Integrated Single-Nucleus Transcriptomic and Epigenomic Profiling of Giant Cell Tumor of Bone

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INTRODUCTION
Giant cell tumor of bone (GCTB) is a locally aggressive osteolytic tumor of intermediate malignancy treated with surgery and/or denosumab, a RANKL inhibitor. Most GCTBs have recently been shown to harbor the H3F3A p.G34W mutation. However, the molecular mechanisms underlying tumor progression and metastasis remain poorly understood. We aimed to characterize intratumoral heterogeneity and molecular alterations associated with treatment and metastasis using integrated single-nucleus multi-omic analysis.

METHODS
Frozen samples from GCTB patients (two pre-treatment samples, one post-treatment sample, and one pulmonary metastasis sample) were analyzed using Chromium Epi Multiome ATAC + Gene Expression (10x Genomics). Libraries were sequenced on Illumina NovaSeq 6000 platform. Transcriptomic clustering and gene set enrichment analyses (GSEA) were performed among tumor and stromal compartments. Chromatin accessibility profiles were compared across tumor cells, giant cells, monocytes, and endothelial cells, with motif enrichment analysis to identify key transcriptional regulators.

RESULTS
Integrated analysis identified 19 clusters, including monocytes, T cells, endothelial cells, B cells, giant cells, and multiple tumor subpopulations (pre-treatment, post-treatment, proliferating, metastatic). Post-treatment clusters showed enrichment of pathways related to protein synthesis (translation initiation/elongation, rRNA processing, nonsense-mediated decay), suggesting translational reprogramming and stress adaptation as mechanisms of persistence. Metastatic clusters were enriched in cell cycle (E2F targets, G2M checkpoint), metabolic reprogramming (glucose metabolism, hypoxia response), and immune signaling (IFN-γ, TNF-α/NF-κB). ATAC-seq revealed open chromatin at RANKL (tumor) and RANK (giant cell) loci, highlighting epigenetic regulation of osteoclast–tumor interactions. Motif analysis identified AP-1, RUNX, NF-κB, TEAD, and KLF family transcription factors as key regulators in tumor chromatin accessibility

DISCUSSION
This integrated single-nucleus transcriptomic and epigenomic profiling demonstrates distinct molecular programs underlying denosumab treatment and metastasis in GCTB. These findings provide new insights into GCTB biology and may inform strategies to overcome recurrence and metastasis.

SIGNIFICANCE/CLINICAL RELEVANCE
This study provides the first integrated single-nucleus transcriptomic and epigenomic profiling of giant cell tumor of bone, revealing distinct molecular programs associated with denosumab treatment response and pulmonary metastasis. These insights may guide the development of novel therapeutic strategies and improve clinical management of GCTB.

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