Title

CAR T cells targeting podoplanin reduce orthotopic glioblastoma in mouse brains.

Key Points

○ We constructed a third generation CAR that targets PDPN and its successful lentivirus-mediated expression on human T cells.

○ We showed that the generated CAR T cells were specific and effective against PDPN-positive GBM cells *in vitro*.

○Systemic injection of the generated T cells significantly increased survival time *in vivo*.

Summary

Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults with a 5-year overall survival rate of less than 10%. Novel therapies are required to improve patient survival. Chimeric antigen receptor (CAR) transduced T cells can recognize predefined tumor surface antigens independent of major histocompatibility complex (MHC) restriction, which is often downregulated in gliomas. Podoplanin (PDPN) is a type I transmembrane mucin-like glycoprotein, expressed in the lymphatic endothelium. It is overexpressed in several solid tumors such as squamous cell carcinoma, testicular seminoma, malignant mesothelioma, and brain tumors including GBM.

Dr. Atsushi Natsume, Department of neurosurgery, Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.), and colleagues generated a lentiviral vector expressing a third generation CAR comprising a PDPN-specific antibody [NZ-1-based single chain variable fragment (scFv)] with CD28, 4-1BB, and CD3ζ intracellular domains. CAR-transduced T cells were immunologically evaluated by calcein-mediated cytotoxic assay, ELISA, tumor size, and overall survival. The generated CAR T cells were specific and effective against PDPN-positive GBM cells *in vitro*. Systemic injection of the CAR T cells into an immunodeficient mouse model inhibited the growth of intracranial glioma xenografts and significantly increased survival time *in vivo*.

CAR T cell therapy that targets PDPN would be a promising adoptive immunotherapy to treat GBM.

Research Background

Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults with a 5-year overall survival rate of less than 10%. Novel therapies are required to improve patient survival.

In recent years, immunotherapy has emerged as a promising strategy for the treatment of GBM. Chimeric antigen receptors (CARs) were recombinant receptors that consisting of an

extracellular domain derived from a single-chain variable fragment (scFv) taken from a tumor antigen-specific monoclonal antibody (mAb), a transmembrane domain, and a cytoplasmic signaling domain CD3 ζ chain (CD3ζ) derived from the T-cell receptor complex. CAR transduced T cells can recognize predefined tumor surface antigens independent of major histocompatibility complex (MHC) restriction, which is often downregulated in gliomas. Third generation CARs, combining two costimulatory domains such as CD28 and 4-1BB, have been described and are highly likely to lyse tumor cells.

We and other groups have generated several CARs against the antigens expressed in GBM, including epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2), and interleukin-13 receptor alpha 2 (IL13Rα2).

Podoplanin (PDPN) is a type I transmembrane mucin-like glycoprotein expressed in the lymphatic endothelium. It is overexpressed in several solid tumors such as squamous cell carcinoma, testicular seminoma, malignant mesothelioma, and brain tumors including GBM. We previously produced a highly reactive mAb to PDPN , called NZ-1, and its recombinant scFv.

We generated a third generation of CAR that targeted PDPN, by using the NZ-1-based scFv. The generated CAR-transduced T cells were immunologically evaluated by calcein-mediated cytotoxic assay, ELISA, tumor size, and overall survival. The CAR T cells were specific and effective against PDPN-positive GBM cells *in vitro*. Systemic injection of the CAR T cells into an immunodeficient mouse model inhibited the growth of intracranial glioma xenografts and significantly increased survival time *in vivo*.

Research Results

We constructed a lentiviral vector tandem linked with the EF1α promoter followed by the leader sequence, NZ-1-based scFv, CD28, 4-1BB, and CD3ζ (Figure 1). The lentiviral vector was used to infect human T cells.

The calcein-based nonradioisotope cytotoxic assay indicated that PDPN-positive LN319 cells and U87MG cells were significantly lysed by NZ-1-CAR transduced T cells in an effector/target (E/T) ratio–dependent manner. In contrast, specific lysis was not observed against PDPN-KO LN319 and PDPN-KO U87MG cells (Figure 2).

NZ-1-CAR transduced T cells cocultured with LN319 or U87MG cells released significantly much IFNγ than mock-transduced T cells (Figure 3). Thus, we successfully generated functional active NZ-1-CAR T cells that recognize PDPN.

