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The Implementation Application of CRISPR Gene-Editing Technology within CAR-T Strategies Targeting Solid Tumors

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Abstract:

With impressive clinical results, The management of hematologic malignancies has evolved as a result of CAR-T treatment. However, its application to solid tumors is restricted by immune-suppressive tumor milieus, antigenic heterogeneity, and insufficient persistence and infiltration of engineered T cells. CRISPR-Cas9, a versatile and precise genome-editing platform, offers new strategies to overcome these challenges through targeted modification of its products. This paper describes the mechanical properties and structural layout of CAR-T treatment, together with important obstacles in the context of solid tumors and CRISPR-driven engineering techniques meant to improve therapeutic efficacy. The development of universal CAR-T products for ready-to-use applications, metabolic reprogramming to adapt to nutrient-limited and hypoxic conditions, cytokine gene integration to support persistence and immune communication, and checkpoint receptor disruption to maintain T-cell activity are examples of representative strategies. Collectively, the convergence of CRISPR and CAR-T technologies enhances precision, durability, and broad applicability, positioning this combined strategy as a promising direction for next-generation immunotherapy against solid cancers.

Keywords: CRISPR-Cas9; CAR-T cells; solid tumors; gene editing.

1. Introduction

Solid tumors remain among the most formidable challenges in oncology due to their complex architecture, antigenic heterogeneity, and highly suppressive tumor microenvironments (TME). In contrast to hematologic cancers, where malignant cells circulate in accessible compartments with relatively uniform antigen expression, solid tumors are embedded in dense stromal networks, display spatiotemporal antigen variability, and actively recruit immunoregulatory pathways to evade immune surveillance [1]. Conventional treatments—including surgery, chemotherapy, and radiotherapy—though indispensable,

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often achieve only partial responses and are frequently accompanied by high recurrence rates, highlighting the urgent demand for innovative therapeutic strategies [2].

Among immunotherapeutic modalities, CAR-T treatment has become a game-changing strategy. By reprogramming patient T lymphocytes with synthetic receptors capable of recognizing tumor-associated antigens, this strategy has delivered remarkable outcomes in hematologic cancers, particularly acute lymphoblastic leukemia and B-cell lymphomas. Well-defined antigen targets are primarily responsible for these achievements, supportive immune contexts, and effective T-cell trafficking. However, extending these benefits to solid tumors has proven more difficult. Physical barriers within the tumor stroma hinder CAR-T infiltration, while immunosuppressive cytokines like TGF-β, Along with checkpoint signals (PD-1 or PD-L1,for example), suppress effector functions and drive T-cell exhaustion. In addition, the rapid loss or mutation of tumor antigens frequently contributes to therapeutic resistance and disease relapse.

The advent of CRISPR-Cas9 genome offers unprecedented opportunities to address these limitations .CRISPR is an RNA-guided DNA endonuclease system that can be programmed to precisely knock out, insert, or modify genes, allowing researchers to enhance CAR-T function at multiple levels. Potential strategies include the deletion of inhibitory checkpoint receptors to sustain cytotoxic activity, insertion of cytokine genes to improve persistence and intercellular communication, metabolic reprogramming to enable CAR-T cells to thrive in nutrient-depleted and hypoxic environments, and the generation of universal allogeneic CAR-T products by disrupting endogenous T-cell receptor and HLA genes. Such modifications aim to create highly potent, long-lasting, and broadly applicable cellular therapeutics capable of overcoming the multifaceted defense mechanisms of solid tumors.

This paper provides a comprehensive review of CAR-T therapy's structural components, underlying mechanisms, and the unique barriers encountered in solid tumor contexts. It further examines how CRISPR-Cas9 can be strategically integrated into CAR-T engineering to overcome these barriers, drawing upon recent preclinical and translational studies. By elucidating these advances, We seek to emphasize the synergistic promise of integrating CAR-T with CRISPR technologies, thereby facilitating the development of next-generation immunotherapies with the potential to transform therapeutic strategies for solid tumors.

