DIFFERENCES IN BONE FORMATION WITH CARTILAGE LOSS DETERMINES OSTEOARTHRITIC SUSCEPTIBILITY

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Purpose: Subchondral bone remodeling is known to play a role in Osteoarthritis (OA), but the precise nature of those changes in initiation and progression is under debate. Generally, in OA, as cartilage is lost trabeculae in subchondral bone thickens. Our previous study determined that a small group of OA patients exhibit slow bone formation and do not fall into this norm. In an effort more accurately define this phenotype we investigated changes in the mean area of trabecular spaces (0.1–1 mm²) with cartilage loss. We also compared this data to the Trabecular Space (TrabSp) data calculated by microCT, which represents the mean TrabSp. We hypothesize that phenotypic differences in subchondral bone formation influences osteoarthritic susceptibility.

Methods: To characterize subchondral bone changes, we performed microCT analysis on femoral condyles of surgically discarded total knee arthroplasty (TKA) tissue. We compared subchondral bone changes in the lateral (full thickness cartilage) to the medial (no cartilage) condyle from 18 medial compartment OA patient and 3 control samples (full thickness cartilage on both condyles). MicroCT data included BV/TV, trabecular thickness, trabecular spacing and mean density. Other data collected included: Age, BMI, OARSI score, average cartilage thickness, average subchondral bone thickness. The percent change between trabecular spaces in full thickness lateral condyle subchondral bone was compared with the subchondral bone of completely denuded medial condyle lesion areas. Additionally, 100 sequential image slices were exported from the microCT for analysis of mean area of small trabecular spaces (0.1–1 mm²) in Image-Pro plus.

Results: A comparison of the percent changes in the mean area of trabecular spaces (0.1–1 mm²) between non-lesion (full cartilage) and lesional (no cartilage) samples in 18 patients resulted in two clearly different cohorts. In one cohort (67%) the trabecular space decreased with cartilage loss and in the second group (33%) the area increased. A group of 3 samples with no visible cartilage damage between the medial and lateral condyles was analyzed as a control and showed no change in trabecular spacing between lateral and medial condyles. These values were compared with the trabecular space data generated by microCT. Our data suggests that during the process of subchondral bone remodelling, some individuals tend to form more bone (% change in area decreased, trabecular spaces became smaller, bone becomes more dense) while others tend to form less (% change in area increased, trabecular spaces became larger, bone becomes less dense). The data from this study indicates that people with increased bone formation associated with cartilage loss have a greater risk of developing OA. This result confirms the inverse relationship between osteoarthrosis and osteoporosis that has been reported. Although our study is preliminary, we speculate that those who form bone more quickly have increased potential for biomechanical stresses in articular chondrocytes, would experience faster cartilage loss and have an increased potential for micro-fracture related acceleration of articular cartilage degeneration. A more in-depth analysis, with a larger sample size will be important to understanding how these variations in subchondral bone formation relates to the pathogenesis of OA and it’s relationship to osteoporosis.

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COMPARISON OF GENE EXPRESSION PROFILES BETWEEN NORMAL AND SCLEROSIS REGIONS OF HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE

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Purpose: Osteoarthritis (OA) is the most prevalent form of arthritis and is characterized primarily by the degeneration of cartilage and subchondral bone remodeling. Recently, increasing evidences support the involvement of subchondral bone in the cartilage destruction. However, methods of extracting good quality RNA from underlying subchondral bone (USB) without cartilage remaining were not available. In our study, a novel RNA extraction protocol was developed to overcome these problems (Figs. 1 & 2) and searching for differentially expressed genes in the USB of osteoarthritic patients. Furthermore, our aim is to investigate the changes in USB sclerosis focusing specifically on the early changes and try to understand what causes this shift.

Methods: Each OA tibia plateau was divided into two parts: one is the outer lateral tibia as relative normal region and the other is the outer media tibia without cartilage remaining as sclerosis region. To detect any alteration in genes between these two areas collected at surgery from the knee tibia plateau of patients undergoing total knee arthroplasty for primary OA, RNA extraction protocol was developed to overcome these problems (Figs. 1 & 2) and searching for differentially expressed genes in the USB of osteoarthritic patients. Furthermore, our aim is to investigate the changes in USB sclerosis focusing specifically on the early changes and try to understand what causes this shift. 

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isolation by a novel method (RIN > 6 = 70%, 260/280: 1.8–2.0 = 100%, 260/230 > 1.0 = 95% (n=50)) and morphometry calculation by μCT (OA n=20, non-OA normal n=2). Illumina microarray was performed (OA n=10, non-OA normal n=2) and real-time quantitative polymerase chain reaction was executed for data validation (OA n=20, non-OA normal n=2).

