

Induction of Tertiary Lymphoid Structures by Immune Checkpoint Therapy in a Murine Model of Triple-Negative Breast Cancer

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Introduction: Though immune checkpoint therapy (ICT) is approved for the treatment of triple-negative breast cancer (TNBC), response rates are low and non-durable. There is thus an urgent need for improved therapeutic biomarkers and deeper understanding of TNBC antitumor immune responses to tailor improved immunotherapies. Tertiary lymphoid structures (TLSs) are intra-tumoral lymphoid tissues composed of B cells and T cells that are associated with improved survival and response to ICT. Despite these associations, it is unclear what impact ICT has on these structures and whether they mediate ICT efficacy. We sought to examine if ICT may promote formation of TLSs in TNBC.

Methods: BALB/c mice were orthotopically implanted with T11-APOBEC murine TNBC tumor cells to generate TNBC tumors. This model genocopies clinical TNBC and is dependent upon B cells and CD4⁺ T cells for response to ICT. Mice were then treated with ICT (anti-PD-1 and anti-CTLA-4 antibodies) twice weekly. TLSs were identified by immunofluorescent staining for B and T cells on tumor sections. Spatial transcriptomics was used to further characterize gene expression profiles of TLSs in murine and human TNBC tumors.

Results: We show that ICT significantly upregulated TLS formation in the periphery of T11-APOBEC tumors, whereas tumors treated with vehicle control show little to no TLS formation ($p = 0.0286$). Using spatial transcriptomics, we show that these TLSs are identified as a spatially and transcriptionally distinct niche in an unbiased manner. TLSs in this model are significantly enriched for four widely used transcriptional signatures of human cancer-associated TLSs, and they also significantly upregulate pathways associated with B cell activation, humoral immunity, and germinal center activity. We then further show that a signature derived from these ICT-induced TLSs are focally enriched in a publicly available cohort of human TNBC samples, suggesting the formation of similar structures in TNBC patients.

Conclusions: These data suggest that ICT promotes the formation of TLS in TNBC, which may mediate enhanced anti-tumor adaptive immune responses. This model also provides a platform to further investigate mechanisms of formation and function of these structures in the context of ICT, which will inform predictive biomarkers and improved immunotherapy approaches for this aggressive disease.