Glioblastoma

Major finding: Intratumoral transcriptional diversity leads to variable signaling and mixed subtypes in glioblastoma.

Approach: Single-cell RNA sequencing identified inherent differences in gene expression programs within tumors.

Impact: Transcriptional variability among tumor cells complicates treatment strategies in glioblastoma.

SINGLE-CELL ANALYSIS REVEALS INTRATUMORAL HETEROGENEITY IN GLIOBLASTOMA

Intratumoral heterogeneity occurs when cells within a single tumor differ according to mutation, phenotype, or epigenetic state, and can lead to complications in clinical diagnosis, treatment, and disease recurrence. Despite advances in targeted therapy, cancers with increased heterogeneity, such as glioblastoma, continue to display high mortality rates, reinforcing the need for individual tumor cell characterization in order to achieve better patient prognosis. To systematically analyze intratumoral heterogeneity, Patel, Tirosh, and colleagues used single-cell RNA sequencing to generate full-length transcriptomes for 430 individual tumor cells isolated from five freshly resected human glioblastomas. Estimation of copy-number variation from expression data in individual tumor cells revealed that tumors largely displayed homogenous chromosomal aberrations. However, in contrast to this large-scale analysis, extreme diversity in transcriptional profiles was observed across individual cells. Multiple receptor tyrosine kinases exhibited mosaic expression, and mutually exclusive expression of oncogenic *EGFR* variants was detected in individual cells. In addition, hierarchical clustering defined four meta-signatures enriched for genes involved in the cell cycle, hypoxia, and immune response that showed intratumoral variation. In contrast to *in vitro* models, only a fraction of individual cells expressed active cell-cycle genes, and intratumoral gradients were observed for the hypoxic and stem-cell signatures, reinforcing the notion of *in vivo* complexity. Of note, a continuous spectrum of stemness and differentiation states was identified across tumor cells and was correlated with the expression of candidate regulatory transcription factors. Furthermore, although the current glioblastoma subtype classification scheme detected dominant subtypes via bulk tumor analysis, scoring individual cells based on this scheme revealed a mixed-subtype population in each tumor. Using this in turn to infer heterogeneity in bulk tumors showed that increased heterogeneity was associated with decreased survival. Together, these findings highlight intratumoral transcriptional variability in glioblastoma and emphasize the need to account for tumor heterogeneity in future therapeutic design. ■

Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 2014;344:1396–401.

Immunology

Major finding: p110δ inactivation alleviates Treg cell–mediated immune suppression and promotes antitumor immunity.

Mechanism: p110δ inhibition impairs Treg-cell function and decreases PMN-MDSCs, enabling $CD8^+$ T-cell activity.

Impact: p110δ blockade may enhance antitumor immune responses in both solid and hematologic cancers.

PI3Kδ REGULATES IMMUNE TOLERANCE TO CANCER

Inhibitors targeting the p110δ isoform of PI3K, which is predominantly expressed in leukocytes, have shown clinical activity in hematologic tumors. However, it remains unclear whether p110δ activity in leukocytes also affects the growth of solid tumors. Ali and colleagues found that expression of an inactivating p110δ mutant suppressed the growth and metastasis of various tumor cell types *in vivo*, prolonged the survival of tumor-bearing mice, and conferred long-term cancer resistance by enabling memory immune responses to develop. This reduction in tumor growth was facilitated through an attenuation in the function of immunosuppressive $FOXP3^+CD4^+$ regulatory T (Treg) cells, as genetic inactivation of p110δ specifically in Treg cells was necessary and sufficient to overcome immune tolerance and restrain tumor growth. Inhibition of tumor growth in response to p110δ inactivation was also dependent on $CD8^+$ T-cell activity; although p110δ loss impaired $CD8^+$ CTL differentiation, suppression of Treg cells shifted the balance between regulatory and effector T cells and promoted CTL-driven antitumor responses. In addition, p110δ inactivation reduced the number of polymorphonuclear (PMN)–myeloid-derived suppressor cells (MDSC) and diminished the ability of PMN-MDSCs to inhibit T-cell proliferation *in vitro*. Furthermore, in a mouse model of pancreatic ductal adenocarcinoma driven by *Kras* and *Trp53* mutations, pharmacologic blockade of p110δ via administration of a selective small-molecule inhibitor decreased metastasis formation and prolonged survival. This therapeutic effect was associated with a reduction in peripheral Treg cells and an increase in infiltrating $CD8^+$ T cells in pancreatic tumors. Although additional work is needed to define the mechanisms by which p110δ inactivation negatively regulates Treg cells and PMN-MDSCs, these findings identify a role for p110δ in promoting immune tolerance and suggest that selective inhibition of this PI3K isoform may also be beneficial in stimulating antitumor immune responses in solid tumors. ■

Ali K, Soond DR, Piñeiro R, Hagemann T, Pearce W, Lim EL, et al. Inactivation of PI(3)K p110δ breaks regulatory T-cell-mediated immune tolerance to cancer. Nature 2014;509:407–11.