Advanced Transcriptomic Analysis of Spp1 in Osteosarcoma: A potential new therapeutic target?

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Significance & Hypothesis

Analysis of the TARGET-OS dataset, a comprehensive genomic resource for pediatric osteosarcoma from the National Cancer Institute, demonstrates that elevated Spp1 expression is associated with decreased overall survival. Secreted phosphoprotein 1 (Spp1), which encodes osteopontin, is an extracellular matrix glycoprotein with established roles in bone remodeling, immune regulation, and cancer progression [1]. Although increased Spp1 expression has been linked to poor prognosis across multiple malignancies, its role in osteosarcoma progression and metastasis remains incompletely defined. We hypothesize that Spp1 promotes osteosarcoma progression through interactions with immune cell populations within the tumor microenvironment.

Innovation

This study integrates single-cell and spatial transcriptomics to define Spp1-driven interactions between tumor cells and the microenvironment in osteosarcoma. This approach reveals spatially distinct signaling and gene expression programs, supporting Spp1 as a potential therapeutic target.

Approach

We integrated single-cell RNA sequencing and Visium V2 spatial transcriptomics to characterize Spp1 expression and signaling in a clinically relevant murine model of osteosarcoma. All samples were derived from Rb1/p53 double-knockout mice and included paired primary tumors and lung metastases from two animals, representing early- and late-stage disease progression. Spatial deconvolution was performed to estimate cell-type composition at each location, and linear regression was used to assess relationships between Spp1 expression and malignant, immune, and stromal populations. CellChat was used to analyze cell–cell communication, Seurat was applied for differential expression analysis, and UCell was used for pathway enrichment.

Results

Single-cell analysis of primary tumors revealed heterogeneous Spp1 expression within malignant populations, with many tumor cells showing little to no expression and others exhibiting variable levels. Distinct differences were observed across osteosarcoma cellular subtypes. Cell–cell communication analysis identified robust, bidirectional Spp1-associated crosstalk between malignant cells and osteoclast populations.

Spatial transcriptomic analysis of primary and lung tumors demonstrated that Spp1 expression consistently correlated with malignant cell populations. In lung tumors, Spp1 also showed a strong association with macrophage-rich regions, a pattern not observed in primary tumors (Figure 1). Linear modeling confirmed that Spp1 expression was strongly correlated with both macrophage and malignant cell populations, with a more pronounced association observed in advanced disease. Differential expression analysis revealed elevated Spp1 expression in more advanced disease. Spp1 was also found in regions that exhibited high epithelial–mesenchymal transition (EMT) signatures, suggesting an important role in tumor progression (Figure 1). Further reclustering analysis revealed that Spp1 expression was higher in osteoblastic than in chondroblastic regions (Figure 2).

Discussion & Impact

In summary, although Spp1 expression has been linked to poor prognosis across multiple cancers, its role in osteosarcoma has remained poorly defined. Our findings identify Spp1 as a mediator of bidirectional crosstalk between malignant cells and osteoclasts within the tumor microenvironment. Notably, Spp1 expression was strongly associated with both macrophage and malignant cell populations, particularly in advanced disease.

Moreover, Spp1 localized to regions enriched for epithelial–mesenchymal transition (EMT) signatures, underscoring its role in driving tumor progression and highlighting its potential as an actionable target.

References

1. Zhang Z, Liu B, Lin Z, Mei L, Chen R, Li Z. SPP1 could be an immunological and prognostic biomarker: From pan-cancer comprehensive analysis to osteosarcoma validation. The FASEB Journal. 2024;38:e23783. doi:10.1096/fj.202400622RR

Figure 1: Spatial transcriptomics reveals enrichment of Spp1 in malignant and macrophage-rich regions of lung metastases and overlay of EMT Pathway

