DUAL TARGETING OF CAR-NK CELLS TO PD-L1 AND ERBB2 FACILITATES SPECIFIC ELIMINATION OF CANCER CELLS OF SOLID TUMOR ORIGIN AND OVERCOMES IMMUNE ESCAPE BY ANTIGEN LOSS

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Background Retargeting of natural killer (NK) cells with chimeric antigen receptors (CARs) can be a powerful approach to overcome NK-cell resistance of tumor cells. However, targeting a single tumor-associated antigen may be insufficient for some tumors to trigger effective NK-cell activation or result in the selection of antigen-loss variants and tumor immune escape.

Methods To overcome this hurdle, here we generated CAR-NK cells carrying two CARs that target the tumor-associated antigens PD-L1 and ErbB2 (HER2), respectively (figure 1A). NK-92 cells were transduced with lentiviral CAR constructs, and their cytotoxicity against cancer cell lines of different solid tumor origins was compared to that of parental NK-92 and corresponding single-target CAR variants.

Results Dual targeting significantly increased in vitro cytotoxicity against PD-L1 and ErbB2 double-positive tumor cell lines including breast, ovarian, pancreatic and gastric cancer cells when compared to single-target CAR variants (Figure 1B,C). These results were also confirmed with 3D spheroid tumor models. Off-target cytotoxicity was not observed. On a molecular level, this enhanced cell killing may be explained by synergistic activation of PLCγ and MAPK pathways. Incubation of cancer cells with IFN-γ further improved killing efficacy due to upregulation of PD-L1 expression. Furthermore, blocking experiments revealed that dual PD-L1/ErbB2-CAR NK-92 cells can overcome immune escape based on loss or inaccessibility of a single target antigen.

Conclusions Altogether, we showed that dual targeting of PD-L1 and ErbB2 improves efficacy of CAR-NK cells against otherwise difficult to treat tumors, and counteracts potential resistance and immune escape mechanisms of cancer cells.

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