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ORAL ABSTRACTS

622.LYMPHOMAS: TRANSLATIONAL-NON-GENETIC

Genome-Scale High-Resolution Spatial Mapping of the Pro-Tumorigenic Cellular Niche in Classic Hodgkin Lymphoma

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Introduction: A fundamental hallmark of cancer is that tumor cells repurpose the tissue microenvironment to promote their own survival. An increased understanding of these mechanisms may lead to improved microenvironment-directed therapies, particularly in lymphoid malignancies. In classic Hodgkin lymphoma (cHL), the rare malignant Hodgkin Reed Sternberg (HRS) cells are surrounded by a CD4+ T-cell and macrophage-rich inflammatory infiltrate. Recent multiplexed immunofluorescence studies suggest that the micron-scale niche around HRS cells is composed of distinct populations of PD-L1+ macrophages and CD4+ T cells, including regulatory CTLA4+ and LAG3+ subsets (Carey et al. Blood 2017, Patel et al. Blood 2019 and Aoki et al. Cancer Discov 2020). However, the topography of the intact tumor microenvironment of cHL requires further definition. Recent single-cell RNA sequencing studies have led to important insights into the biology of cHL; however, they do not adequately capture myeloid cells, fibroblasts, and HRS cells, likely due to the relative fragility of these cells in conventional tissue dissociation protocols. In this study, we use tandem single nucleus and spatially resolved RNA sequencing to systematically dissect the pro-tumorigenic cellular niche of cHL to define potentially targetable microenvironmental dependencies.

Methods: Using single nucleus (10X Chromium V3.1 gene expression) and spatially resolved RNA sequencing (Slide-seqV2 and 10X Visium), we generated a dataset of 12 newly diagnosed cHL and 7 reactive lymphoid tissues comprising over 324,855 nuclei and 2.5 million spatially resolved gene expression profiles to construct a genome-scale single cell and spatially resolved atlas of cHL. To validate the expression of ligands/receptors on proximal cell types within the HRS cell niche at single-cell resolution, we also generated an orthogonal 1000-plex image-based spatial transcriptomic dataset (CosMx Spatial Molecular Imager). Additionally, we developed a novel Bayesian consensus tensor factorization approach (C-ZIPTF) to infer shared cancer-associated immune signatures and ligand-receptor interactions.

Results: Single nucleus RNA sequencing captures a diversity of cell types and cell states in cHL, including distinct myeloid and stromal cell subsets and HRS cells. Copy number inference analysis identifies the HRS cells, and unsupervised consensus tensor factorization reveals recurrent HRS cell transcriptional states, including aberrant myeloid, stromal, neuronal, and secretory/cytokine programs. Of all clinical and histological parameters, EBV infection status showed the strongest associa-

tion with HRS cell transcriptional state. Regulatory T cells, macrophages, and fibroblasts are quantitatively increased in cHL compared to reactive lymphoid tissues and additionally exhibit qualitatively altered expression programs. Using C-ZIPTE, we identify disease-defining and prognostically relevant immunosuppressive regulatory T cell and myeloid cell programs and an activated pro-fibrotic myofibroblast-like fibroblast signature. Spatial analysis provides strong evidence for a "niche" model, i.e., the spatial organization of cell types and states around HRS cells. Specifically, CD4+ T cells and myeloid cells are spatially enriched in the immediate proximity of HRS cells and, plasma cells are depleted. Moreover, the CD4+ T cells and myeloid cells exhibit a specialized pro-tumorigenic gene expression program with increased expression of multiple immunosuppressive cytokines, checkpoint molecules, and putative growth factors for HRS cells. Spatially-aware ligand-receptor interaction analysis enables systematic identification and prioritization of novel growth factors for HRS cells. Functional testing of these putative growth factors using an ex vivo 3D cell culture system confirms their growth-promoting effect on primary HRS cells.

Conclusions: The HRS cells in cHL are surrounded by a distinctive pro-tumorigenic cellular niche that not only mediates immune evasion but may also directly promote tumor cell growth. These observations provide a rich set of hypotheses for preclinical model development and microenvironment-directed therapies. More broadly, our study demonstrates the power of spatial genomic approaches to deconvolute the molecular architecture of pro-tumorigenic cellular niches in tumor ecosystems.

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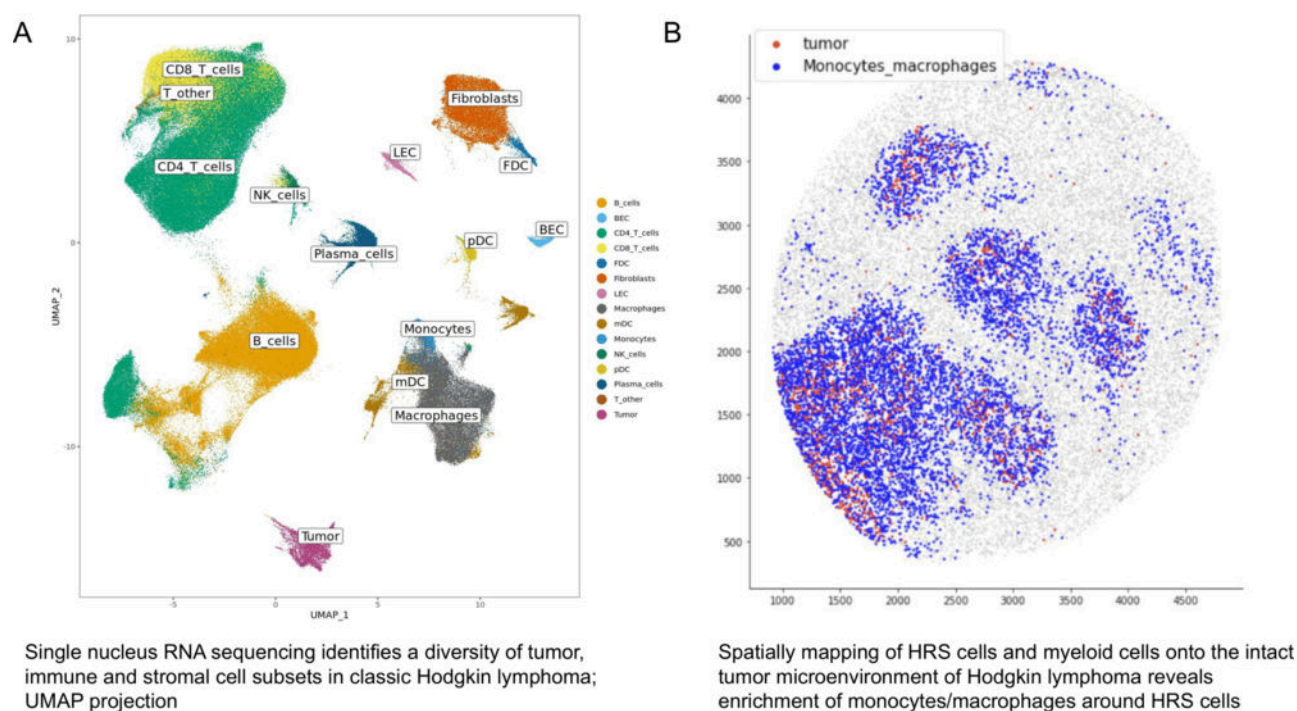


Figure 1

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