Longitudinal Single-Cell Analysis of Osteosarcoma Defines Pre-treatment Chemoresistance and an Actionable PI3K Target

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Significance & Hypothesis

Neoadjuvant chemotherapy (NAC) resistance remains the central obstacle to improving osteosarcoma survival. The only reliable measure of chemotherapy response—the Huvos histologic necrosis rate—is available only after definitive resection, precluding pre-treatment risk stratification or early therapeutic adaptation. Bulk transcriptomic studies have identified resistance-associated patterns but cannot resolve whether signals originate from tumor cells, stromal remodeling, or immune shifts. We aimed to map the transcriptional rewiring accompanying NAC resistance at single-cell resolution and derive a clinically applicable gene signature capable of identifying refractory tumors from pre-treatment biopsies.

Innovation

Paired single-cell RNA sequencing (scRNA-seq) of matched pre- and post-NAC specimens from the same patient enables longitudinal tracking of resistance dynamics at cellular resolution while controlling for inter-patient heterogeneity. This study uniquely integrates resistance trajectory reconstruction with drug sensitivity profiling to identify actionable therapeutic vulnerabilities linked to the resistance phenotype.

Approach (Materials & Methods)

We performed scRNA-seq on six matched pre- and post-NAC specimens from three osteosarcoma patients (16,272 cells total), enabling within-patient longitudinal tracking of resistance evolution. Cell-type composition changes were quantified across treatment timepoints. Resistance trajectories were reconstructed using Monocle 3 pseudotime analysis. A candidate gene signature was derived from genes progressively activated along the resistance trajectory. The signature was validated in two independent cohorts: an in-house PKUPH cohort (n=70) with Huvos grading and clinical follow-up, and the TARGET-OS database (n=87). Drug sensitivity profiling was performed using the oncoPredict pipeline to identify actionable therapeutic vulnerabilities associated with resistance.

Results

Paired analysis revealed profound microenvironmental remodeling after NAC (Figure 1): malignant cell fractions decreased from 26.2% to 9.9%, while cancer-associated fibroblasts (CAFs) expanded from 2.2% to 25.2%. Residual tumor cells transitioned to a drug-tolerant persister state characterized by upregulated epithelial–mesenchymal transition and extracellular matrix programs. Pseudotime analysis identified a nine-gene resistance signature (KCNMA1, KIF21A, MIR181A1HG, RPS27, PDPN, ADIRF, PRELP, PHEX, COL9A2) that progressively activated along the resistance trajectory. The signature score correlated inversely with Huvos necrosis rate ($r=-0.35$, $P=0.006$), confirming its biological relevance to chemosensitivity. High-score patients had significantly worse progression-free survival in the institutional cohort (HR=2.4, 95% CI: 1.2–4.8, $P=0.01$), replicated in the TARGET cohort (PFS: HR=2.1; OS: HR=3.1, $P<0.01$) (Figure 2). Drug sensitivity profiling revealed that high-score resistant tumors exhibited significantly greater predicted sensitivity to Pictilisib, a PI3K inhibitor, across both cohorts ($P<0.05$), identifying an actionable therapeutic vulnerability.

Discussion

This paired single-cell analysis identifies a nine-gene signature that predicts NAC resistance from pre-treatment osteosarcoma biopsies, enabling early risk stratification before definitive surgery. The finding that resistant tumors

harbor selective vulnerability to PI3K inhibition offers a biomarker-guided therapeutic strategy for patients predicted to respond poorly to standard multi-agent chemotherapy. For the limb salvage community, pre-treatment identification of refractory disease could inform surgical timing, guide neoadjuvant protocol modification, and support rational integration of targeted agents into multimodal treatment. These results warrant prospective multi-institutional validation.

