Identification of a Metastatic Clone in Human Undifferentiated Pleomorphic Sarcoma Reveals a Metastasis-Suppressing Therapy

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INTRODUCTION:

Undifferentiated pleomorphic sarcoma (UPS) exhibits substantial intratumoral heterogeneity, a key factor contributing to treatment failure and metastasis. Prior studies in mouse models demonstrated that a subpopulation of tumor cells—termed the metastatic clone (MC)—drives metastasis. Here, we aimed to determine whether a similar population exists in human UPS and to identify actionable targets for therapeutic intervention. We combined single-cell RNA sequencing (scRNA-seq) with functional analysis of single-cell–derived clones from primary human UPS tumors to assess metastatic potential and therapeutic vulnerabilities.

METHODS:

Primary tumor samples from ten untreated UPS patients were dissociated and depleted of immune cells. Single tumor cells were clonally expanded, genetically barcoded with fluorescent lentiviral markers, and pooled for orthotopic injection into immunodeficient (NSG) mice. Eight weeks post-injection, primary tumors were resected, and animals were monitored for metastasis. Metastatic potential was confirmed via tail vein injection and an in vivo imaging system (IVIS). Bulk RNA-seq was used to compare transcriptomes of metastatic and non-metastatic clones from three patients (Fig. 1). Key candidate genes were validated by RT-PCR and western blotting. scRNA-seq was performed on the original tumors, and tumor cell clusters were defined after excluding non-tumor cells. Therapeutic targeting of one the upregulated genes in the MC, Angiotensin II Receptor (AGTR2) was tested in a xenograft model using a pharmacologic approach with Azilsartan (an AGTR2 inhibitor already in patient use for hypertension). Eight primary UPS tumors were tested in this mouse model after xenografts were established in the quadriceps muscle. Animals were followed for survival and metastatic disease of 120 days.

RESULTS: Metastases consistently arose from a single, fluorescently labeled clone per tumor, confirming the presence of a metastasis-competent subpopulation. Tail vein assays showed only MCs formed lung tumors despite similar initial seeding (Fig. 2). RNA-seq of metastatic clones revealed consistent upregulation of the genes, *ESM1* and *AGTR2* across the MC from the tumors. scRNA-seq identified a distinct tumor cell cluster highly expressing ESM1, present only in tumors that metastasized (Fig. 3). Pharmacologic inhibition of AGTR2 using Azilsartan in tumors established as xenografts significantly improved survival and reduced lung metastases in treated mice compared to controls, with minimal impact on primary tumor growth (Fig. 4).

DISCUSSION AND CONCLUSION:

This study provides direct evidence that a metastatic-clone subpopulation exists in human UPS tumors and is responsible for metastatic dissemination. Targeting AGTR2, a gene selectively expressed in this population, effectively suppresses metastasis in preclinical models. These findings suggest that therapies directed at metastatic subpopulations—using FDA-approved agents like Azilsartan—may represent a promising strategy to improve outcomes for patients with this otherwise difficult to treat sarcoma. Combining this therapy with conventional and newer therapies, such as the use of Immune checkpoint inhibitors, could improve overall survival.

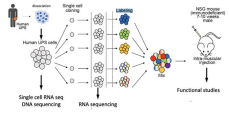

Fig. 1: Diagrammatic repression of our process to generate fluorescently labeled clones from single cells from UPS tumors. The tumor cells were mixed reconstitute a tumor containing all clones for functional studies

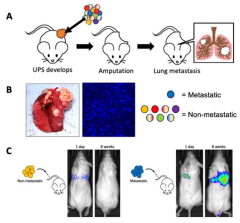

Fig. 2: Human UPS tumors contain an MC population with an enhanced capacity to form lung metastases. A) Diagrammatic repression of our experimental plan. Reconstituted tumors as in Figure 1 containing all clones were injected into the hind limb in immunodeficient mice. **B)** Lung metastases that developed expressed a single clone (one fluorescent label). The same labeled clone forms metastases from each tumor in multiple mice. **C)** MC and non-MC cells injected into the tail vein showing that only the MC cells formed tumors in the lungs (IVIS imaging).

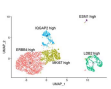

Fig. 3: Single cell RNA sequencing from UPS tumors analyzed via UMAP. Tumor cell segregated into five clusters, one of which was an ESM1 high closure (see cluster at the top of the UMAP). Non-metastatic tumors did not include this cluster. This UMAP combines all of the metastatic primary tumor samples.

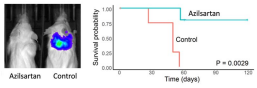

Fig. 4: AGTR2 blockade with Azilsartan inhibits metastasis and improved survival in UPS. of Representative IVIS imaging showing a lack of cells in the lung in MC cell cells with Azilsartan. Survival curves showing significant improvement with Azilsartan treatment. Eight animals in each group.