Preclinical efficacy and safety profile of *in vivo* generated claudin 18.2 specific chimeric antigen receptor t cells in gastric cancer models

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Introduction

Autologous CAR-T cell therapy has made significant progress in hematologic malignancies and shown promise in solid tumors. However, the complex manufacturing process and high costs have limited its broader application. To address these challenges, we developed the iMagic platform, a novel lentiviral-based *in vivo* CAR-T system incorporating a mutated MxV glycoprotein and a T cell–targeting module (TCM3). This platform enables selective transduction of T cells and direct generation of CAR-T cells *in vivo* and has demonstrated potent antitumor efficacy in blood cancer models. Here, we present the preclinical data of *in vivo* generated CLDN18.2 CAR-T in gastric cancer models.

Methods

The specificity of lentiviral vector expressing CLDN18.2 CAR (iMagic-18.2) was assessed by in vitro transduction assays using primary cells and multiple tumor cell lines. T cell activation, CAR-T generation were analyzed after PBMCs were co-incubated with iMagic-18.2. In vitro cytotoxicity assays were evaluated by co-culturing of iMagic-18.2 or conventional CLDN18.2 CAR-T cells with CLDN18.2-positive tumor cells at various effector-to-target ratios, cytokine secretion were also analyzed following target cell stimulation. For *in vivo* evaluation, MHCII/B2M-dKO immunodeficient mice were reconstituted with human PBMCs and engrafted with NUGC4-Luc gastric tumors. Mice were treated with either iMagic-18.2 (5E6 TU) or conventional CLDN18.2 CAR-T cells (2E6 CAR+ cells), and tumor growth, CAR-T expansion, body weight were monitored

Results

In vitro, iMagic-18.2 selectively transduced T cells with negligible transduction in human primary cells or tumor cell lines, demonstrating high specificity. Co-incubation of PBMCs with the iMagic-18.2 led to efficient T cell activation and CAR-T cell generation. When co-cultured with CLDN18.2-positive tumor cells, iMagic-18.2 induced CAR-T cells exhibited comparable tumor-killing activity versus conventional CLDN18.2 CAR-T cells. Furthermore, cytokines such as IFN-γ, IL-2, and TNF-α, were secreted by iMagic-18.2 induced CAR-T cells upon tumor cells stimulation.

In vivo, The iMagic-18.2 showed potent and even better anti-tumor efficacy in mice than the conventional CLDN18.2 CAR-T groups. The pharmacokinetic profile of CAR-T cells in peripheral blood demonstrated that the iMagic-18.2 group achieved a comparable Cmax but exhibited greater persistence than the conventional CLDN18.2 CAR-T group. Moreover, iMagic-18.2 was well tolerated, with no observed abnormalities or body weight loss throughout the study.

Conclusions

The iMagic platform enables efficient and selective *in vivo* generation of functional CAR-T cells and demonstrated potent antitumor activity with a favorable safety profile in gastric cancer

preclinical models. These findings underscore the strong potential of our *in vivo* CAR-T platform to extend into clinical development for solid tumor therapy.