Identification and characterization of tumor-infiltrating regulatory T cell subsets

Sang-Jun Ha
河 相 準

Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea

Tumor-infiltrating regulatory T cells (Tregs) represent a major barrier to effective anti-tumor immunity and immune checkpoint blockade therapy. However, the differentiation pathways and functional heterogeneity of Tregs within the tumor microenvironment (TME) remain poorly understood. In this study, we investigated the accumulation, differentiation, and spatial organization of tumor-infiltrating Tregs during tumor progression using mouse syngeneic tumor models. We observed progressive enrichment of Tregs in tumor tissues, strongly correlating with tumor growth. Single-cell RNA sequencing identified five distinct Treg subsets—naïve, proliferating, intermediate, effector, and terminally differentiated states—forming a linear developmental trajectory. These subsets were further validated by flow cytometry using CCR7, CD69, PD-1, and CCRL2 as key markers. Differentiation was accompanied by gradual upregulation of suppressive and activation-associated molecules, including CCR8, CD39, and PD-1. Functional assays demonstrated that effector Tregs exhibited maximal suppressive activity, whereas terminal Tregs displayed apoptotic features and reduced function. Spatial imaging revealed that effector and terminal Tregs occupy distinct tumor niches, with effector Tregs enriched in fibroblast- and M2 macrophage–associated suppressive regions, while terminal Tregs localized to immune-inflamed areas dominated by M1 macrophages. Importantly, analogous Treg subsets were also detected in human lung cancer single-cell datasets, supporting clinical relevance. Together, these findings provide a framework for selectively targeting tumor-specific Treg subsets to improve cancer immunotherapy outcomes.