POLYFUNCTIONAL ACTIVITY OF GD3CAR T-CELLS AGAINST TUMORS IN TUBEROUS SCLEROSIS COMPLEX

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Background Gangliosides are glycosphingolipids that are involved in cellular functions, including signal transduction, cell proliferation, differentiation, adhesion, and angiogenesis. We confirmed marked overexpression of GD3 in tumors associated with Tuberous Sclerosis complex (TSC) and proposed to evaluate the use of T cells expressing a second-generation chimeric antigen receptor (GD3CAR T-cells) for patient treatment. To evaluate the potency of GD3CAR T-cells targeting solid tumor cells, we performed in vitro assays using Tsc2 knockout, GD3 overexpressing tumor cells isolated from mice heterozygous for Tsc2. HEK293 cells transfected or not with an expression plasmid encoding the enzyme SIAT8, responsible for converting GM3 to GD3, were used as controls. Cell were subjected to cytotoxicity assays using live cell imaging, and single cell cytokine secreteme analysis among CD4 or CD8 CAR T-cells.

Methods GD3CAR construct generated includes an anti-GD3 antibody single-chain antibody fragment and intracellular sequence of CD28 and CD3 zeta chain. Mouse T cells were transduced, and transduction was established by flow cytometry. GD3 expressing Tsc2/- tumor cells or HEK cells were co-cultured with untransduced or GD3CAR T-cells and cytotoxicity was measured using the Incucyte S3 system. Cytokine secretion patterns of CD4 and CD8 subpopulations of CAR T-cells were measured after coculture in a single cell polyfunctional strength mouse Isocode Chip (IsoPlexis). Secretory profiles of single cells were analyzed by IsoSpeak Software. IFNγ secretion was quantified by ELISA as a functional readout of T cell activity. Results Transduction efficiencies observed were upward of 70% live GD3 CAR T-cells with 96% transduction efficiency of CD4 T cells and 90% of CD8 T cells. The cytotoxicity assay in the Incucyte live-cell imaging system indicated 4-fold increased apoptosis (p=0.038) when target cells were co-cultured with GD3CAR T-cells. Both CD8 and CD4 T cells were efficiently transduced to express the GD3CAR. In single cell cytokine analysis, both T cell subsets showed enhanced polyfunctionality with increased polyfunctional strength index (PSI) by 9 and 10-fold in the GD3CAR T-cells in the CD4 and CD8 populations, respectively. This was mainly attributed to effector, chemo-attractive and stimulatory cytokines IFNγ production was increased significantly in response to target cells expressing GD3. Conclusions Both CD4 and CD8 GD3CAR T-cells express polyfunctional cytokine profiles in response to GD3 expressing tumor cells, and CAR T-cells were selectively cytotoxic to relevant tumor cells. The data suggests that GD3CAR T-cells may reduce tumor growth observed in patients with TSC.

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