

CRISPR/Cas9-Mediated PD-1 Engineered T Cells: New Strategy to Block Tumor Immune Escape

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Abstract. At present, overexpression of immune-related genes is one of the key mechanisms of tumor immune evasion. As a tumor cell-mediated immune escape core checkpoint molecule, the PD-L1 (programmed death ligand 1) will recruit SHP-1/SHP-2 (Src homology phosphotyrosyl phosphatase 1/ Src homology phosphotyrosyl phosphatase 2) phosphatase by interacting with PD-1 (Programmed Death 1) on T cells' surface, dephosphorylation of key molecules of the downstream signaling pathway of T cell receptors (TCR) (such as CD3 ζ , ZAP70, PI3K/Akt), thereby inhibiting T cell expansion, cytokine secretion, cytotoxic particle release, and metabolic reprogramming. Eventually, it leads to T cell failure or apoptosis and directly inhibiting the anti-tumor function of T cells. Today, existing immune checkpoint blockade still has some problems during treatment, such as some patients do not respond or lose their efficacy due to drug resistance. According to the basic principles of PD-1 immune escape and the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9) system, this paper explores the mechanism and advantages and disadvantages of the CRISPR/Cas9 technique in PD-1 expression inhibition combined with existing research, so as to provide a theoretical basis, summarize the shortcomings and future prospects for tumor immunotherapy Page layout.

Keywords: PD-L1, PD-1, CRISPR/Cas9, tumor immune evasion

1. Introduction

Traditional tumor treatments (like radiotherapy and chemotherapy) still have significant side effects and potential genetic mutation risks, so it is important to explore and study new treatment strategies. At present, immunotherapy, as an important method of cancer treatment, has made significant progress in recent years, especially immune checkpoint inhibitors targeting PD-1 (Programmed Death 1), blocking PD-1/PD-L1 (Programmed Death Ligand 1), reversing T cell depletion, and restoring its tumor-killing ability [1].

However, existing immune checkpoint inhibitors still have some problems during treatment, such as non-response in some patients or loss of efficacy due to drug resistance [1]. This makes it an urgent need to develop new strategies to enhance PD-1 inhibition. After the breakthrough of the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9) system, it offers an effective tool to boost the inherent ability of human T cells to combat cancer. The latest clinical trials confirm that by precisely editing the T-cell genome and transporting engineered T cells into patients, engraftment can be achieved to enhance their antitumor ability [2].

This article will review the PD-1/PD-L1 and explore an application of CRISPR/Cas9-mediated PD-1 engineered T cells in blocking tumor immune escape, analyze its research progress and challenges, and look forward to future research directions and clinical application potential.

2. PD-1/PD-L1 pathway mechanism

PD-1 acts as an adverse immune regulator that is categorized as the CD28 family of type I transmembrane proteins. PD-1 was obtained and named in apoptotic T cell hybridomas by Ishida et al. In 1992, and is an immunosuppressive receptor mainly localized to the membrane of activated B cells, T cells, and myeloid cells [3]. Its ligands PD-L1 and PD-1 can be localized to a variety of tumor

cells, antigen-presenting cells, and cancer cells, especially when stimulated by inflammatory factors such as interferon- γ [4].

When PD-1 binds to PD-L1 on the surface of tumor or antigen-presenting cells, SHP-1/SHP-2 phosphatase is recruited to dephosphorylate key molecules of the T cell receptor (TCR) downstream signaling pathway (eg. CD3 ζ , ZAP70, PI3K/Akt), thereby inhibiting T cell expansion, cytokine (eg. IL-2, IFN- γ , TNF- α) secretion, cytotoxic particle release, and metabolic reprogramming, ultimately leading to T cell failure or apoptosis [5].

Tumor cells use this physiological negative feedback regulatory mechanism to express PD-L1 in addition to actively inhibiting the attack of tumor-specific T cells, thereby achieving immune escape. Therefore, PD-1/PD-L1 is widely recognized as a key target for tumor immunotherapy [6].

3. CRISPR/Cas9 technology and advantages

CRISPR/Cas9 system evolved from the bacterial adaptive immunity [6]. This technology is currently widely applied in various clinical fields. CRISPR/Cas9 system consists of guide RNA (sgRNA) and Cas9 endonuclease. The sgRNA targets specific genomic DNA sites through approximately 20 base sequences (spacers) at its 5' terminus, guiding the Cas9 to undergo DNA DSBs (double-strand breaks) near the PAM (Protospacer Adjacent Motif) sequence. Cells primarily repair DSBs through the activity of prone to errors NHEJs (non-homologous end junctions), resulting in insertion or deletion of small fragments at target sites, resulting in gene knockout [7]. According to research findings, the production of engineered T cells using this technique not only improves the tumor immune microenvironment but also overcomes chemotherapy resistance in tumors [7].

