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Perspective

Multiplexed Genome Editing in CAR-T Cells for Enhanced Therapeutic Efficacy against Solid Tumors

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DESCRIPTION

Chimeric Antigen Receptor T-cell (CAR-T) therapy has achieved remarkable success in treating hematological malignancies, but efficacy against solid tumors remains limited due to immunosuppressive tumor microenvironments and T-cell exhaustion. This investigation employs multiplexed genome editing strategies to simultaneously enhance CAR-T cell persistence, overcome inhibitory signals, and improve tumor penetration. The approach combines Clustered Regularly Interspaced Short Palindromic Repeats associated with protein 9 (CRISPR-Cas9) knockout of exhaustion-associated genes with targeted integration of enhancing factors.

Primary human T cells were isolated from healthy donors and activated using CD3/CD28 beads in the presence of IL-2 and IL-15. CAR constructs targeting the tumor-associated antigen mesothelin were introduced *via* lentiviral transduction, incorporating 4-1BB costimulatory domains and CD3 signaling domains. Simultaneous editing targeted multiple genes: Programmed cell Death protein 1 (PD-1) and T cell Immunoglobulin (TIM-3) knockout to reduce exhaustion.

Chimeric Antigen Receptor (CAR) T cell therapy has shown remarkable success in treating hematologic malignancies, yet its efficacy against solid tumors remains limited due to several biological and immunological barriers. These challenges include the immunosuppressive Tumor Microenvironment (TME), antigen heterogeneity, limited T cell persistence, and inhibitory immune checkpoint signaling. To overcome these hurdles and improve therapeutic outcomes, multiplexed genome editing has emerged as a promising strategy to endow CAR-T cells with enhanced functionalities and resistance to tumor-induced suppression.

Advancements in genome engineering technologies-especially CRISPR/Cas9-have enabled precise and simultaneous modification of multiple genomic loci in T cells. Through multiplexed editing, it is now feasible to knock out genes that encode inhibitory receptors (such as PD-1 or CTLA-4), disrupt endogenous T Cell Receptor (TCR) components to reduce graft-versus-host risk, and insert transgenes that boost CAR-T cell proliferation, persistence, or trafficking. Additionally, synthetic gene circuits and safety switches can be incorporated to increase control and minimize off-target effects.

This approach not only expands the therapeutic potential of CAR-T cells in solid tumors but also represents a paradigm shift toward next-generation, programmable cell therapies. As the field evolves, multiplexed editing holds promise for creating "armored" CAR-T cells capable of overcoming immune resistance, targeting multiple tumor antigens, and maintaining durable anti-tumor responses. This introduction explores the principles, methodologies, and translational potential of multiplexed genome editing in CAR-T cell engineering for more effective treatment of solid tumors.

Electroporation-mediated delivery of multiple gRNA-Cas9 ribonucleoprotein complexes achieved editing efficiencies of 67% for PD-1, 59% for TIM-3, and 52%, with 34% of cells showing simultaneous editing of all three targets. Homology-directed repair using Adeno-Associated Virus (AAV) vectors enabled targeted integration of IL-12 expression cassettes at the T-cell Receptor Alpha Constant (TRAC) locus in 28% of cells. Flow cytometric analysis confirmed successful CAR expression and loss of target proteins in edited cells.

Functional characterization revealed enhanced cytotoxic activity against mesothelin-positive tumor cells, with multiplexed-edited CAR-T cells showing 3.2-fold increase in specific lysis compared to conventional CAR-T cells. Cytokine production analysis demonstrated elevated IL-2, IFN-γ, and IL-12 secretion, indicating enhanced activation states. Importantly, edited cells maintained proliferative capacity and showed reduced expression of exhaustion markers following repeated antigen stimulation.

In vivo efficacy studies using patient-derived xenograft models of mesothelioma demonstrated superior antitumor activity of multiplexed-edited CAR-T cells. Treated mice showed 73% reduction in tumor burden compared to conventional CAR-T therapy, with significantly improved survival outcomes.

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CONCLUSION

Multiplexed genome editing significantly enhances CAR-T cell therapeutic efficacy against solid tumors by addressing multiple resistance mechanisms simultaneously. The combined knockout of exhaustion genes and integration of enhancing factors demonstrates the power of precision engineering for immunotherapy applications. This work establishes multiplexed editing as a valuable approach for next-generation CAR-T cell therapies. Histological analysis revealed enhanced T-cell infiltration and sustained CAR-T persistence within tumors. Notably, the IL-12 expression promoted recruitment of endogenous immune cells, creating a more favorable tumor microenvironment.