice underwent compressive loading (9N for 60 cycles) to produce an articular cartilage defect (1) and CldU was injected (10 mg/mL) at multiple time points after injury (0, 5, 9 days, N=4 in each group) in both strains following by in drinking water (1 mg/mL) for 4-5 days (to label rapidly dividing cells). Contralateral left knees served as controls. Knee joints were harvested 5, 9 and 14 days following injury and processed for histology. Immunohistochemistry for aggrecan and cartilage oligomeric matrix protein (COMP) was performed to observe the pattern and location of these extracellular matrix molecules synovium and cartilage. To detect cell apoptosis, we used TUNEL (Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling) assay.

Results: Our loading regimen (i.e. 9N) resulted in transection of anterior cruciate ligament, thickening of synovial lining, as well as in a severe cartilage injury on the lateral femoral condyle. The latter was characterized by loss of Safranin-O staining, chondrocyte apoptosis and change in the pattern of extracellular matrix molecules namely aggrecan and COMP. No such changes were observed in the contralateral non-loaded knees.

While, slow-cycling IdU-positive cells were detected in cartilage and synovium in both strains at all time-points, a marked proliferation of fast-cycling CldU-positive cells was only observed in the synovium. No fast-responding cells were detected in the cartilage.

We also observed an interesting pattern of aggrecan and COMP expression in the loaded knees. For example, both of these proteins were present around the chondrocytes in the intact area while at the site of injury these molecules became internalized in the apoptosed chondrocytes. We did not find any significant differences between the two mouse strains for the above parameters. However, we have
noticed that LGXSM-33 showed ectopic synovial chondrogenesis 14-days post-injury. These nodules were positive for Safranin-O, aggrecan, COMP, and Type II collagen staining. No such nodules were observed in LGXSM-6 mice or in the non-loaded contralateral knee.

Discussion: It appears that there are no differences in cell apoptosis and pattern of matrix distribution between the two mouse strains at early phase (within 14 days) after loading. However, we did identify a difference in the synovial cell response. We also identified that the location of cells responding to injury by proliferation was only in the synovium. Thus, cartilage with apoptosis and a lack of cell proliferation is likely to degenerate in both genetic types of mice; however, the participation of the synovium is suggested to be differential.

Significance: The ability to heal cartilage has been associated with relative resistance to Osteoarthritis. This study was designed to determine whether mice that can heal cartilage respond differently to impact injury.

ORS 2015 Annual Meeting
Poster No: 1203