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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Efficacy of Human iPSC-Derived CAR-NK Cells Targeting Multiple Myeloma Cells

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NK cells armed with chimeric antigen receptors (CAR) enable NK cells specifically to target cancer cells by recognizing tumor associated antigens. Previous studies have proven that human iPSC-derived CAR-NK cells have enhanced anti-tumor activity. Targeted B-cell maturation antigen (BCMA) therapy for relapsed refractory MM (rrMM) has been widely used with antibody-drug conjugate (ADC), bispecific antibodies, and CART cells, which has produced a rapid response, but relapse is common. Recent work has revealed GPRC5D as an alternative target in MM for immunotherapy and patients who have previously received anti-BCMA therapy have responded to GPRC5D CART cells. To assess target GPRC5D therapy with CAR-NK cells, which may in future serve as allogeneic immunotherapy, we transduced anti-GPRC5D CAR into an iPSC line derived from a healthy donor using a piggybac transposon system. More than 97% of CAR inserted-iPSCs (CAR-iPSCs) expressed anti-GPRC5D scFV with a similar expression level identified in CAR-iPSC-derived-NK cells (CAR-iNK, 92.7%). The anti-GPRC5D CAR-iNK demonstrated high purity (>99.9% for CD45 $^{+}$ and CD56 $^{+}$) and expressed a high level of CD16 (63.6%), the NK cell activating receptor NKG2D (96.3%) and NKp30 (98.7%), and the co-stimulatory receptors CD244 (99.6%) and CD226 (97.8%). Nonetheless expression of TCR$\alpha\beta$ and TCRΥ- was relatively low (<2%). Cytotoxicity assay revealed that anti-GPRC5D CAR-iNK had similar cytotoxicity against K562 cells (No GPRC5D antigen) to cord blood-derived NK cells (CB-NK), wide type (WT) iNK and anti-BCMA CAR-iNK. Cytotoxicity was dose-dependent indicating that these NK cells had similar innate anti-cancer cytotoxicity. When incubated at a ratio of 4:1 with NCI-H929 cells (MM) for 4 hours, which express a high level of both BCMA and GPRC5D, anti-GPRC5D CAR-iNK demonstrated an overwhelming advantage (approximately 90% killing rate) over the CB-NK (no killing) and WT iNK (around 10% killing rate). Furthermore, the anti-GPRC5D CAR-iNK also showed superior cytotoxicity to anti-BCMA CAR-iNK (Figure 1), indicating that GPRC5D may be a more efficient target for CAR-NK-based immunotherapy in MM. We further confirmed that anti-GPRC5D CAR-NK have acceptable cytotoxicity to NCI-H929 and OPM-2 cells, even when transported over a long distance (more than 2000 km) and following cryopreservation. The lower expression by OPM-2 cells of BCMA and GPRC5D compared with NCI-H929 indicates that both fresh and cryopreserved anti-GPRC5D CAR-iNK can specifically target MM with a higher and lower expression of GPRC5D. In addition, cryopreserved anti-GPRC5D CAR-iNK cells maintain comparable antigen-specific cytotoxicity in vitro, and demonstrate the ability to reduce tumor burden in an antigen-specific manner in an OPM-2 xenograft model. Our results demonstrate that genome-engineered human iPSCs can provide an unlimited source for manufacturing scalable, off-the-shelf, and cost-effective CAR-NK cells, and GPRC5D is a potent immunotherapeutic target in multiple myeloma.

Disclosures Fu: *Cell Inspire Biotechnology:* Current Employment. **Jiang:** *Cell Inspire Biotechnology:* Current Employment. **Zhu:** *Cell Inspire Biotechnology:* Current Employment. **Yan:** *Cell Inspire Biotechnology:* Current Employment. **Wu:** *KYinno Biotechnology (Beijing) Co., Ltd.:* Current Employment. **Wei:** *Cell Inspire Biotechnology:* Current Employment. **Ning:** *KYinno Biotechnology (Beijing) Co., Ltd.:* Current Employment, Current equity holder in private company. **Yang:** *Cell Inspire Therapeutics:* Current Employment; *Cell Inspire Biotechnology:* Current equity holder in private company.

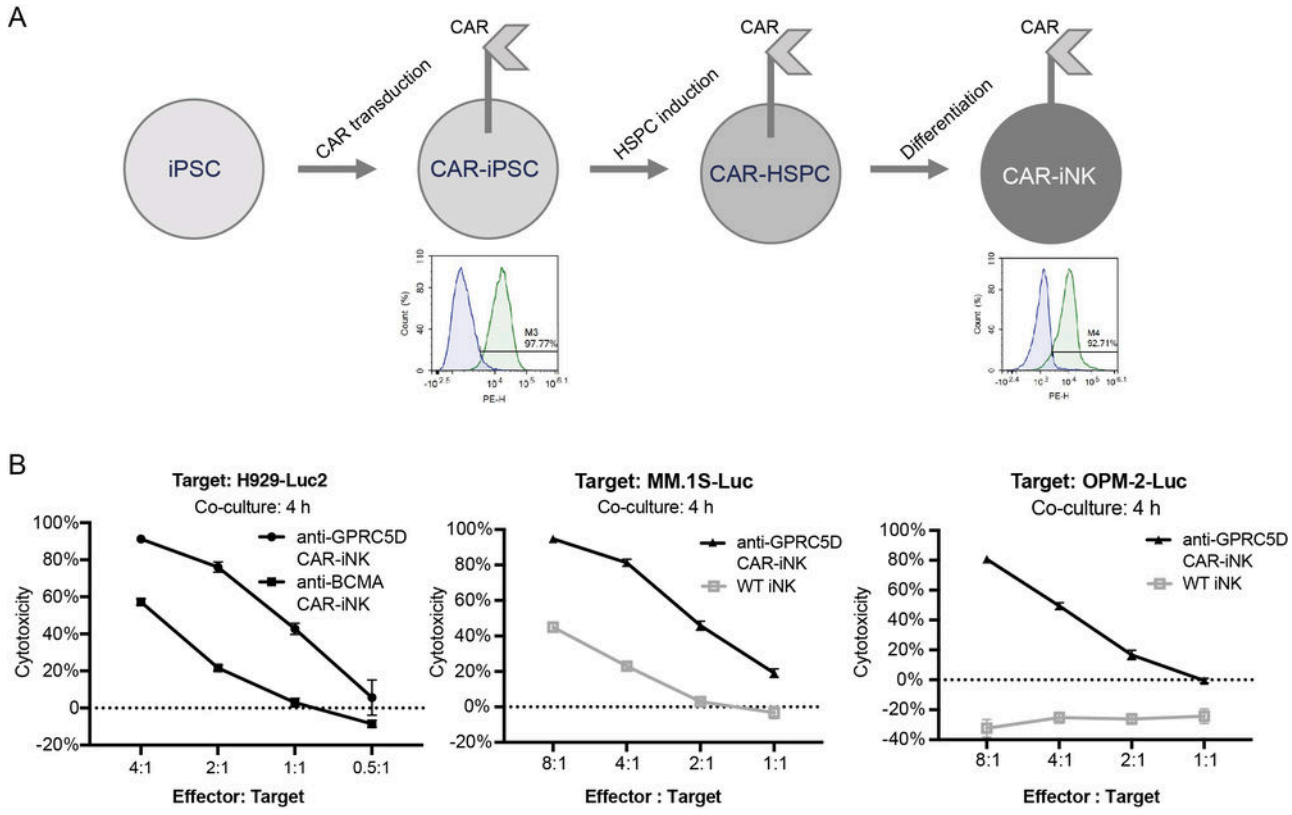

Figure 1

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