Single-cell Atlas Reveals Lymph Node-Associated Immune Microenvironment Remodeling and FKBP5-Mediated CD8+ T cell Dysfunction in Non-Small Cell Lung Cancer Immunotherapy

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Abstract:

Despite the clinical success of immune checkpoint inhibitors in non-small cell lung cancer, a substantial proportion of patients exhibit limited therapeutic responses. The mechanisms underlying differential immunotherapy outcomes, particularly the role of tumor-draining lymph nodes in shaping systemic anti-tumor immunity, remain incompletely understood. We constructed a comprehensive single-cell RNA sequencing atlas comprising 528,010 cells from 78 samples across five independent cohorts, including treatment-naïve and post-immunotherapy non-small cell lung cancer tumors with or without major pathological response and matched lymph nodes. Integrated analysis combined with functional validation experiments was performed to dissect immune cell heterogeneity and microenvironmental determinants. We identified distinct immune phenotypes in both tumor and lymph node compartments. Lymph nodes paired with major pathological response tumors exhibited enhanced CD8+ T cell cytotoxicity and ATP metabolic activation, whereas lymph nodes from hot tumors displayed elevated protein folding stress signatures associated with poor prognosis. Mechanistically, fibroblast-enriched lymph nodes from cold tumors exhibited excessive collagen deposition and THBS1-CD47 inhibitory signaling, creating physical and functional barriers to CD8+ T cell infiltration. FKBP5 emerged as a critical regulator linking lymph node dysfunction to T cell exhaustion, with high expression correlating with poor survival across multiple cancer types. Functional studies demonstrated that Safit2 promotes CD8+ T cell differentiation toward cytotoxic effector phenotypes, attenuates TIM-3-mediated exhaustion, and synergistically enhances anti-tumor efficacy when combined with PD-1 blockade in both organoid co-culture systems and murine Lewis lung carcinoma models. Our study establishes lymph node immune status as a determinant of immunotherapy efficacy in non-small cell lung cancer and identifies FKBP5-mediated T cell dysfunction as a therapeutic vulnerability. These findings provide a mechanistic rationale for combining metabolic or stromal modulators with checkpoint inhibitors to overcome immunotherapy resistance.

Keywords:

Non-small cell lung cancer, Single-cell RNA sequencing, Immunotherapy, Lymph node, CD8+ T cell exhaustion, FKBP5, TIM-3, Safit2, PD-1 blockade.