Armoring CAR-T Therapy with PD-1 Blockade: A Powerful Strategy for Enhancing Anti-Tumor Effects in Pancreatic Cancer

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Abstract

Chimeric antigen receptor-modified T cells (CAR-T) have been widely applied in the treatment of hematological malignancies and are currently under investigation for their potential effectiveness against solid tumors. Nevertheless, the effectiveness of CAR-T in treating solid tumors remains unsatisfactory due to a range of factors, including CAR-T cell infiltration and heterogeneity. The efficacy of CAR-T therapy is substantially influenced by the tumor microenvironment. This is particularly evident when PD-L1 is activated through CAR-T, leading to the inhibition of CAR-T cell activity via the PD-1 pathway.

In this study, we engineered a CAR-T therapy that targets mesothelin (MSLN) and concurrently expresses a membrane-bound PD-1 receptor. This design blocks the PD-1 on CAR-T cells, effectively counteracting the suppression of CAR-T function mediated by PD-L1. We observed a substantial upregulation of PD-L1 on AsPC-1 tumor cells after 4 hours of IFN-γ stimulation in vitro. CAR-T cells engineered to co-express PD-1 antibody (aMSLN-28BBz-mPD-1) exhibited similar short-term cytotoxicity and cytokine secretion profiles to those of the traditional third-generation CAR-T (aMSLN-28BBz). Notably, aMSLN-28BBz-mPD-1 displayed significantly improved long-term target cell killing. Furthermore, in an in vivo model of pancreatic cancer, aMSLN-28BBz-mPD-1 demonstrated enhanced anti-tumor activity when compared to the traditional aMSLN-28BBz, as supported by immunofluorescence analysis and immunofluorescence assays of tumor tissues. These analyses revealed a marked upregulation of PD-L1 expression and enhanced CAR-T cell infiltration in the aMSLN-28bbz-mPD-1 treatment group. In summary, our study demonstrates that co-expression of a membrane-bound PD-1 antibody overcomes PD-L1 mediated CAR-T suppression, as observed in both *in vitro* and *in vivo*. This innovative strategy underscores its therapeutic potential and value in treating solid tumors.

Methods

In vitro, CAR T cells were collected for surface marker expression analysis after second round of stimulation, enriched via bead-based method, then co-cultured with luciferase-transduced tumor cells for re-challenge cytotoxicity assay.

In vivo, an AsPC-1 cell line with overexpression of human MSLN was inoculated subcutaneously into NCG mice. When the tumor volume reached about 100 mm3, grouping and T cell dosing were carried out. Tumor volume and animal body weight were continuously monitored until the end of pharmacodynamics study. The bio-distribution of CAR was detected by QPCR on day 0 (2 hours), day 4, day 7, day 11, day 14, day 21, day 28, and day 35 after T cell treatment in pharmacokinetics study. The distribution of hCD3+, hPD-1+ or hPD-L1+ cells in tumor on day 14 was detected by immunofluorescence (IF) .

Results

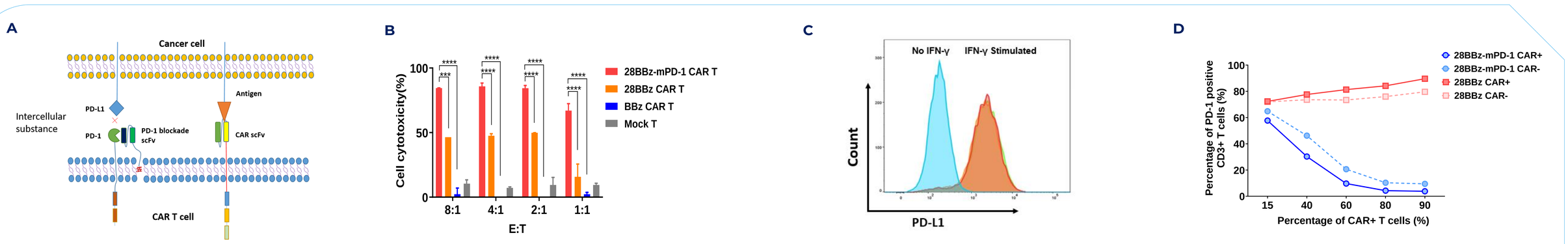


Figure 1. 28BBz-mPD-1 CAR enhances the cytotoxicity of CAR-T cells *in vitro*.
(A) Schematic diagram of 28BBz-mPD-1 CAR. (B) *In vitro* long-term cytotoxicity assay, two-way ANOVA, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001. (C) *In vitro* PD-L1 expression in AsPC-1 after 4 hours FN-γ stimulation. (D) CAR-T cell PD-1 self-blocking detection.