2. CAR-T Treatments

A new immunotherapy technique called CAR-T rewires the immune system to target cancers. The engineered receptors are made up of three parts, drug that recognizes the cancer markers, another living part that attaches the receptor to the cell's outer membrane; and the third causes the immunological response within the cell to become active. The overall design is illustrated in Fig. 1 [3].

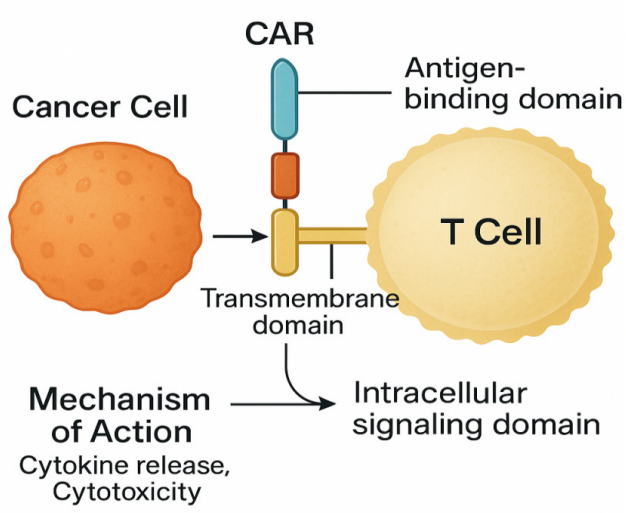

Fig1. Schematic Illustration of CAR-T Cell Functional Mechanism: Antigen Binding and Intracellular Signal Transduction. Picture credit: Original

2.1 Structure Components and Mechanism of CAR-T Therapy

The effectiveness of CAR-T cells relies on the receptor's structure, which includes three main components. Firstly, The antigen-binding domain, typically from a monoclonal antibody's single-chain variable fragment (scFv), binds specifically to tumor-associated antigens like CD19 in B-cell cancers or BCMA in multiple myeloma, enabling tumor targeting without dependence on MHC presentation [4].

Second, the transmembrane domain, typically derived from proteins such as CD8α or CD28, serves to firmly anchor the receptor within the T-cell membrane. This structural component not only stabilizes the CAR construct but also ensures proper spatial orientation, thereby facilitating efficient downstream signal transduction.

Thirdly, the intracellular signaling system of T cells includes the CD3ζ chain - this is the core component of TCR-related signal transduction, as well as co-stimulatory motifs such as CD28 or CD41B (CD137). Together, these intracellular domains drive T-cell activation after antigen engagement, promoting vigorous proliferation, sustained issuance of cytokines like IL-2, enhanced cytotoxic activi-

ty, CAR-modified T cell long-term viability in vivo [1]. Through this modular organization, the CAR-T constructs simultaneously achieve targeted specificity and effective immune activation, thereby shaping its therapeutic performance and clinical outcomes [1]. After genetic modification and expansion in vitro, CAR-T cells are infused into patients, triggering a strong and lasting anti-tumor response. A subset develops memory phenotypes, supporting long-term immune surveillance and reducing the chance of tumor recurrence.

2.2 CAR-T Clinical Uses in Cancer Treatment

2.2.1 Hematological Malignancies

CAR-T therapies have significantly enhanced treatment options for hematologic cancers like ALL and B-cell lymphomas, showing high clinical efficacy due to clear antigen targets and supportive immune environments, which promote effective CAR-T infiltration, prolonged activity, and reliable therapeutic outcomes[5] [6].

2.2.2 Solid Tumors

By contrast, a number of obstacles make it difficult to deploy CAR-T techniques to solid tumors, like suppressive tumor milieus, heterogeneous antigen expression, insufficient infiltration, and functional exhaustion of T cells [7]. Within these hostile environments, factors including TGF-β, IL-10, and tumor-associated macrophages substantially dampen CAR-T activity by restricting activation and penetration [8]. In addition, the architectural complexity and disrupted signaling networks of tumors like pancreatic and liver cancers further limit effective infiltration. Antigenic diversity contributes to inconsistent target recognition, thereby weakening therapeutic outcomes [9]. Moreover, utilizing checkpoint pathways (PD-1/PD-L1, for example) to inhibit CAR-T activity and encourage immune evasion, malignant cells [1].

