1049 RECONSTRUCTION OF GENE REGULATORY NETWORKS DISSECTS TRANSCRIPTIONAL CONTROL OF INTRATUMORAL REGULATORY T CELLS

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Background Regulatory T cells (T_{reg}) -targeted therapy exhibit clinical benefit and has been reported as a promising strategy. However, many gaps remain in our understanding of T_{reg} biology within the context of tumor microenvironment (TME). The autoimmune toxicity and restricted efficacy are major limitations of T_{reg} therapies in the clinic, when T_{reg} depletion occurred not only in the tumor but in other organ systems, or concurrent downregulation of antitumor effector T cells.[1, 2]

Methods We profiled 51,195 single-cell transcriptomes of $CD4^+$ T cells in tumors and peripheral blood from patients with head and neck squamous cell carcinomas (HNSCC)[3], in inflamed tonsil tissues and in healthy peripheral blood. Canonical genes, gene sets and RNA Velocity[4] were used to define cell states of T_{reg}. Cibersortx[5] and bulk RNA sequencing data in The Cancer Genome Atlas were used to infer the association between the enrichment of T_{reg} subpopulations and progression-free survival of patients with solid tumors. SCENIC[6] and Causal mixed graphical modeling[7] were used to reconstruct the gene regulatory network (GRN). Knockout of *BATF* with CRISPR-Cas9[8] in conjunction with bulk RNA sequencing, immunophenotyping and in vitro functional assays were used to interrogate the roles of BATF in human activated T_{reg}.

Results We identified an activated subpopulation of T_{reg} expressing multiple tumor necrosis factor receptor (TNFR) genes, including *OX40* and *4-1BB*, which is highly enriched in solid TME compared with non-tumor tissues. These TNFR-activated T_{reg} were associated with worse prognosis across solid tumors. Notably, we found *BATF* is a central component of a GRN that controls the transcriptional signature of TNFR-activated T_{reg}. Consistent with single-cell analyses, BATF was co-expressed with 4-1BB, OX40, CD96 and CD39 that highly enriched in HNSCC intratumoral T_{reg} at protein level. CRISPR-editing results revealed an enhancement of immunosuppression in *BATF* KO T_{reg} and activation in *BATF* KO T_{reg} accompanied with increased expression of genes including 4-1BB, OX40, ICOS, LAG3 and neuropilin-1, indicating that BATF functions as a transcriptional nexus in human activated T_{reg} that essential for T_{reg} activation, function and stability.

Conclusions We identify a unique intertumoral subpopulation of T_{reg} characterized by BATF-driven expression of tumor necrosis factor receptor family expression and associated with survival across solid tumors, suggesting a possibility to target suppressive intratumoral T_{reg} without causing overt autoimmunity in normal tissues. A deeper understanding of transcriptional network in T_{reg} biology will provide novel mechanisms for immunotherapies in cancer, but also for T_{reg} engineering in autoimmunity.

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Ethics Approval All patients provided informed written consent, and this study was approved by our Institutional Review Board (University of Pittsburgh Cancer Institute, Tissue Collection Protocol 99-069).

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