AN IMMUNOSUPPRESSIVE TUMOR POPULATION DRIVES GLOBAL AND REGIONAL PATTERNS OF IMMUNE INFILTRATION IN HETEROGENEOUS TUMORS

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Background Intratumoral heterogeneity (ITH) is cellular and molecular diversity within a tumor. ITH is linked to failure of immunotherapy in multiple cancer types. One of the suggested mechanisms of this failure is the absence of a productive immune response, which can be driven by the dominance of a tumor population that creates immunosuppressive microenvironment. However, the molecular mechanisms that mediate such dominant immunosuppressive effects and how a dominant immunosuppressive tumor population affects the efficacy of immune checkpoint blockade (ICB) therapy are poorly understood.

Methods We generated a library of squamous skin cell carcinoma cell lines derived from DMBA+TPA carcinogen-treated mice. Upon transplantation into immunocompetent mice, two cell lines (CF6 and CF9) gave rise to highly and poorly immune-infiltrated tumors, respectively. These two cell lines were tagged with YFP and RFP, respectively, mixed at a 1:1 ratio and injected subcutaneously. The resulting mixed tumors contained a patchwork of distinct regions that are predominantly occupied with YFP (CF6) cells, occupied with RFP (CF9) cells, or are a mixture of YFP and RFP cells. These mixed tumors were microdissected into YFP, RFP, and mixed regions, and the immune infiltrate in each region was analyzed by flow cytometry.

Results We found mixed tumors were "cold" on a whole-tumor level. However, regional analysis showed a higher frequency of total and CD4 T cells in YFP regions compared to RFP regions. In contrast, macrophages exhibited a preferential localization to RFP regions. This suggests that local factors unique to each region drive the immune infiltration patterns of CD4 T cells and macrophages. Interestingly, although CD8 T cells showed a low frequency in all regions, a majority of CD8 T cells from YFP regions produced IFN γ in response to PMA/Ionomycin stimulation while IFN γ +CD8 T cells from RFP regions made up a small fraction. This suggests that the presence of CF9-RFP regions inhibits infiltration of CD8 T cells throughout the tumor, but may not affect the anti-tumoral potential of CD8 T cells that succeed in infiltrating CF6-YFP regions.

Conclusions An immunosuppressive tumor population rendered our model heterogeneous tumors "cold" on the whole. Nonetheless, we observed regional patterns in the quantity and quality of immune infiltrates, with local immune infiltration being shaped by the tumor cells present in that region. Understanding how these immune infiltration patterns impact the response of each region to ICB therapy will provide insights into the mechanism by which ITH poses barriers to immunotherapy.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.317>