3. CRISPR-Cas9–Enhanced CAR-T Therapy

RNA-guided CRISPR-Cas9 machinery serves as a programmable genome-editing toolkit to enhance CAR-T efficacy. By leveraging RNA-guided DNA cleavage via the Cas9 enzyme, CRISPR allows targeted genomic editing (knockouts or insertions) to optimize CAR-T function and overcome solid tumor-associated barriers [10].

3.1 CRISPR-Cas9: Mechanism and Genome Editing Principle

Originally discovered in bacteria as a natural defense against viral DNA, short guide RNAs are used by the CRISPR-Cas9 machinery to lead Cas9 to complementary genomic regions, where double-strand breaks are induced. Non-homologous end joining (NHEJ) and homology-directed repair (HDR), As internal cell repair mechanisms, subsequently fix these DNA double-strand breaks, enabling site-specific genomic modifications [11].

This process, by precisely targeting specific locations in the genome, allows scientists to alter genetic information with remarkable accuracy. By either joining the broken DNA ends or using a template to guide the repair, cells can either introduce or correct genetic sequences. The efficiency of this repair process is critical, as it determines whether the desired genetic change will be successfully incorporated. These advancements are vital in applications such as gene therapy, where introducing specific mutations or correcting defective genes can have therapeutic benefits.

3.2 Strategic Applications in CAR-T Engineering

3.2.1 Immune Checkpoint Removal

A major challenge in solid tumor immunotherapy is the immune evasion mediated by inhibitory checkpoint pathways. Tumors exploit receptors such as PD-1 and CTLA-4 to suppress the T-cell responses, promoting immune tolerance and therapeutic resistance [12]. When these checkpoints are engaged by ligands like PD-L1 or CD80/CD86 in the tumor microenvironment, T cells become exhausted, reducing their proliferation, cytotoxic function, and survival [13].

CRISPR-Cas9 facilitates the targeted removal of inhibitory elements in CAR-T cells, supporting continuous T-cell activation despite an immunosuppressive environment, as demonstrated in Fig. 2. PD-1 knockout enhances cytokine production (e.g., IFN-γ), prolongs T-cell persistence, and increases tumor infiltration [14]. CTLA-4 deletion further strengthens immune activation. Dual checkpoint deletion has demonstrated synergistic effects in preclinical models of ovarian and pancreatic cancer, leading to significantly improved tumor clearance. Additionally, targeting emerging checkpoints such as TIM-3, LAG-3, and TIGIT using CRISPR is under exploration and may produce next-generation CAR-T therapies resistant to tumor-induced suppression [15][16].

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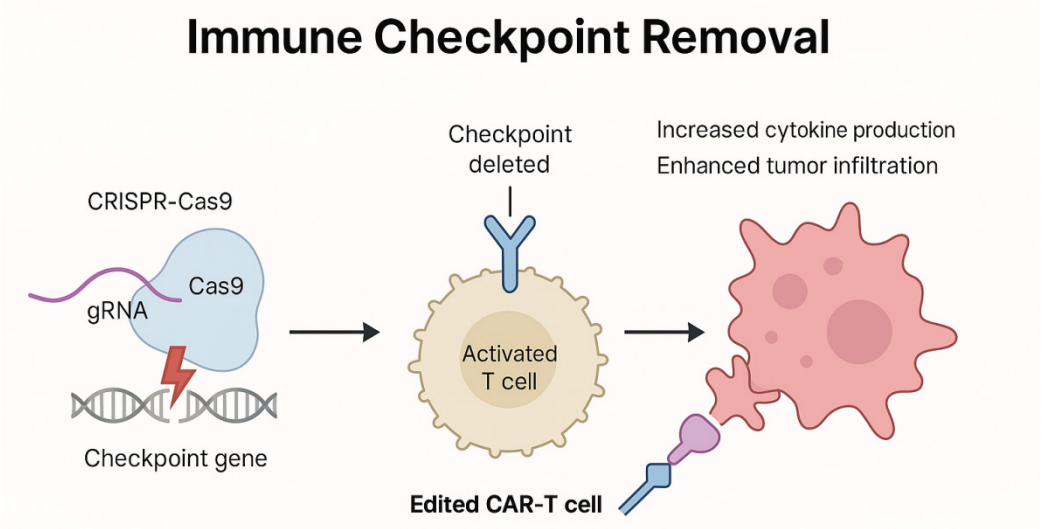