U87MG cells were stereotactically implanted into an immunodeficient mouse brain. Seven days after tumor implantation, NZ-1-CAR transduced T cells or mock-transduced T cells were infused intravenously via the tail vein. The non-treated mice were infused with PBS alone. The volume of gadolinium-enhanced tumors was evaluated by 3T-MRI sequentially. In approximately 60% of the mice treated with NZ-1-CAR T cells, the tumor grew markedly slower (Figure 4) and the mice survived significantly longer than that in the other two groups (Figure 5).
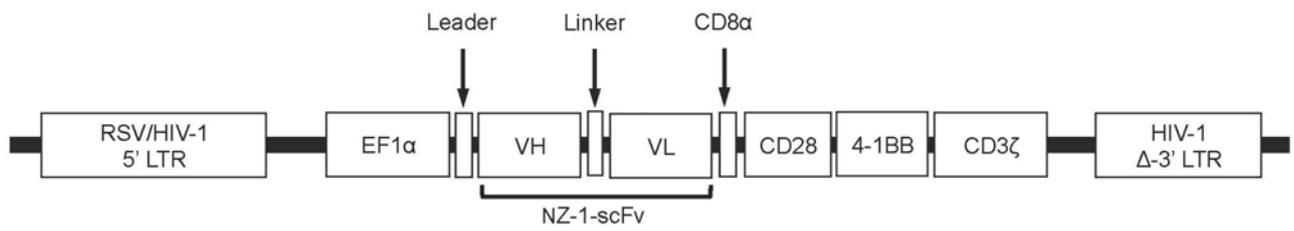
Figure 1 : Structure of NZ-1-CAR
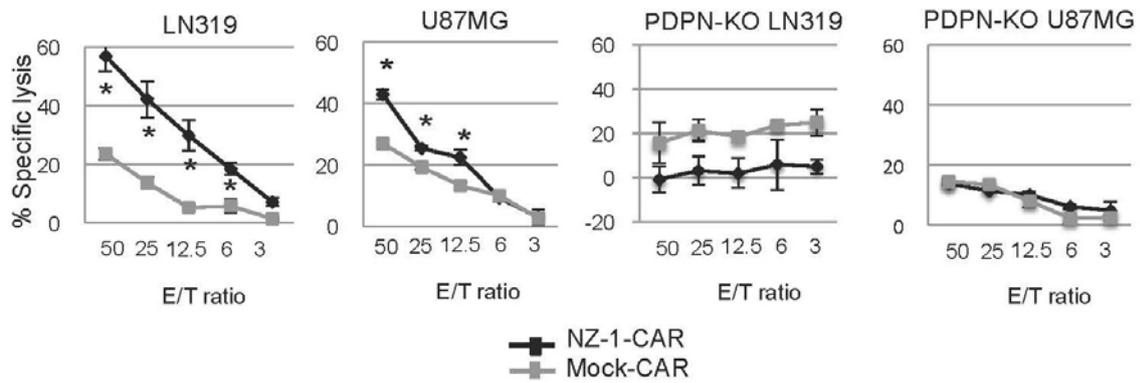
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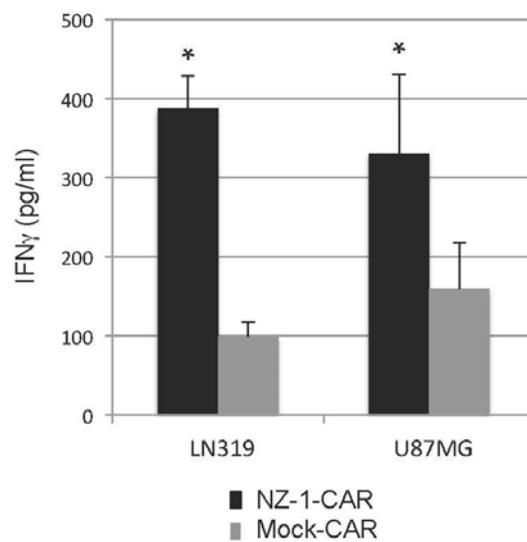
Figure 3 : IFNγ production
