Neuropilin-2 as a Therapeutic Target in Osteosarcoma: Spatial Transcriptomics and In Vivo Testing of a Clinic-Ready Anti-Neuropilin-2 Antibody

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Significance & Hypothesis

Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents and is associated with poor clinical outcomes, largely due to a high incidence of pulmonary metastasis. We previously demonstrated that the transmembrane co-receptor neuropilin-2 (NRP2) plays a role in OS angiogenesis and metastasis [1]. We hypothesize that NRP2 promotes osteosarcoma progression by mediating interactions between malignant cells and the tumor microenvironment, and therapeutic inhibition with an anti-NRP2 antibody will suppress metastatic spread.

Innovation

To further evaluate NRP2 as a therapeutic target and delineate its contribution to OS biology, we combined spatial transcriptomic analyses with antibody-based therapeutic approaches in clinically relevant models. This approach allows us not only to understand how gene expression programs are spatially organized, but also to conduct preclinical testing of a novel, clinic-ready NRP2-blocking antibody.

Approach

Single-cell RNA sequencing and Visium V2 spatial transcriptomics were performed on OS samples collected from Rb1/Trp53 double-knockout mice. Spatial transcriptomics data comprised of paired primary tumors and lung metastases derived from two animals representing distinct disease progression states. Spatial deconvolution methods were used to estimate cell-type proportions within individual Visium spots. Linear regression models were applied to evaluate associations between NRP2 expression and malignant, immune, and stromal cell populations. Therapeutic targeting of NRP2 was evaluated using two orthotopic mouse models. Human OS cells were implanted into the tibiae of SCID mice using either the 143B cell line or a patient-derived xenograft (PDX). We utilized the humanized anti-NRP2 antibody ATYR2810 (aTyr Pharma, San Diego, CA), which has been developed for clinical trials. Tumor-bearing mice were treated with 10mg/kg of ATYR2810, administered via intraperitoneal injections twice weekly. Tumor growth was assessed by serial caliper measurements and lungs were stained with Bouin's solution to visualize metastatic nodules. All animals were monitored for treatment-related toxicities.

Results

Spatial transcriptomic analyses revealed that NRP2 expression was enriched in malignant cell-dense regions and was associated with osteoclast-related niches. Linear modeling confirmed osteoclast correlated with NRP2 across most tumor contexts. Notably, a stronger correlation between NRP2 and malignant cell populations was observed in the metastatic, advanced-stage sample (Figure 1). In vivo, ATYR2810 treatment significantly reduced tumor growth in 143B (p-value) and PDX (p-value) models, with therapeutic effects evident after the first week and sustained over time (Figure 2). Treated animals also exhibited fewer pulmonary nodules (Figure 2). No overt toxicity or treatment-related morbidity was observed.

Discussion & Impact

NRP2 targeting with ATYR2810 was associated with reduced tumor growth and metastasis in preclinical OS models, without evidence of overt toxicity. Integrated spatial and single-cell transcriptomic analyses further implicate NRP2 as a key mediator of interactions between malignant cells and osteoclasts, highlighting a potential mechanism driving disease progression. Given that ATYR2810 is a clinically advanced, trial-ready antibody, these findings provide a strong rationale for the translation of NRP2 inhibition into clinical studies.

Reference

1. Ji T, Guo Y, Kim K, McQueen P, Ghaffar S, Christ A, Lin C, Eskander R, Zi X, Hoang BH. Neuropilin-2 expression is inhibited by secreted Wnt antagonists and its down-regulation is associated with reduced tumor growth and metastasis in osteosarcoma. Mol Cancer. 2015 Apr 17;14:86. doi: 10.1186/s12943-015-0359-4. PMID: 25890345; PMCID: PMC4411772.

Figure 1: Spatial mapping of different celltypes in tumor samples reveal NRP2 enrichment in malignant and osteoclast-rich regions of osteosarcoma

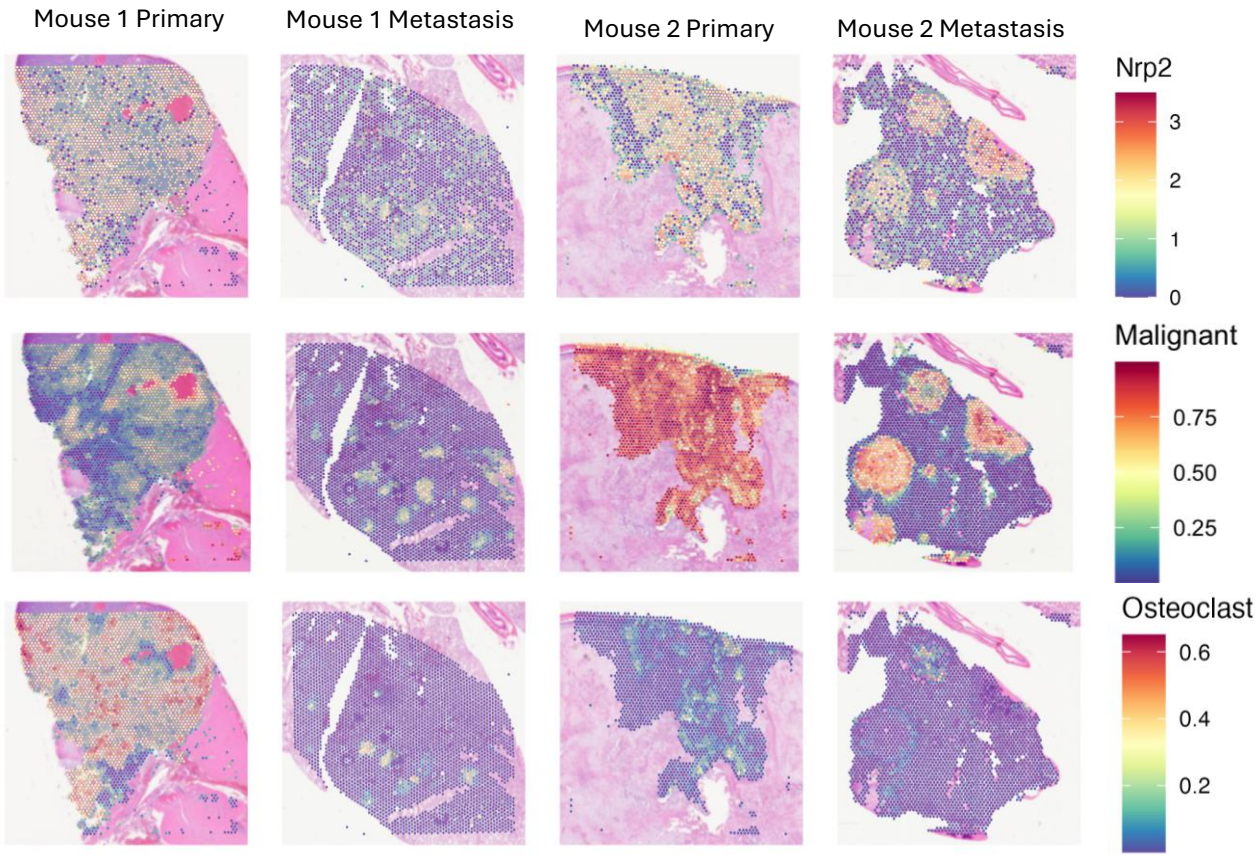

Figure 2: Anti-NRP2 treatment reduces OS tumor growth and lung metastasis