Comparative analysis of syngeneic oral cancer development at the murine heterotopic and orthotopic sites

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Objectives:

Subcutaneous heterotopic tumors in murine models are often utilized for simplicity of implantation and ease of tumor access. However, orthotopic models, implanted into relevant organ-specific environments, are widely considered more translationally relevant. While both models may be used to study head and neck squamous cell carcinomas (HNSCC), few studies have directly compared the tumor immune microenvironments (TIME) of orthotopic and heterotopic tumors. Moreover, tumor draining lymph nodes (tdLNs), sites critical for antigen presentation and T cell activation, are commonly resected in patients with HNSCC. Recent studies have increasingly shown key implications tdLN loss in anti-tumor response. Herein, we investigated locoregional TIME differences in a carcinogen-induced, HPV-negative preclinical oral cancer model, unresponsive to traditional immunotherapy. We hypothesize there will be no differences in immune cell populations between heterotopic and orthotopic sites in this model.

Experimental Methods:

ROC1 cells were maintained as published and tumors established in the murine flank and oral cavity. Tumor growth kinetics were assessed at each site. At distinct tumor growth stages, tumors and their respective (inguinal, cervical) tdLNs were harvested. Multi-parameter spectral flow cytometry was performed to analyze immune cell populations at each site.

Results:

Both sites displayed initial periods of delayed tumor growth followed by rapid tumor progression. Comprehensive analyses revealed low T cell infiltration in both models, with greater increases over time in most cell types in the flank compared to the oral cavity. In both models, increases in myeloid cell types over time, specifically non-M1 macrophages and conventional type 2 dendritic cells (DCs) were observed. Regulatory T cells and macrophages were increased in inguinal tdLNs, while cervical tdLNs had increased DCs. Immune checkpoint marker (PD-1, PD-L1, CTLA-4) expression also increased over time.

Conclusions:

Our results indicate similar “cold” immune profiles between ROC1 TIMEs in the flank and oral cavity, confirming our hypothesis. However, observed tissue-specific TIME differences may impact anti-tumor treatment and response. Moreover, differences between corresponding tdLNs indicate

changes in immunosuppressive phenotypes, which may further impact response. Understanding of critical immune microenvironment differences between orthotopic and heterotopic models will enable tailoring of future therapeutic strategies and provide insight into model selection and data interpretation from translational studies.

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References

1. Shi, Y, Xie, T, Wang, B, et al. Mutant p53 drives an immune cold tumor immune microenvironment in oral squamous cell carcinoma. Commun Biol. 2022 Jul;5(757).