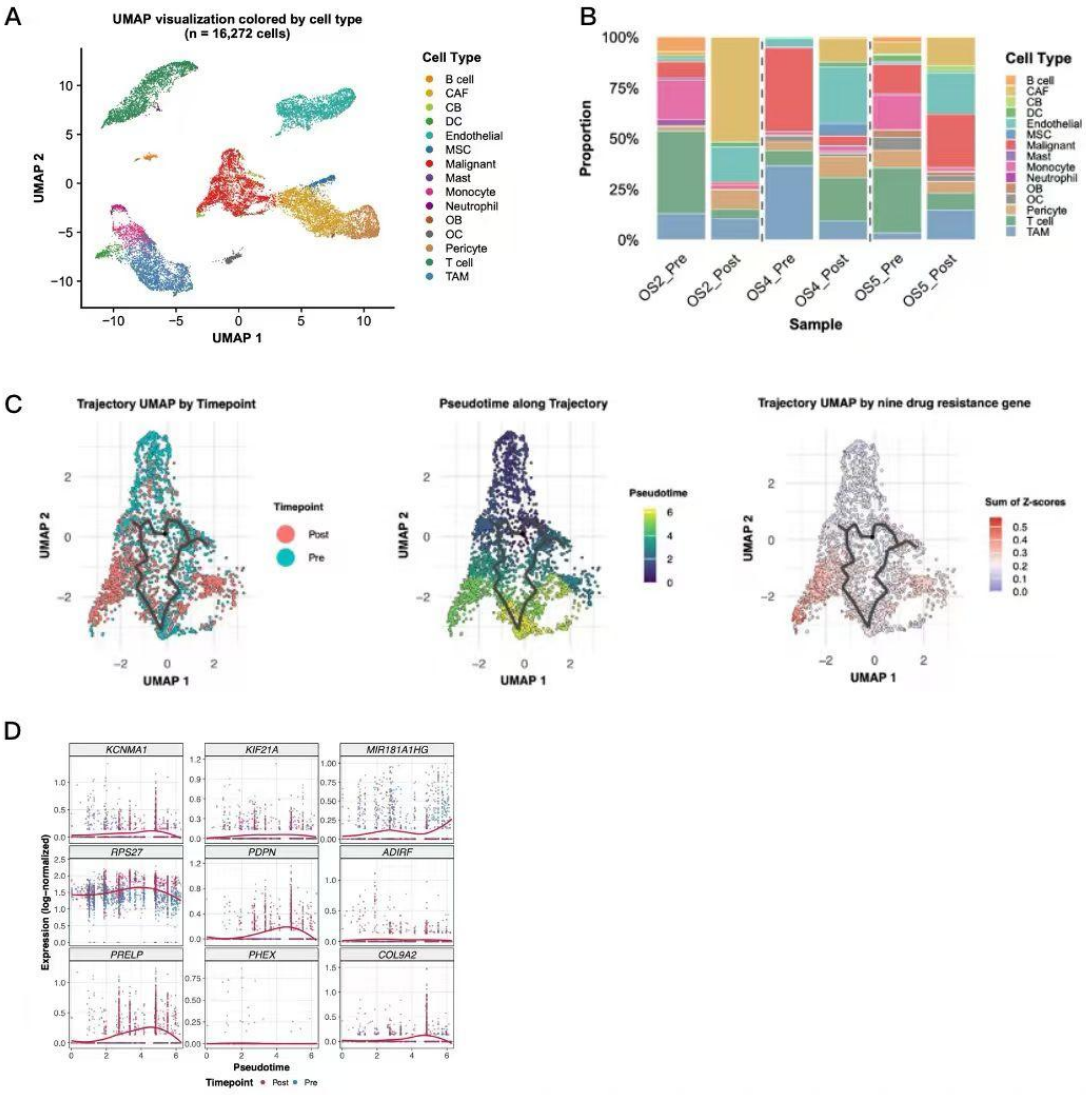

Figure 1. Single-cell landscape and transcriptional evolution of chemoresistance in osteosarcoma. (A) UMAP visualization identifying 15 distinct cell lineages across paired specimens. (B) Relative proportions highlighting the ten-fold expansion of cancer-associated fibroblasts (CAFs) following neoadjuvant chemotherapy (NAC). (C) Pseudotime trajectory reconstructing the evolutionary continuum from NAC-sensitive to resistant malignant cell states. (D) Dynamic expression profiles showing the progressive upregulation of the nine-gene signature along the resistance trajectory.

