SYNNOTCH-CAR T CELLS DEMONSTRATE POTENT ANTI-TUMOR EFFICACY IN A PRECLINICAL IMMUNOCOMPETENT MOUSE MODEL FOR GliOBLASTOMA


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Background Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor. Recent immunotherapy studies, including CAR T cells, have shown limited success in GBM due to multiple barriers, such as on-target off-tumor toxicity, the blood-brain barrier, and the immunosuppressive tumor microenvironment (TME). To overcome these challenges, we recently adopted a novel synthetic Notch ‘syn-Notch’ receptor system and developed innovative T cell (‘synNotch-CART’) circuits based on the ‘prime-and-kill’ strategy. Here, we investigated the GBM-homing and efficacy of SynNotch-CAR T cells using a preclinical immunocompetent mouse model, which, similar to GBM patients, is poorly immunogenic and unresponsive to immune checkpoint blockade.

Methods Epidermal growth factor receptor (EGFR) amplification and overexpression occur in approximately 40–60% of GBM patients. Therefore, we first expressed the extracellular domain of mouse EGFR in a syngeneic glioma cell line, SB28 (SB28-mEGFR). Next, we developed a novel synNotch-CAR (mBSYNC) circuit in which the brain-specific antigen, brevican (BCAN) primes the T cells to induce the expression of a CAR that recognizes and kills EGFR-positive cells (mBSYNC synNotch-CAR T cells). 8-week-old C57BL/6 mice bearing the SB28-mEGFR cells in the frontal lobe received a single intravenous infusion of mBSYNC synNotch-CAR or conventional CAR T cells with constitutive CAR expression on day 8 following the tumor inoculation. Trafficking of CAR T cells and host immune cells into the brains was evaluated using high-dimensional flow cytometry.

Results Constitutive α-EGFR CAR and mBSYNC synNotch-CAR T cells mediated antigen-specific cytotoxicity against SB28-mEGFR cells in vitro. In an in vivo prospective study, on day 12 after T cell infusion, mBSYNC synNotch-CAR T cells demonstrated a higher persistence, increased infiltration of endogenous CD86+ and MHC-IIhi macrophages and decreased infiltration of CD206+ macrophages into the GBM TME compared to either untransduced or constitutive α-EGFR CAR T cells. Lastly, a single intravenous infusion of mBSYNC synNotch-CAR T cells significantly (p < 0.01) prolonged the survival and completely eradicated the aggressive tumor in 3 of 10 mice bearing SB28-mEGFR gliomas.

Conclusions mBSYNC synNotch-CAR T cells, but not conventional CAR T cells, effectively home into the brain TME, persist, and eliminate the tumor in an immunocompetent setting. The induced pro-inflammatory activity of endogenous myeloid cells could contribute to the anti-tumor efficacy mediated by synNotch-CAR T cells. Ongoing studies are aimed at the thorough characterization of tumor cells, host immune cells, and synNotch-CAR T cells in the brain TME.

REFERENCES

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