Fig.2 Schematic of CRISPR-Cas9–Facilitated Genome Editing in T Cells Picture credit:Original

3.2.2 Cytokine Support Integration

CRISPR-Cas9 enables precise insertion of cytokine genes such as pro-inflammatory IL-12 and homeostatic IL-15 into CAR-engineered T cell constructs, thereby enhancing therapeutic potency. IL-12 strengthens communication between engineered T lymphocytes and innate immune effectors(e.g., NK cells, DCs) , amplifying the overall antitumor effect. IL-15 supports proliferation, survival, and the formation of memory phenotypes critical for durable tumor surveillance [17][18]. Preclinical evidence shows that CAR-T cells engineered to Emit IL-12 exhibit superior infiltration and reduced exhaustion in hostile solid tumor niches. Additionally, the secretion of IL-15 enhances CAR-T cell survival, further improving Therapeutic outcomes.

3.2.3 Metabolic Reprogramming

Solid tumor microenvironments are often characterized by hypoxia, nutrient scarcity, and acidic pH, all of which impair CAR-T cell survival and function. By using CRISPR-Cas9 to edit genes involved in glucose uptake, fatty acid oxidation, and amino acid metabolism, CAR-T cells can be reprogrammed to better utilize available nutrients under stress conditions. For instance, enhancing glycolytic and oxidative phosphorylation pathways can boost energy production, while modifying lipid metabolism may improve membrane flexibility and motilit [19]. Additionally, reprogramming amino acid metabolism, such as arginine and glutamine utilization, supports sustained proliferation and effector function. Preclinical studies indicate that metabolically enhanced CAR-T products exhibit greater tumor penetration, reduced susceptibility to exhaustion, and elevated cytotoxic activity within nutrient-deprived solid tumor microenvironments [20].

3.2.4 Development of Universal CAR-T Cells

To overcome donor–recipient compatibility issues, CRISPR can be applied to interfere with modified T cells' natural expression of TCR and HLA. The production of universal allogeneic CAR-T products is made possible by such alterations, lowering the risk of immune rejection and supporting the development of readily available, off-the-shelf therapies [21][22].

4. Conclusion

Despite the fact that CAR-T treatment has changed the way that hematologic malignancies are treated, extending its use to solid tumors is still an emerging challenge. Integrating CRISPR-Cas9 technology addresses key limitations by enhancing CAR-T cell precision, persistence, and adaptability. Strategic genome editing, including checkpoint deletion, cytokine insertion, and metabolic reprogramming, significantly boosts therapeutic potential.

As clinical trials continue to evaluate safety and efficacy, ongoing research should focus on refining editing accuracy, minimizing off-target effects, and ensuring scalable manufacturing. These advancements will be crucial in ensuring that the advantages of CAR-T therapy are accessible to a broader patient group. Moreover, it is essential to improve the cost-efficiency of these therapies to make them viable for routine clinical use. By overcoming these challenges, CAR-T and CRISPR technologies together represent a powerful, complementary approach, capable of reshaping cancer immunotherapy. The future of cancer treatment is found in harnessing the full potential of this

synergistic combination, with the promise of more personalized, effective, and lasting therapeutic options for patients suffering from solid tumors.

References

[1] Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer Journal, 2021, 11: 69.

[2] Zugasti I, Espinosa-Aroca L, Fidyt K, et al. CAR-T cell therapy for cancer: current challenges and future directions. Signal Transduction and Targeted Therapy, 2025, 10: 210.