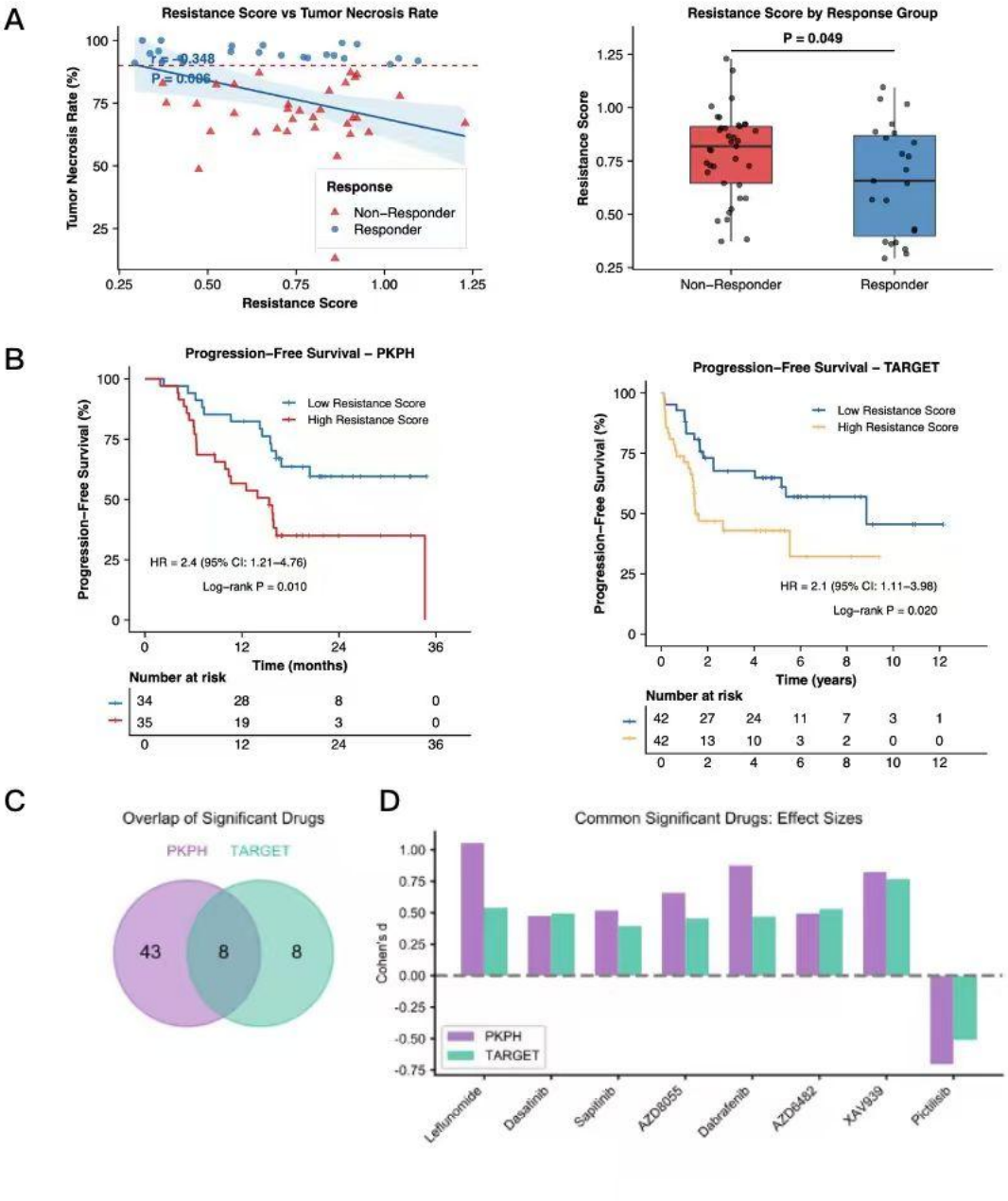

Figure 2. Clinical validation of the resistance signature and targeted therapy guidance. (A) Correlation and box plot analysis demonstrating the inverse association between the resistance score and tumor necrosis rate (Huvos grade). (B) Kaplan-Meier curves for progression-free survival in the PKUPH cohort (n=70) and TARGET-OS (n=87) cohorts, stratified by the resistance score. (C) Venn diagram showing the intersection of significant drug candidates across independent validation cohorts. (D) Identification of Pictilisib (PI3K inhibitor) as a potent therapeutic candidate for chemotherapy-refractory osteosarcoma based on effect size analysis.