Tissue Microarray Analysis of Osteoarthritic Samples Indicate Subchondral Trabecular Bone Changes Define Molecular Pathology

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INTRODUCTION: Subchondral bone remodeling is known to play a role in Osteoarthritis (OA), but the precise nature of the subchondral bone changes in initiation and progression is under debate. Generally, in OA, subchondral bone thickens as cartilage is lost. The turnover of bone is reported to be a coupled reaction; which means as bone is resorbed new bone replaces it. However, new studies indicate that there can be differences in each of these two processes occur, resulting in imbalances between the two. In our previous work using femoral condyles from TKA patients with OA, we determined that trabecular bone under the subchondral bone plate responds to cartilage loss. These changes can vary within and between groups. One group showed no change or loss in bone volume/total volume (BV/TV) while the other group has increased BV/TV. To investigate the role of subchondral trabecular bone remodeling subtypes in osteoarthritis pathology we used tissue microarray analysis to determine whether these two groups differed in eight different proteins known to be associated with OA. Additionally, tissue was collected from all tissues within the joint. We found that variations in subchondral trabecular bone remodeling between patients may be associated with unique molecular pathologies in OA development and progression.

METHODS: To characterize subchondral bone changes, we performed microCT analysis on surgically discarded TKA samples of femoral condyles. We compared subchondral trabecular bone changes in the lateral (full cartilage) versus the medial (no cartilage) condyle from 15 medial compartment OA patients. 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 difference between subchondral trabecular bone BV/TV in full thickness lateral condyle and differences in the rates at which these two processes occur, resulting in the patients into two groups. Tissue microarray slides were created (Figure 1) and immunohistochemistry was performed, imaged and quantitated for all tissue cores on the slide using a custom macro in Image ProPlus 6.2 (MediaCybernetics, Silver Springs, MD). Using this information coupled with patient info and morphometric data, we compiled a data matrix for each sample and applied principal components analysis to determine a profile of all collected data. We then segregated the data by subchondral trabecular BV/TV response to cartilage loss and reapplied the principal component analysis.

RESULTS: To investigate the individual tissue properties with respect to the eight proteins the entire data set was analyzed with principal components and Pearson correlation coefficients analysis. We then reanalyzed the data separating it into the two groups identified by change in BV/TV. Analysis of the whole data set as a single entity yielded a large number of protein interactions. When this data is segregated by individual tissue contributions, a more specific profile is obtained. Furthermore, comparison of each tissue type with another yielded additional insights. For example, when lateral articular cartilage (early OA) is compared with medial articular cartilage (late stage OA), interactions between VEGF, IHH and FGF are observed in the early OA sample, but VEGF is associated with IL-6 in the late OA sample. The use of principal component and Pearson correlation coefficient analysis of the tissue microarray protein data combined with patient data identifies which proteins or patient data variables are statistically significant in each tissue and in high and low bone density groups (Figure 2).

DISCUSSION: This study demonstrated for the first time how tissue microarray technology can be used to identify the molecular pathology of individual tissues within the osteoarthritic joint. It also showed that subchondral bone characteristics play a key role in osteoarthritis, affecting each tissue within the osteoarthritic joint. When comparing results from tissues of the lateral versus medial condyle, these analyses allow the identification of protein localization and interactions specifically associated with early and late disease states; which have the potential to act as biomarkers. Additionally, the correlation of proteins with specific patient data attributes, such as age or BMI, provides insight into how these characteristics contribute to mechanism of action in disease progression.

SIGNIFICANCE: The results reported in this study provide a large amount of data and insight into osteoarthritis, however the potential of tissue microarray analysis of this data set and of OA has yet to be realized.