Title: Oncolytic Adenovirus Therapy Modulates Immune Response in an Immunocompetent Breast Cancer Model.

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Breast cancer remains a major clinical challenge, with recurrence and metastasis often driven by therapy-resistant breast cancer stem cells (BCSCs). Despite effective treatments, up to 41% of estrogen receptor-positive (ER+) breast cancer patients develop resistance, and the immunosuppressive tumor microenvironment further limits therapeutic success. This underscores the need for innovative therapies targeting BCSCs while overcoming immune evasion.

We previously developed OAd5/3-Cox2-NIS, an infectivity-enhanced, tumor-selective oncolytic adenovirus (OAd) encoding the sodium iodide symporter (NIS), which enables radioiodine imaging and therapy. Our studies demonstrated that OAd5/3-Cox2-NIS effectively induces oncolysis and enhances NIS expression in paclitaxel-resistant ER+ BCSCs, including chemoresistant tumorsphere models. The vector displayed strong selectivity, eliminating cancer cells at low viral titers while sparing normal mammary epithelial cells. However, a relevant immunocompetent, adenovirus-permissive preclinical model has been lacking, limiting the study of OAd-driven immune responses.

To address this, we developed a novel Mammary INtra Ductal (MIND) model in immunocompetent, Ad-permissive hamsters using the HMAM5 cell line. This model mimics key features of human ER+ breast cancer, including tumor growth within mammary ducts, estrogen receptor expression, epithelial marker retention, and lymphatic metastases. OAd replication was confirmed, overcoming limitations of non-permissive rodent models. Therapeutic studies revealed a ten-fold tumor volume reduction, robust viral replication, and minimal off-target presence in liver, lung, and spleen.

We are now evaluating OAd-driven immunomodulation in this model, including abscopal effects in a bilateral tumor setting to determine whether OAd-mediated immune activation can suppress distant, untreated tumors. Additionally, immune profiling will assess tumor-infiltrating immune cells, cytokine secretion, and immune checkpoint expression. Single-cell RNA sequencing will further reveal OAd-induced gene expression changes in pathways related to innate/adaptive immunity, tumor antigen presentation, and immune evasion.

Our MIND model provides a clinically relevant system for evaluating OAd-based viroimmunotherapy in ER+ breast cancer. These findings support the clinical translation of OAd for advanced and metastatic breast cancer, offering insights into immune modulation and combination therapy potential.