Title: *Single-Cell RNA Sequencing and Proteomic Spatial Profiling Reveal Complex Tumor Microenvironment and Novel Immune Interactions in Chondrosarcoma*

Background: Chondrosarcoma (CSA) is a primary bone malignancy with few therapeutic options. Discovery of new therapies is hampered by the paucity of data on the biology of CSA and its tumor microenvironment (TME). The TME significantly influences CSA progression, yet its cellular composition and critical intercellular interactions remain poorly understood. This study aimed to dissect the CSA TME using integrated single-cell and spatial approaches to identify novel interactions that could be leveraged for future therapeutic targets.

Methods: Fresh tumor tissues from 10 CSA patients (Grades 2, 3, dedifferentiated) underwent single-cell RNA sequencing (scRNA-seq). Computational analysis (including SCEVAN for tumor cell identification) defined cell populations. CellChat explored intercellular communication. Key findings were validated using PhenoCycler, a multiplexed spatial proteomics technique.

Results: scRNA-seq of CSA tumors revealed diverse tumor, cancer-associated fibroblast, and immune cell populations, plus osteoclast-like cells in dedifferentiated CSA. PhenoCycler spatial profiling validated these, visualizing Sox9+ tumor cells, T cells (CD4+, CD8+), dendritic cells, and monocyte/macrophages. Cell-cell interaction analysis inferred key tumor-immune interactions, including TNF signaling, MIF-CD74 axis, and SPP1-integrin signaling, pathways implicated in macrophage polarization, tumor progression, and immune evasion.

Conclusions: Chondrosarcomas exhibit a complex ecology of cellular phenotypes within their TME. Our combined single-cell and spatial proteomics approach provides an unprecedented atlas of the CSA cellular landscape and organization, detailing the tumor-immune interface. These findings, including the identification of specific intercellular signaling pathways, offer new insights into CSA pathobiology and identify specific cell populations and their interactions that could be exploited for novel therapeutic interventions.

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