(B048) Neutrophil extracellular traps-targeting DNase I enhances the therapeutic efficacy of CAR-T cell adoptive immunotherapy in a syngeneic murine metastasis model.

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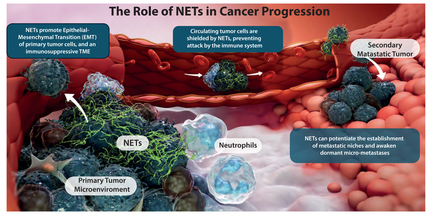
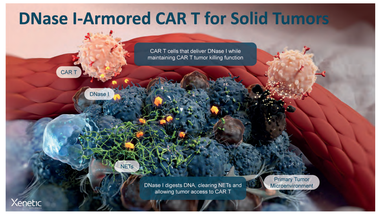
Background

In recent years, chimeric antigen receptor (CAR) T cell therapy has emerged as a promising approach for treating various malignancies, most notably in hematologic cancers. However, its efficacy in solid tumors is often hindered by an immunosuppressive tumor microenvironment (TME), which, in particular, is frequently characterized by the presence of tumor-associated cell-free DNA (cfDNA), especially in the form of neutrophil extracellular traps (NETs). In adoptive immunotherapies NETs, composed primarily of neutrophil-derived DNA, histones and neutrophil granule enzymes (e.g., MPO, NE), act as physical barriers and induce the secretion of immunosuppressive factors that impair CAR T cell function.



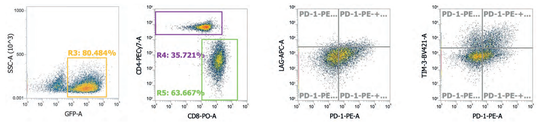


Materials&Methods

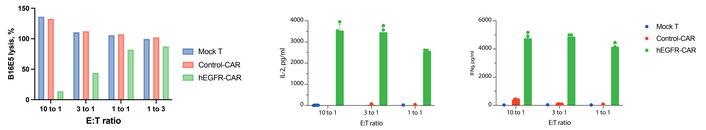
1.Female C57BL/6 mice 7-8 weeks old at start.
2.0.8*10^6 B16-hEGFR tumor cells intravenously (i.v.).
3.Day before CAR T cells injection mice preconditioned by 5Gy irradiation or cyclophosphomide.
4.Day 7 Injection of 2*10^6 hEGFR-CAR T cells i.v.
5.Day 7 rhDNaseI treament 10 mg/kg started.
6.For TILs and Metastatic index analysis lungs colected at day 3 and 6 after CAR T cells infusion.

hEGFR-CAR sctucture
a-hEGFR scFc.28.z

CAR T cells were manufactured from C57BL/6 splenocytes



hEGFR-CAR T cells specifically lyse B16-EGFR cells in vitro and secrete proinflammatory cytokines



Summary

This research highlights the critical role of the NETs in modulating CAR T cell efficacy and provides a compelling rationale for incorporating DNase I as an adjunctive treatment to improve therapeutic responses in patients undergoing CAR T cell therapy. Further clinical studies are warranted to validate these findings and explore the translational potential of this combinatorial approach in enhancing cancer treatment.

Results

This study investigates the potential of co-administration of deoxyribonuclease I (DNase I) with CAR T cells in a syngeneic B16 melanoma murine model of lung metastasis. Bioluminescent imaging of melanoma metastatic processes has shown that a single injection of DNase I (10 mg/kg) suppresses B16-EGFR lung metastasis at early stages in comparison to the vehicle control group but does not improve survival. However, co-administration of DNase I with murine EGFR-CAR T cells significantly suppresses tumor burden, decreases the number of metastatic foci, and substantially prolongs survival compared to the CAR T cell monotherapy group. Degrading of NETs by DNase I increases the amount of tumor-infiltrating T and CAR T cells and reduces the immunosuppressive effects of the TME. This is reflected in the CD8 population of tumor-infiltrating CAR T cells from the DNase I treated group, which have lower expression of PD-1 and TIM-3 exhaustion markers.

