Osteoclastomics of the Human Giant Cell Tumors (GCT) of Bone: First-Time Spatial Whole-Transcriptomic Deciphering of Novel Genes Accountable for Massive Osteoclastogenesis and Bone Destruction

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INTRODUCTION: Osteoclasts are the main drivers of bone destruction on bone tumors. Among those, GCT also known as osteoclastoma stands out as the most predominant osteolytic tumor. GCT is well known for its pathognomonic crater-like bone destruction and massively gigantic osteoclastogenesis. While past studies using gene expression comparison identified RANKL, RANK, and OPG about 30 years ago, the pathophysiological mechanism by which gigantic osteoclastogenesis takes in the confined bone has not yet been elucidated. Furthermore, the molecular drive that propels the fusion of stimulated macrophages and aggressive bone resorption is unknown. 'Spatial Transcriptomics' is the most state-of-the-art molecular approach that enables detection of 20,000 human genes with 2 micrometer resolution in the human pathology specimens as opposed to single gene in situ hybridization 30 years ago. Furthermore, unlike RNAsequencing of the suspended cells, 'Spatial Transcriptomics' allows the analyses of the dynamic gene expressions in each cell. Given the fact, the size of the giant cells is 50 - 200 micrometers, 'Spatial Transcriptomics' allows the detection of gene expression dynamics within the single giant cell. The purpose of this study is to delineate genes that are highly expressed in the giant cells in comparison to surrounding macrophages that are known to be osteoclast precursors. By identifying such osteoclastogenic transcriptomics (Osteoclastomics), new drugs can be developed to treat tumor-induced bone loss and osteoporosis.

METHODS: Visium HD spatial transcriptomic analyses were performed in duplicates of 10 different human GCT pathological specimens (total N=20) after verifying the intactness of RNAs in the paraffin-embedded samples. All GCT cases showed characteristic osteolytic appearance on radiographs. GCTs were from 4 femurs, 3 fibulas, 2 radius, and other bones in 3 male and 7 female patients with age ranging 18 to 75. All patients underwent extended curettage and cementation and were followed for longer than 2 years. RNA-preserved paraffin embedded histological sections were processed with batch-effect correction, sample integration, normalization, and dimensionality reduction. Intercellular network analysis was performed using 10x Loupe Ranger, Single Cell Sequencing Analyzer, R Program, and CellChat were performed (**Figure 1**). Differentially expressed mRNAs were validated with single-cell RNA sequencing and *in vitro* cell-culture assay.

RESULTS: Osteoclasts in the GCT exhibited distinctive differential transcriptomic zones with dynamic functional heterogeneity in macrophage lineage cells (**Figure 2**). Strikingly, for the first time, giant cells show 3 distinct gene-expression clusters in 3 radially separated rings. The peripheral transcriptomic ring cluster was seen macrophages fusing with the pre-formed osteoclasts. The middle transcriptomic ring cluster showed evidence of enzymatic degradation. The central transcriptomic ring cluster showed genes that are known to drive the multinuclear osteoclast fusion (**Figure 3**). In addition, osteoclasts show more anaerobic glycolysis compared to surrounding mononuclear cells. We also identified and verified over 50 novel genes many of which showed strong temporal correlation with the RANKL induced osteoclastogenesis in vitro.

DISCUSSION AND CONCLUSION: In this study, we unexpectedly identified that osteoclasts in GCT have geographically distinct subcellular gene expression clusters, implying the stratified novel therapies can be designed for specific targeting of bone resorption, cell fusion, and maintenance of osteoclasts. In addition, macrophage lineage cells of giant cell tumor hold tremendous functional and gene expression-level heterogeneity. Furthermore, osteoclasts in GCT are the products of dynamic interactions of thousands of genes. Novel genes that are differentially upregulated in osteoclasts and that show temporal upregulation during osteoclastogenesis will be logical novel therapeutic targets for osteoclast-related diseases such as tumor-induced bone loss and osteoporosis.

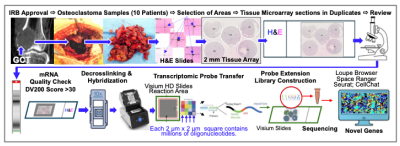

Figure 1. Spatial Transcriptomic Analyses of Human GCT Samples. A schematic workflow of whole spatial transcriptomic sample preparation of the human osteoclastoma (GCT). Duplicates of 10 biopsy-proven GCT pathology slides were reviewed after the IRB Approval. Spatial transcriptomic unbiased screening analyses of 20 different samples showed clustering of genes that are known to be expressed in the mesenchymal stromal cells, macrophages, and osteoclasts

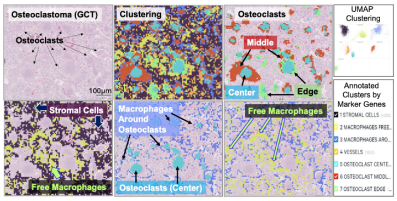

Figure 2. Differential clustering patterns seen in giant cell tumor of bone. Distinctive gene clustering generated via annotation with known macrophage markers (CD14 and CD74), osteoclast markers (TRAP and cathepsin K), and stromal cell markers (SDF1, PTHR1). Osteoclasts show evidence of highly-distinctive populations based upon proximity to the giant cell.

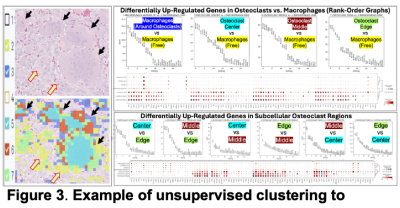

Figure 3. Example of unsupervised clustering to examine distinct gene expression profiling in osteoclast and macrophage populations. Sub-cellular analysis of the giant cell reveals distinctive gene expression patterns depending on osteoclast and macrophage regions (periphery vs center).