

**951 SPATIAL ATLASES OF IMMUNOLOGICAL DEVELOPMENT
WITHIN THE LYMPH NODE**

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Background Immunological mechanisms regulating detection and clearance of cancer, including CTLA-4 and PD-L1, were discovered by studying the natural physiological processes regulating immune cell maturation, attenuation, and dissemination throughout the body. Single-cell atlases mapping immune cells provide hints to these aspects of immunology but lack essential spatio-temporal relationships between cells. With the advent of spatial 'omics we can resolve thousands of RNA or more than 60 protein molecules simultaneously *in situ*, enabling direct insight into the dynamics occurring as immune cells mature and migrate through tissue.

Methods We profiled lymph node samples using complementary spatial 'omics platforms: the GeoMx[®] Digital Spatial Profiler and the CosMx[™] Spatial Molecular Imager. With GeoMx, we profiled whole transcriptomes from 5 patients focusing on key structures within the lymph node including the germinal center, mantle zones, medulla, and paracortex. With CosMx, we examine one FFPE lymph node with clear germinal center zonation, analyzing 1000 genes and 55 proteins across serial sections covering >100mm² and >1.4 million cells/section. We used a novel, semi-supervised clustering algorithm to map cells to CosMx from lymph node scRNAseq.

Results Across structures profiled with GeoMx we observed 11,316 genes above background in >10% of tissue regions profiled. We identified 2,618 genes (FDR<0.05) associated with distinct functional regions, and 928 significant pathways (FDR<0.05). Profiling with CosMx identified 27 cell types, 6 of which were not captured in the dissociated reference. Integrating results from GeoMx and CosMx, we observed 643 pathways enriched (FDR<0.05) in dark (n=283) and light (n=206) zones of the germinal center or at their interface (n=154), as well as 139 key ligand-receptor interactions driving such pathways. For example, we found co-stimulation of CD28 was enriched within the light zone (FDR<7.5e-06, GeoMx). CosMx confirmed that CD86 ligands within light zone B cells were significantly colocalized with the CD28 receptors of the T_H cells of the germinal center (FDR<1.5e-05). We also found IL18 signaling between macrophages and B cells within the germinal center (FDR<5.6e-09), which was confirmed with protein profiling with CosMx.

Conclusions The spatial ecosystem provided by the GeoMx and CosMx platforms captures an unprecedented view into architecture, hard-to-profile cells, and immunological processes happening within tissue. These findings shed light on novel interactions happening at key immunological interfaces, which can be compared to immune infiltrate into tumors to identify perturbed interactions active during tumor immune escape.

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