

DEPLOYING SPATIAL TRANSCRIPTOMICS TO INFORM ON INTRATUMORAL HETEROGENEITY IN LATE-STAGE UVEAL MELANOMA LEVERAGING ADVANCED PRECLINICAL MODELING AND CLINICAL SAMPLES

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Background Uveal melanoma (UM) is a rare tumor characterized by mutually exclusive activating mutations in GNAQ in GNA11, followed by secondary events in BAP1, SF3B1 and EIF1AX. Notably, a large subset of patients presents with copy-loss of chromosome 3 (monosomy 3), which is highly associated with metastatic progression in late-stage disease. Monosomy 3 tumors demonstrate a marked resistance to chemotherapy, targeted therapeutics, and immunotherapy, despite successes observed in cutaneous melanoma.

Informing on the biology underlying chromosome 3 copy-loss, and its impact on the tumor microenvironment, is critical towards directing future efforts in targeted therapeutics and immunotherapy. Current limited insight can be attributed to both lack of: (a) preclinical models, and (b) in-depth characterization of paired primary and metastatic tumors in patients.

Methods To address this, we first induced chromosome 3 copy-loss through CRISPR-based centromere targeting in a well characterized Disomy 3 UM cell line. Disomy 3 (D3) and Monosomy 3 (M3) clones derived from these efforts have enabled us to develop patient derived xenograft (PDX) models to compare D3 and M3 behavior in paired primary and metastatic settings.

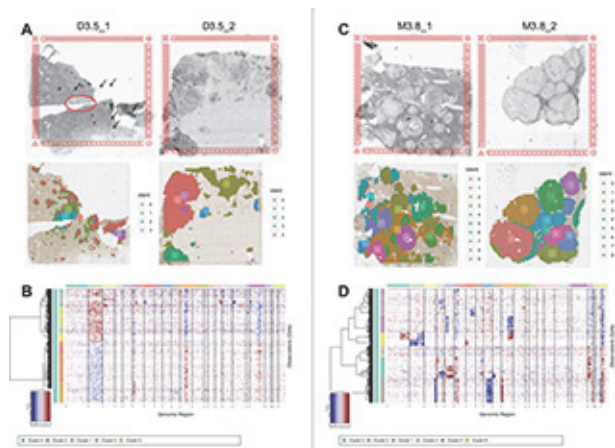
Additionally, we identified and collected a range of matched (primary and metastatic) clinical UM samples across multiple patients.

Leveraging these unique samples, we applied spatial transcriptomics to inform on tumor intrinsic and extrinsic features of progressive UM, identifying gene signatures of disease advancement and deconvoluting the evolving tumor microenvironment.

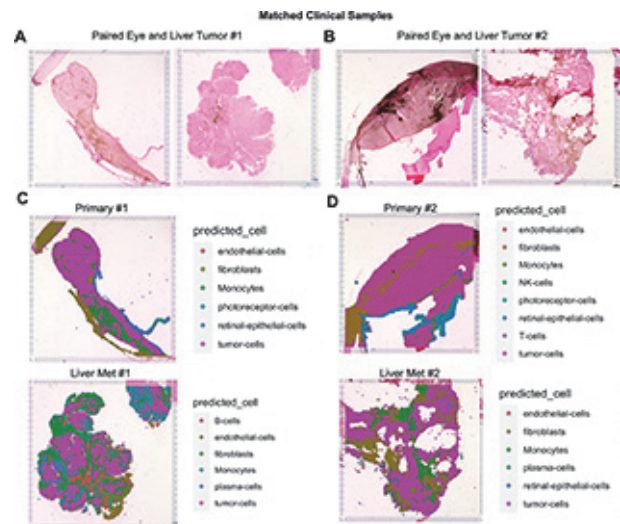
Results Investigation of metastatic UM tumor heterogeneity in our models enables us to characterize unique features of phenotypically transformed clones (e.g. depigmentation and growth advantage) (figure 1). We also adapted existing methodology to infer chromosomal copy number events from spatial transcriptomics data to our PDX system, overcoming the lack of same-species microenvironment controls. We complement our preclinical analyses with an investigation of spatial heterogeneity in a patient cohort of paired primary and metastatic tumors (figure 2). Leveraging single-cell deconvolution in this paired dataset, we captured unique immune microenvironments in primary vs. metastatic tumors. Additionally, we integrated our preclinical tumor intrinsic signatures to pair differential gene expression signatures of tumor sub-clones with differential immune cell populations.

Conclusions Our methodology allows for deep characterization of sub-clonal heterogeneity in primary and metastatic settings and informs on the unique microenvironmental heterogeneity underlying invasiveness and outgrowth of M3 tumors. More broadly, comparing these preclinical and patient tumors provides an opportunity to expand on our knowledge of metastatic disease drivers and derive prognostic signatures

associated with poor survival and lack of response to immunotherapy.



Abstract 1498 Figure 1 Spatial Transcriptomics Informs on Liver Outgrowth Phenotypes in Engineered UM Preclinical Models. (A) Spatial slides of two Disomy 3 tumors in the mouse liver and matched clustering following the removal of mouse background demonstrated with differential pigmentation and tumor size. (B) Corresponding copy number inference of the D3.5_2 slide demonstrates our ability to identify subclones based on copy number heterogeneity. (C) Spatial slides of two Monosomy 3 tumors in the mouse liver and matched clustering following the removal of mouse background. (D) Corresponding copy number inference of the M3.8_2 slide unable to accurately capture copy number in the absence of microenvironment as a normal control demonstrated by chromosome 3 being inferred as copy neutral



Abstract 1498 Figure 2 Spatial Transcriptomics on Uveal Melanoma Clinical Samples — Paired Primary Eye and Liver Metastases. (A) Spatial slide with matched primary eye and liver sections from Patient #1. (B) Spatial slide with matched primary eye and liver sections from Patient #2. (C) Predicted cell types observed in matched primary and liver tumors of Patient #1. (D) Predicted cell types in matched primary and liver tumors of Patient #2

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