**Preparation and Characterization of Cell Membranes for Cancer Immunotherapy**

Current efforts in cancer immunotherapy focus on eliciting cytotoxic T lymphocyte (CTL) responses against tumor-associated antigens (TAAs) and neo-antigens. Commonly-generated tumor cell lysates contain antigen-rich membrane vesicles, which can serve as a potent vaccine delivery vehicle. However, co-delivery of these antigens and adjuvants to dendritic cells (DCs) is crucial for effective responses. We aimed to incorporate adjuvants into membrane vesicles, thus expanding tumor-specific CTLs and eliciting humoral responses against tumor cell surface. Vesicles were generated by freeze-thawing and sonication of B16F10 OVA murine melanoma cells, expressing transmembrane model antigen ovalbumin. The vesicles were aggregated using calcium, washed, and then modified with DSPE-PEG by post-synthesis insertion method allowing for effective dispersion. Commonly-used adjuvants, MPLA and cholesterol-modified CpG, were also incorporated allowing co-delivery with membrane-associated antigens. Compared to soluble cytosolic proteins, membrane vesicles were taken up 3-fold more efficiently by DCs and led to cross-presentation and expansion of OVA-specific T cells in vitro. PEGylation allowed for increased stability during storage and led to 2.5-fold increased draining to inguinal lymph nodes eliciting OVA-specific CTL responses and IgG responses against tumor cell lysates. C57BL/6 mice were immunized prophylactically (two doses with two-week interval) and challenged with B16F10 OVA subcutaneously resulting in 67% protection (animals remained tumor-free for 80 days). In comparison, naïve mice succumbed to tumor burden within 20 days. Additionally, immunized mice challenged intravenously with melanoma displayed a 25-fold reduction in the number of metastatic nodules on the lungs compared to naïve mice. In a therapeutic setting, mice were challenged subcutaneously with melanoma and immunized on days 5 and 12 leading to decreased tumor growth and increased median survival from 20 to 29 days (p = 0.0042) The results of these studies demonstrate that PEGylated tumor membrane vesicles can effectively drain to lymph nodes and generate effective adoptive immune response against melanoma.