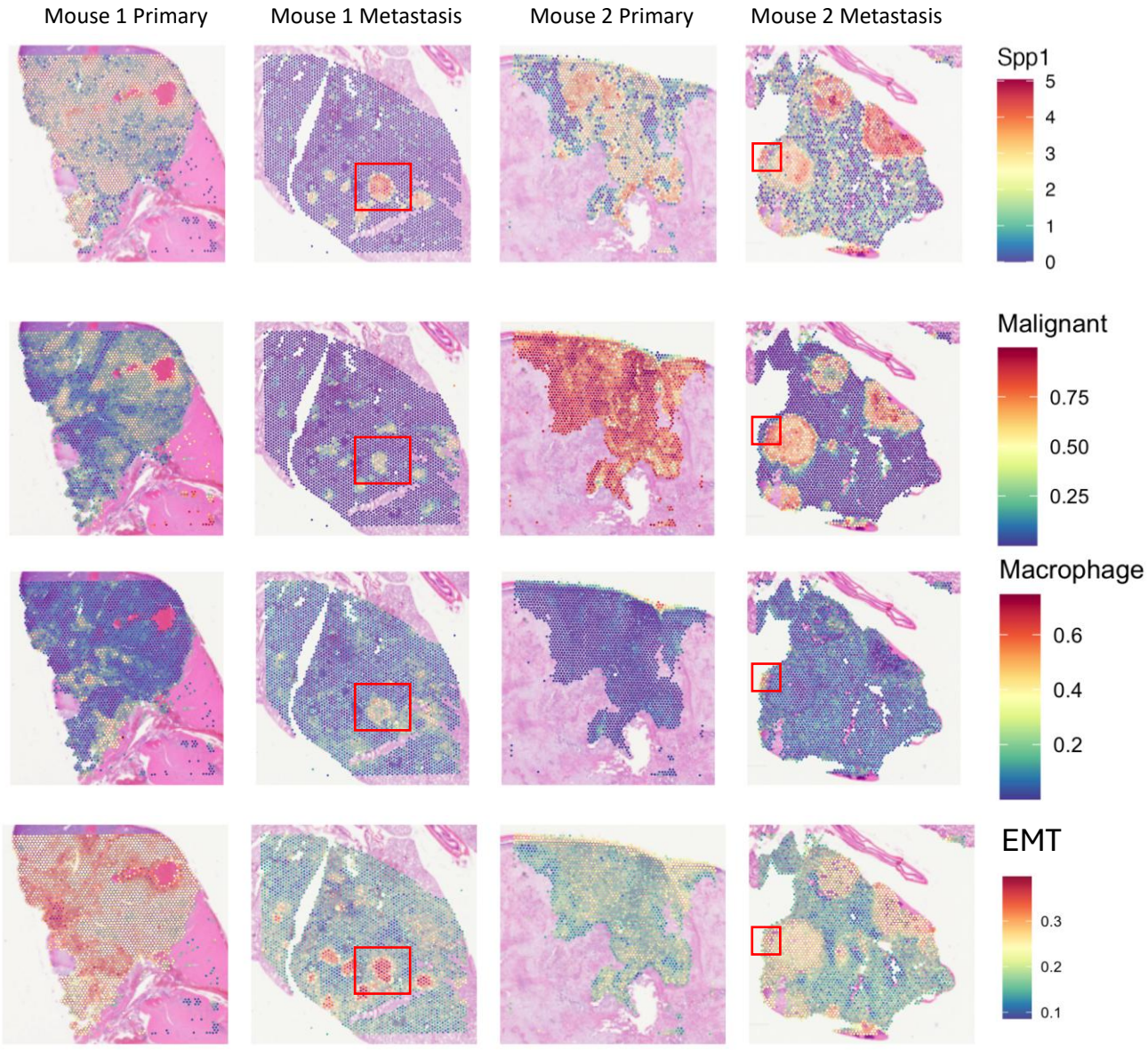

Figure 2: SPP1 preferentially marks osteoblastic-like lung metastases in osteosarcoma