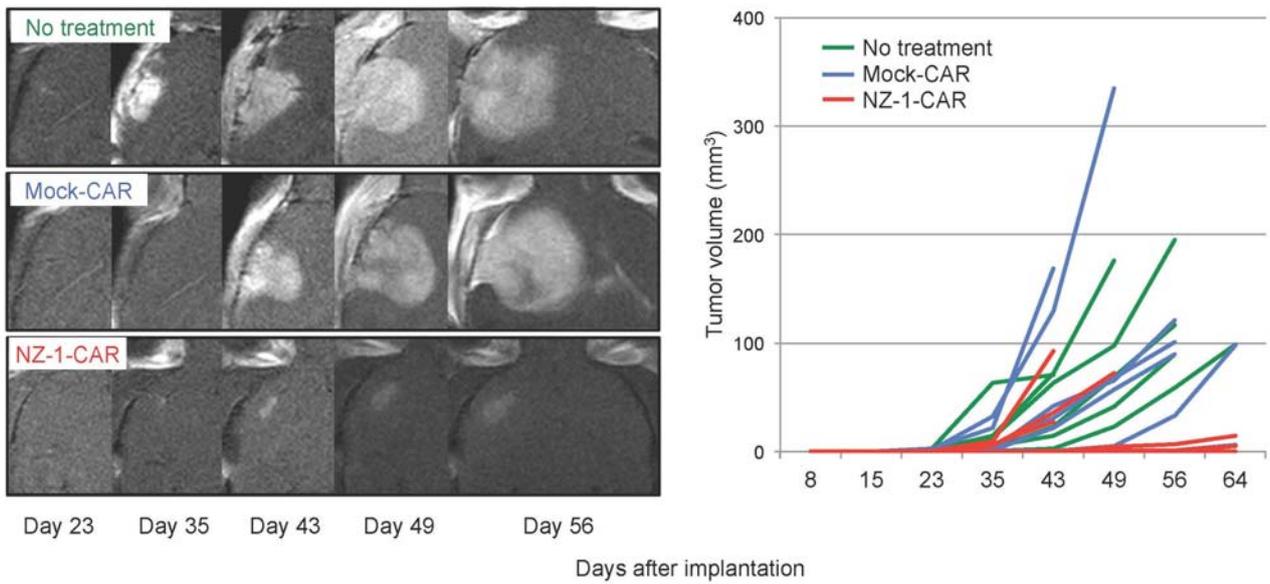
Figure 4 : Changes of contrast MRI and tumor volume
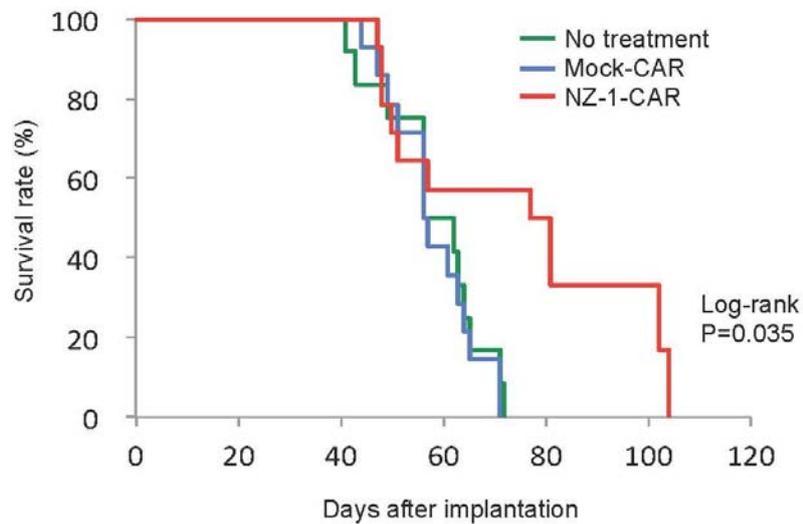
Figure 5 : Survival curve

Research Summary and Future Perspective

CAR T cell therapy that targets PDPN would be a promising adoptive immunotherapy to treat GBM.

One of the concerns with PDPN-targeted CAR therapy is that PDPN is expressed in normal

tissues, including the lymphatic endothelium, lung type I alveolar cells. We established a cancer-specific mAb (CasMab) to human PDPN. The CasMab can react with PDPN-expressing cancer cells, but not with normal cells such as lymphatic cells and type I alveolar cells. It may be useful to produce a new CAR using the CasMab for CAR therapy targeting PDPN.

Article

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