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Decoding Microenvironmental Drivers of HLA-I Silencing and ICB Resistance in Melanoma

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Background and central scientific questions or problem: Cytotoxic CD8+ T lymphocytes are key mediators of clinical responses to immune checkpoint blockade (ICB), eliminating tumor cells upon recognition of cognate HLA class I (HLA-I) tumor antigen peptide complexes. The surface presentation of these complexes depends on the coordinated expression of components involved in antigen processing and peptide loading onto HLA-I molecules, collectively referred to as the antigen processing and presentation machinery (APM).

Previously, we demonstrated that melanoma cells can evade T cell recognition through genetic alterations in distinct components of the HLA-I APM, linking these defects to ICB resistance. In addition to genetic changes, we also showed that melanoma cells evade T cell-mediated killing through coordinated transcriptional silencing of HLA-I APM components. Notably, within metastatic lesions, we observed spatial segregation of HLA-I-negative and HLA-I-positive melanoma cells, suggesting that local microenvironmental cues contribute to HLA-I APM silencing. However, the nature of these cues and the underlying regulatory mechanisms remain poorly understood.

Using longitudinal tissue sampling, this project aims to define the mechanisms driving HLA-I APM silencing, focusing on the interplay between tumor-intrinsic factors and microenvironmental signals, and their role in resistance to ICB

Technical and conceptual approach to address the research question: Using patient-derived melanoma models, our lab has long-standing expertise in studying tumor–T cell interactions. We have 27

access to longitudinal patient tissue samples (both cryopreserved and FFPE), enabling us to investigate HLA-I APM silencing in melanoma cells over the course of disease and during ICB treatment. Established techniques such as single-cell RNA sequencing (scRNA-seq) of tumor cell suspensions, combined with multiplex staining, will allow for comparative analyses of transcriptional programs and the spatial cellular composition of HLA-I APM-positive versus HLA-I APM-negative tumor regions. Ex vivo and in vitro cell culture models will be employed to validate functional mechanisms. Together, these approaches will enable us to identify the microenvironmental drivers of HLA-I APM silencing.

Scientific expertise within the group: Tumor immunology, melanoma, T cells, immune evasion, therapy resistance, multiplex tissue staining (Phenocycler), flow cytometry, scRNAseq, CRISPR/Cas9 genome editing