Results: A total of 205 transcripts were found be expressed at significantly different level among the categorizations. Most of these genes related to the extracellular matrix synthesis, cell proliferation, bone cell differentiation. These genes might play key roles in breakdown of subchondral bone homeostasis in OA.

Conclusions: The novel USB RNA isolation methodical approach reported here not only allows to determine area-specific gene profiles in knee specimen. In addition, this study also show some genes might play a role in OA bone remodeling.

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CHARACTERIZATION OF BONE CELLS FROM HEALTHY AND OSTEOARTHRITIS PATIENTS

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Human bone cells derived from bone marrow (BM) aspirates or trabecular bone (TB) tissue have been used for studies of bone biology. However, an in-vitro characterization of these cells is necessary not only for basic studies in health and disease but also for cellular therapies for tissue repair. Bone tissue is often discarded during joint replacement or bone correction surgery and this tissues could represent a good potential alternative source of osteoprogenitor cells for tissue engineering purposes. The study aims to validate the potential of different bone cells obtained from the same anatomical site. Therefore, we isolated MSCs from BM and osteoblasts (OBs) from tibia of healthy and OA patients. MSCs and OBs were compared for growth condition, cell proliferation, phenotype and osteogenic potential.

The study aims to validate the potential of different bone cells obtained from the same anatomical site. Therefore, we isolated MSCs from BM and osteoblasts (OBs) from tibia of healthy and OA patients. MSCs and OBs were compared for growth conditions, cell proliferation, phenotype and osteogenic potential. MSCs and OBs were grown both in α-MEM and DMEM/F12 medium. We found that MSCs were able to grow only in α-MEM while OBs could survive and proliferate in both medium tested. The proliferation of healthy MSC and OB grown in α-MEM was significantly higher than OA MSC and OB, while no significant differences were observed in OB grown in DMEM/F12. Flow cytometric analysis for CD73, CD90, CD105, CD146, alkaline phosphatase, bone sialoprotein (BSP) and collagen type I did not show differences among the groups analysed. The osteogenic potential of MSC and OB grown in α-MEM were next tested inducing the cells to differentiate along the osteoblast lineage in osteogenic medium and demonstrating the positive staining for extracellular calcium deposition at day 28. Real time PCR analysis demonstrated a decrease expression of alkaline phosphatase and collagen type I associated with an increase of collagen type XV from Day 0 to day 28, while BSP expression was significantly increased only in healthy MSC and OA OB but not in OA MSC. Osteogenic control experiments were also performed using MSC from iliac crest. Here, we found a decreased expression of alkaline phosphatase and collagen type 1 from day 0 to day 28 and a significant increase in collagen type XV and BSP expression. These data suggest that even if osteogenic differentiation occurs in MSC and OB from OA patients, OA OB were skewed towards a pattern of expression of specific osteogenic markers more similar to that found in canonical BM MSC.

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SMAD2/3 SIGNALING IS CRUCIAL FOR CARTILAGE MAINTENANCE, WHEREAS SMAD1/5/8 SIGNALING DETERMINES CHONDROCYTE TERMINAL DIFFERENTIATION

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Purpose: In osteoarthritis (OA), chondrocyte behavior seems a recapitulation of the developmental process. Chondrocytes loose their articular cartilage phenotype and progress into a state highly similar to chondrocyte hypertrophy, overexpressing MMP13. We have previously shown that during OA, the balance between the TGF-beta receptors ALK1 and ALK5 shifts, favoring the ALK1 receptor. Downstream this leads to a shift from signaling via Smad2/3 towards Smad1/5/8, thereby favoring the signaling pathway that is important in terminal differentiation. As a consequence, blocking of the Smad1/5/8 pathway in differentiating chondrocytes might be a tool to inhibit chondrocytes terminal differentiation.

Results: We show that Smad1/5/8P expression is correlated with MMP13 expression in limb formation and in terminally differentiated pellet cultures. Moreover, whereas blocking Smad2/3P halted further chondrogenesis, we show that blocking the Smad1/5/8 pathway blocked terminal differentiation and therefore inhibited chondrocyte hypertrophy.

Methods: BMSC were chondrogenically differentiated in pellet culture in chondrogenic medium including TGF-β. SB-505124 was added to inhibit Smad2/3 phosphorylation, while dorsomorphin was added to inhibit Smad1/5/8 phosphorylation.

Conclusion: Blocking Smad2/3 phosphorylation from day 14-35 resulted in a halt in collagen II production, whereas blocking Smad1/5/8 phosphorylation decreased the expression of MMP13, collagen X and alkaline phosphatase without inhibiting further collagen II production. Blocking Smad1/5/8 phosphorylation completely prevented mineralization (Figure 1).