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Development of CAR T-cells for future treatment of NETs

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Background

Neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs). We investigated the antitumor activity of chimeric antigen receptor (CAR) T cells directed against SSTRs.

Methods

A second-generation CAR-like construct containing two molecules of octreotide in the extracellular moiety and CD28 as costimulatory module was cloned in a pMSGV1-28Z retroviral vector and then transduced in human T cells. Luciferase$^+$ (Luc$^+$) BON1, CM, QGP1, CNDT2.5 and H727 NET cell lines were screened for membrane $SSTR_{2/5}$ expression by Western blot (WB) and flow cytometry. Co-culture experiments were performed at effector:target (E:T) ratios ranging from 50:1 to 1:50 for up to 72 hrs. Tumor cell cytotoxicity was assessed by bioluminescence imaging. The release of IFN-γ and IL-2 by activated CAR T cells was investigated by ELISA. NSG female mice (n=11/group) were subcutaneously injected with $2x10^6$ Luc$^+$ BON1 or CM cells, and were then intravenously treated either with $7x10^6$ anti-SSTR CAR T cells, or untransduced (UT) T cells. Excised tumors were subjected to PCR to assess the infiltration of CAR T cells. Potential on-target off-tumor toxicities of anti-SSTR CAR T cells were investigated by pathological analysis of mouse brain and pancreas.

Results

All NET cell lines expressed $SSTR_{2/5,}$ although at variable levels. Following WB confirmation of the CAR expression by transduced lymphocytes, anti-SSTR CAR T cells were co-incubated with target cells. Tumor cell death was induced in approximately 40% (±8%) of CM and BON1 cells at E:T ratio of 1:1. The tumoricidal effect of CAR T cells was time-dependent and peaked at 72 hrs. Compared with UT T cells, CAR T cells secreted significantly higher levels ($p<0.01$) of IFN-γ and IL-2 after co-incubation with NET cells. Anti-SSTR CAR T cells effectively infiltrated tumors and significantly reduced the growth of subcutaneous CM ($p=0.01$) and BON1 xenografts ($p=0.02$) in mice by *in vivo* bioluminescence imaging. No pathological alterations were seen in the brain and pancreas of mice treated with CAR T cells.

Conclusions

Anti-SSTR CAR T cells exert antitumor activity against SSTR$^+$ NET cell lines, both in vitro and in vivo.

Legal entity responsible for the study

The authors.

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Disclosure

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