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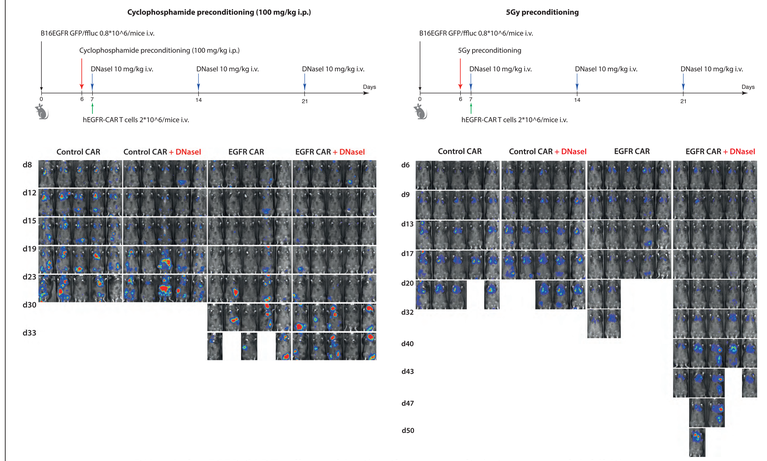
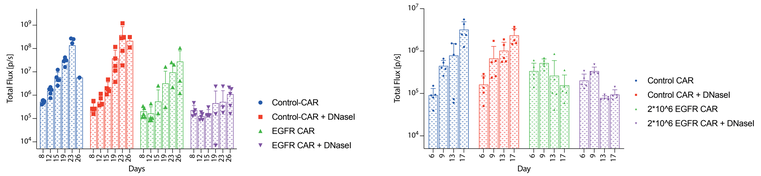
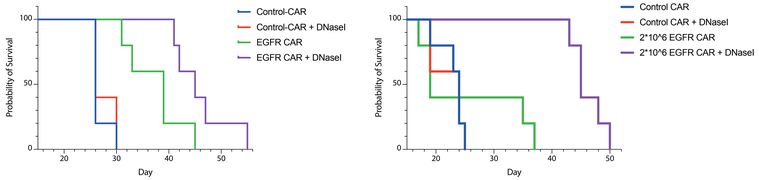
DNaseI plus EGFR-CAR T cells combination therapy results in tumor growh inhibition and enhanced survival in mice bearing B16-EGFR lung metastatic melanoma tumors.



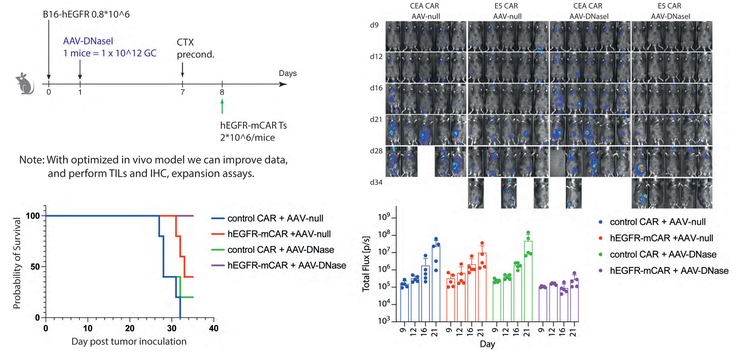
DNaseI plus EGFR-CAR T cells combination therapy results in tumor growh inhibition



DNaseI plus EGFR-CAR T cells combination therapy results in enhanced survival



AAV-DNaseI plus EGFR-CAR T cells combination therapy results in tumor growh inhibition and enhanced survival in mice bearing B16-EGFR lung metastatic melanoma tumors.



hEGFR-CAR T cells combination with i.v. DNase I supress the lung metastases in melanoma murine model.
Increase proportion of tumor infiltrating CD4 and CD8 cels, and delay exhaustion of the effector CD8 cells.