Developing Patient-Derived Spheroid Models for Preclinical Testing in Head and Neck Cancers

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Introduction: Head and neck cancer (HNC), the sixth most common cancer globally, remains a highly morbid disease with high rates of relapse and treatment resistance, despite the evolving landscape of cancer therapies. While immune checkpoint inhibitors, such as pembrolizumab, have improved outcomes for some patients, the overall response rate remains low, and predictors of treatment response are poorly understood. Current preclinical models, such as 2D cell cultures and patient-derived xenografts, often lose genetic similarity to the original patient tumors and fail to accurately mimic tumor-immune interactions. We sought to develop a patient-derived spheroid model using primary keratinocyte cell lines cultured from resected HNC tumors to better preserve original tumor characteristics and serve as a preclinical model for immunotherapy treatment.

Methods: Primary keratinocyte cell lines derived from three HNC tumors (HNC 208, 285, 365) were cultured as 2D monolayers and 3D spheroids. Spheroid morphology was examined microscopically. Western Blotting assessed expression of commonly mutated proteins in HNC: PD-L1, B7-H3, p21, p-STAT3, and p-AKT. Spheroids were co-cultured with healthy donor peripheral blood mononuclear cells (PBMCs) ± pembrolizumab, and immune cell infiltration was assessed via flow cytometry. Another co-culture experiment was set up for immunofluorescence, however, staining was not completed yet due to tissue processing delays. Spheroids were also injected into immunocompromised mice to evaluate tumorigenicity and treatment response with PBMCs ± pembrolizumab. Lastly, retrospective chart review collected patient tumor mutation reports.

Results: All three cell lines formed spheroids in vitro, though HNC 208 appeared morphologically more like normal epithelia than cancerous. Western blotting revealed high PD-L1 expression in each line, B7-H3 and p21 were highly expressed in 208, and p-AKT was highly expressed in HNC 285. Flow cytometry showed T-cell infiltration into spheroids for lines 208 and 365, with increased infiltration in groups receiving pembrolizumab treatment. In vivo, HNC 208 failed to form tumors, though both HNC 285 and 365 formed tumors with a slight decrease in tumor volume in pembrolizumab-treated mice. Patient chart review showed mutations in TP53 in all 3 cell lines.

Conclusion: This patient-derived spheroid model shows promise for preserving original tumor characteristics and demonstrating tumor-immune interactions. We hope this model may serve as a strong alternative to current options for assessing immunotherapy treatment responses for patients with HNC. Ultimately, further expansion of the model to other cell lines and patient tumor samples as well as replication of the above experiments, will enable further analysis and validation of the spheroid model.

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