[3] Ramos CA, Dotti G. Chimeric antigen receptor (CAR)-engineered lymphocytes for cancer therapy. Expert Opinion on Biological Therapy, 2011, 11(7): 855-873.

[4] Zhang C, Liu J, Zhong JF, Zhang X. Engineering CAR-T cells. Biomarker Research, 2017, 5: 22.

[5] Zhou Delian, Zhu Xiaojian, Xiao Yi. CAR-T cell combination therapies in hematologic malignancies. Experimental Hematology & Oncology, 2024, 13: 69.

[6] Junru Lu,Guan Jiang. The journey of CAR-T therapy in hematological malignancies. Molecular Cancer, 2022, 21: 194.

[7] Tu, Z., Chen, Y., Zhang, Z. et al. Barriers and solutions for CAR-T therapy in solid tumors. Cancer Gene Ther (2025).

[8] Ting Yan, Lingfeng Zhu, Jin Chen. Current advances and challenges in CAR T-Cell therapy for solid tumors: tumor-associated antigens and the tumor microenvironment. Experimental Hematology & Oncology, 2023, 12: 14.

[9] Muhammadul-Awwal Irodatullah Bisola, Ikponmwosa Jude Ogieuhi, Victor Oluwatomiwa Ajekigbe, et al. Next-generation cancer treatment: exploring the tumor microenvironment and CAR-T cell therapy potential. Discover Medicine, 2025, 2: 113.

[10] Alexander Dimitri, Friederike Herbst, Joseph A. Fraietta. Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Molecular Cancer, 2022, 21: 78.

[11] Jinek M, Chylinski K, Fonfara I, et al. A programmable dualRNA–guided DNA endonuclease in adaptive bacterial immunity. Science, 2012, 337(6096): 816–821.

[12] Xu Liangliang, Zou Chang, Zhang Shanshan, et al. Reshaping the systemic tumor immune environment (STIE) and tumor immune microenvironment (TIME) to enhance immunotherapy efficacy in solid tumors. Journal of Hematology & Oncology, 2022, 15: 87.

[13] Vandenborre K, Van Gool S W, Kasran A, et al. Interaction of CTLA-4 (CD152) with CD80 or CD86 inhibits human T-cell activation. J Immunol, 1999, 163(3): 1646-1653.

[14] Wei W, Chen ZN, Wang K. CRISPR/Cas9: A Powerful Strategy to Improve CAR-T Cell Persistence. Int J Mol Sci, 2023, 24(15): 12317.

[15] Park CH. Making Potent CAR T Cells Using Genetic Engineering and Synergistic Agents. Cancers, 2021, 13(13): 3236.

[16] Cai LT, Li YC, Tan JX, et al. Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy. J Hematol Oncol, 2023, 16: 101.

[17] Yang Z, Pietrobon V, Bobbin M, et al. Nanoscale, antigen encounter-dependent, IL-12 delivery by CAR T cells plus PD-L1 blockade for cancer treatment. J Transl Med, 2023, 21: 158.

[18] Burga RA, Aksoy BA, Ao Z, et al. IL-2-independent expansion, persistence, and antitumor activity in TIL expressing regulatable membrane-bound IL-15. Mol Ther. 2025.

[19] Li WS, Pan XX, Chen LR, et al. Cell metabolism-based optimization strategy of CAR-T cell function in cancer therapy. Front Immunol, 2023, 14: 1186383.

[20] Chen J, Zhao LF, Li WY, et al. Glutamine-driven metabolic reprogramming promotes CAR-T cell function through mTOR-SREBP2 mediated HMGCS1 upregulation in ovarian cancer. J Transl Med, 2025, 23: 803.

[21] Li WL, Zhu XX, Xu YM, et al. Simultaneous editing of TCR, HLA-I/II and HLA-E resulted in enhanced universal CAR-T resistance to allo-rejection. Front Immunol, 2022, 13: 1052717.

[22] Lee J, Sheen JH, Lim O, et al. Abrogation of HLA surface expression using CRISPR/Cas9 genome editing: a step toward universal T cell therapy. Sci Rep, 2020, 10: 17753.