CRISPR Gene Edited Glioblastoma Multiforme Cell Line as a Model for Testing Antigen Specificity of CAR T-Cell Therapy

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Glioblastoma Multiforme (GBM) is an aggressive and fast-growing form of brain cancer with a high rate of mortality. It is the most common malignant brain tumor in adults, accounting for 47.7% of all brain tumor cases. Currently, the main form of treatment is surgery followed by radiation and chemotherapy. PD-L1 is an immunoinhibitory molecule located on the trans-membrane of a tumor cell. PD-L1 suppresses T-cell activation, thereby enabling tumor cells to proliferate. PD-L1 is rarely expressed on normal tissue but is over-expressed on GBM tumor cells which makes it a selective target for antigen specific therapy. Chimeric antigen receptor T (CAR-T) cells are engineered T-cells that can target surface antigens and cause antigen-specific killing of tumor cells. A PD-L1 specific CAR-T cell (MC9999) contains an anti-PD-L1 antibody on its cell surface, which can recognize PD-L1 and cause antigen-specific T-cell proliferation to cause cancer killing. To demonstrate PD-L1 antigen specific killing of tumor cells, this project generated a PD-L1 knock out (KO) GBM cell line model. This KO cell line model was made using clustered regularly interspaced short palindromic repeats (CRISPR) cas-mediated gene editing platform. Using nucleofection the CRISPR RNP complex was delivered into the nucleus of the GBM cells and the PD-L1 gene was disrupted. The cells expanded over several weeks. All single clones were tested using flow cytometry and a single PD-L1 KO cell clone that had 0.2% PD-L1 expression remaining was selected. The KO of PD-L1 using CRISPR was successful. This engineered cell line model will be instrumental in proving MC9999 CAR-T's antigen-specific killing mechanism against glioblastoma.

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