239 CSPG4-TARGETED CAR T CELLS DEMONSTRATE POTENT ANTITUMOR ACTIVITY IN AN ORTHOTOPIC MURINE MODEL OF ANAPLASTIC THYROID CANCER

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Background Anaplastic thyroid cancer (ATC) is a rare and lethal malignancy. Once the tumor has developed, local disease progression and distant metastases rapidly occur, and all ATC patients ultimately succumb to their disease, with a median survival rate of 6 months. Here, we developed a Chimeric Antigen Receptor (CAR) T cell-based immunotherapy targeting the Chondroitin Sulfate Proteoglycan 4 (CSPG4) tumor antigen. We selected CSPG4, since it is highly expressed on ATC, with limited distribution in normal tissues. Additionally, increased CSPG4 expression has been associated with shorter overall survival in ATC patients.

Methods CSPG4 expression on human thyroid cancer cell lines was assessed by flow cytometry. CSPG4-CAR T cells were generated from healthy donors' peripheral blood mononuclear cells by transduction with a retroviral vector encoding a second generation CSPG4-specific CAR comprised of CD28 costimulatory domain. Antitumor activity of CSPG4-CAR T cells was tested *in vitro* by coculturing them with thyroid cancer cells at different effector to target (E:T) ratios, and in an orthotopic murine model of ATC.

Results Membrane CSPG4 expression was found to be high and homogeneous on several types of thyroid cancer cell lines, including ATC, Hurthle Cell Carcinoma (HCC), and Papillary Thyroid Cancer (PTC), with an average of 84% positive cells. CSPG4-CAR T cells were successfully generated from three healthy donors, with a mean transduction efficiency of 70%. CSPG4-CAR T cells demonstrated complete, or near complete *in vitro* eradication of cancer cells at high E:T ratio (68±18% killing at 1:1) and dose-dependent antitumor activity at lower E:T ratios (30±11% and 16±13% killing at 1:4 and 1:8, respectively) against ATC, HCC, and PTC cell lines. Specificity of CAR-engagement was confirmed by the intense release of effector cytokines (IFN-γ, Granzyme A/B, IL-2, IL-6) by CSPG4-CAR T cells cocultured with ATC cell lines. CSPG4-CAR T cells demonstrated strong expansion ability after systemic administration to NSG mice, which resulted in significant control (60% of mice) or complete eradication (40% mice) of ATC tumors. CSPG4-CAR T cell administration prolonged survival of mice compared to control groups.

Conclusions Here we demonstrate the preclinical efficacy of a CSPG4-CAR T cell-based immunotherapy for the treatment of ATC. Our findings provide the rationale to design clinical studies for ATC patients not responding to currently available therapies, and patients with BRAF wild type tumors who are not eligible for treatment with the Dabrafenib/Trametinib combination, the only FDA-approved treatment for ATC.

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