

SPATIAL CLUSTERING REVEALS IMMUNE HUB INTERACTION WITH RESERVOIR OF STEM-LIKE CD8 T CELLS AND PREDICTS IMMUNOTHERAPY RESPONSE IN LUNG CANCER PATIENTS

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Background Immunologic tumor control involves coordination of multiple cell types, but the organization of these interactions in human tumors is poorly understood. We recently reported that immunogenic tumors contain spatially-localized multicellular immunity hubs characterized by expression of interferon-stimulated genes, including *CXCL10/CXCL11* (T cell attracting chemokines), and the presence of *IFNG*+ T cells.¹ This suggested a positive feedback loop where T cell-derived IFN γ stimulates production of CXCR3 ligands, thereby attracting more T cells. However, we did not know (1) whether these hubs predict response to immunotherapy and (2) how hubs intersect with the various CD8 T cell states which play central roles in anti-tumor immunity.

Methods To understand the composition of this immunity hub and its potential association with immunotherapy response, we performed multiplex RNA FISH to visualize hub components in NSCLC tissue from 68 patients prior to PD1-blockade. Cells were segmented and phenotyped automatically. We additionally imaged serial sections with a second panel for markers of CD8 T cell state. We then computationally registered sequential images, integrating our panels. To identify hubs in an unbiased manner, we employed kmeans clustering.

Results We found that the presence of the immunity hub is predictive of subsequent response to PD1-blockade. Image registration revealed that hubs are enriched for CD8 T cells in multiple states, confirming their role as key sites of anti-tumor T cell activity. Furthermore, immunity hubs in responders contained more activated CD8 T cells and *IFNG*+ cells than those in nonresponders. To determine how hub heterogeneity may influence hub functions, we subclustered hubs by phenotypic composition. This analysis uncovered a ‘hybrid hub’ subclass that spatially overlaps with structures containing stem-like TCF7+ CD8 T cells, resembling the interfollicular zone of lymph nodes. The presence of a single hybrid hub was strongly associated with RECIST response ($p = 0.0005$), found in 85% of responders and only 24% of non-responders. Hybrid hubs also showed a striking association with patient PFS ($p = 0.0014$).

Conclusions Our study provides insight into the multicellular networks that underlie anti-tumor immunity. Immunity hubs are predictive of response to immunotherapy in human lung cancer and organize intratumoral CD8 T cell activity. Moreover, hybrid hubs may represent an active intra-tumoral niche for tumor-specific stem-like T cells that sustain anti-tumor immunity. These multicellular networks are excellent candidates for biomarker development and targets for immunotherapy.

REFERENCE

1. Pelka K, et al. Spatially organized multicellular immune hubs in human colorectal cancer. *Cell* 2021;**184**:4734–4752.e20.

Ethics Approval This study was approved by the Massachusetts General Hospital Institutional Review Board as a discard tissue protocol (#2019P002829).