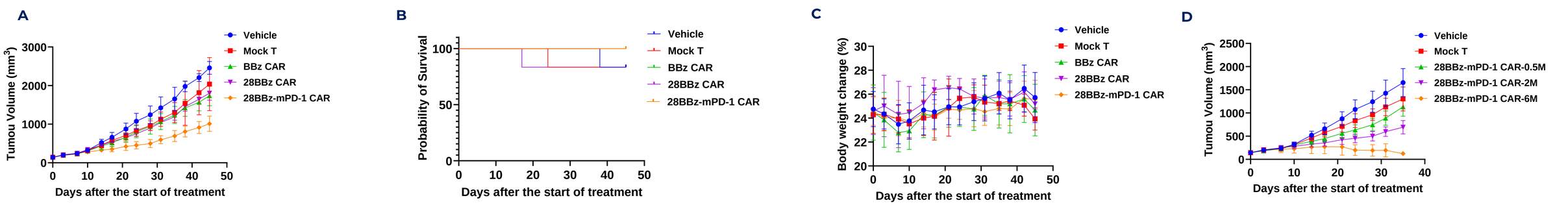


Figure 2. 28BBz-mPD-1 CART enhances the anti-tumor suppression *in vivo*.
(A) Tumor growth curve of CAR with different costimulatory domains. (B) Survival curve of CAR with different costimulatory domains. (C) Body weight change of CAR with different costimulatory domains.(D) Tumor growth curve of 28BBz-mPD-1 CAR at different dosages.

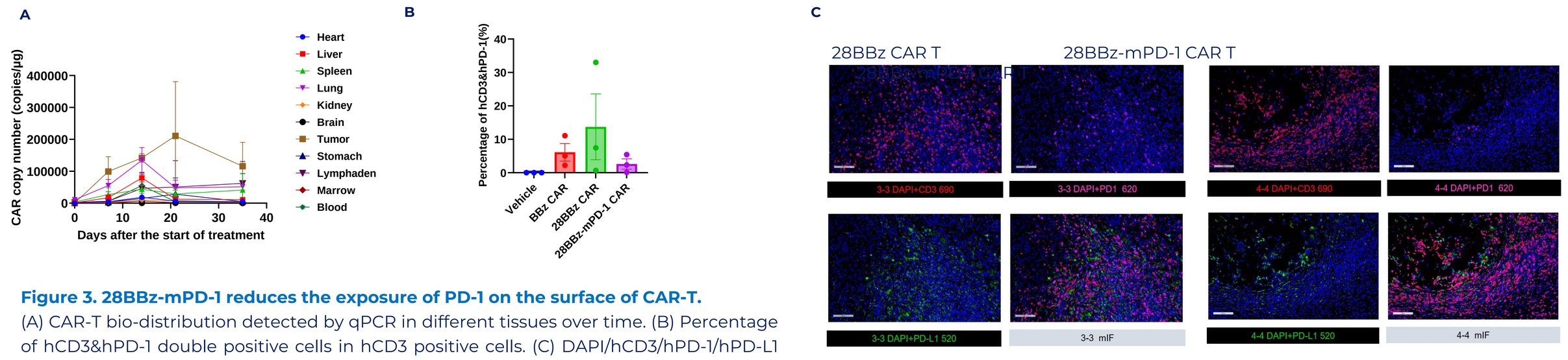
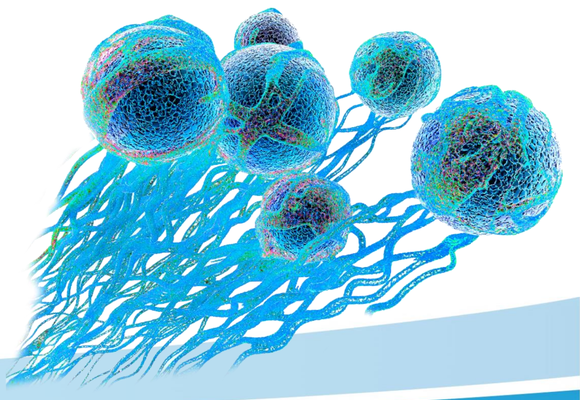


Figure 3. 28BBz-mPD-1 reduces the exposure of PD-1 on the surface of CAR-T.
(A) CAR-T bio-distribution detected by qPCR in different tissues over time. (B) Percentage of hCD3&hPD-1 double positive cells in hCD3 positive cells. (C) DAPI/hCD3/hPD-1/hPD-L1 multiplex expression on tumor tissue (200x).

Conclusion

The inclusion of PD-1 immune checkpoint blockade protein enhances and prolongs CAR-T cells tumor suppression effect both in vitro and in vivo. This opens up the possibility of adding an immune checkpoint inhibitor transgene to improve the tumor microenvironment and extend the survival of CAR T cells.



References

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2. Wu P, Wu D, Li L, Chai Y, Huang K. PD-L1 and Survival in Solid Tumors: A Meta-Analysis. PLoS ONE. 2015; 10:e0131403

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