A key step in building engineered T cells is the formation and delivery of CRISPR-Cas9 RNP complexes. Using S.p. HiFi Cas9 Nuclease V3, single guide RNAs, and electroporation enhancer from IDT's Alt-R CRISPR-Cas9 catalog, Chamberlain CA et al generated engineered T cells [8]. CRISPR/Cas9 has the strengths of simple design, low cost, and multiple editing.

Efficient and safe delivery is key to technology. Currently, electroporation delivery of RNP is the mainstream method due to its high editing efficiency and short duration of Cas9 protein presence in cells, which can decrease the off-target risk and immunogenicity [8]. According to the study, three patients with treatment-resistant advanced cancer received deliveries of engineered T cells. The infusion displayed a favorable tolerability profile, with no serious adverse events, and the engineered T cells endured for up to 9 months, reflecting negligible immunogenicity in this setting and confirming that CRISPR-based gene editing is feasible in cancer immunotherapy [2].

4. Functional benefits of PD-1 engineered T cells

PD-1 engineered T cells demonstrate sustained antineoplastic activity by disrupting the PD-1/PD-L1 axis, thereby effectively relieving immunosuppressive state imposed on effector T cells in the tumor microenvironment. This disruption restores T cell receptor (TCR)-mediated proliferation and cytotoxic function, while also promoting the establishment of long-term immune memory responses, which are critical for preventing tumor recurrence and metastasis. Furthermore, these engineered cells demonstrate enhanced adaptability to the tumor microenvironment, characterized by the reversal of T cell dysfunctional phenotypes and improved infiltration capacity into solid tumors [9].

In chimeric antigen receptor (CAR)-T cell therapy, CRISPR/Cas9-mediated PD-1 deletion has been demonstrated to substantially enhance the secretory profile and multifunctionality of CAR-T cells in vitro, leading to superior antitumor efficacy in vivo. Transcriptomic analyses further reveal that PD-1 editing leads to the downregulation of gene expression profiles associated with T cell dysfunction, suggesting a reprogramming toward a more persistent and functional state. Additionally, when compared to unedited counterparts, PD-1-edited tumor-infiltrating lymphocyte (TIL)-derived T cells possess a markedly enhanced capacity for autologous tumor cell elimination. This improved efficacy is accompanied by the retention of a greater percentage of tumor-specific T cell receptor

(TCR) clones, underscoring the potential of PD-1 editing to preserve and amplify pre-existing anti-tumor immunity [9].

5. Clinical Research and Advances

Based on current research, the field has seen major strides in both animal experiments and human trials. This engineered T cell has the opportunity to be an excellent means of clinical treatment.

5.1. Knockout of PD-1 enhances T cell antitumor activity

According to recent studies, PD-1-deficient T cells undergo multiple steady-state proliferations when transplanted into lymphopenic mice. In lymphoproliferative mice receiving PD-1-deficient T cell infusion, the quantity of IFN- γ CTLs (Cytotoxic T Lymphocytes) was markedly increased, and tumor progression was significantly suppressed. PD-L1 expression was significantly elevated on tumor cells of lymphorepenic mice that underwent steady-state proliferation. Disruption of PD-L1 in B16 melanoma cells strengthens T cell-mediated antitumor immunity during steady-state proliferation and significantly restricts tumor progression [10]. In preclinical model studies of glioblastoma, CRISPR-Cas9 served to eliminate endogenous T cell receptors, β -2 microglobulin, and PD-1 to generate allogeneic CAR T cells with endurance to PD-1 inhibition. CAR-T cells with three gene edits show enhanced antitumor activity in glioma preclinical studies. Although intravenous injection was not achieved, it was observed that gene-edited EGFRvIII (Epidermal Growth Factor Receptor variant III) CAR T cells enhanced their antitumor efficacy by targeting PD-1 in the preclinical model of GBM, verifying the feasibility of PD-1 disruption using the CRISPR/Cas9 system to strengthen the antitumor functionality of T cells [11].

5.2. Treatment with favorable tolerability and facile editing

Among those suffering from advanced-stage non-small cell lung carcinoma, the initial phase of a phase I clinical trial involving CRISPR/Cas9-mediated PD-1 engineered T cells, peripheral blood analysis detected edited T cells throughout the treatment period and post-treatment. Side effects were limited to grade 1 or 2 in all patients receiving PD-1-edited T cells, indicating favorable tolerability. The clinical use of CRISPR-Cas9 PD-1 engineered T cell therapy has been validated with an acceptable safety profile and feasibility [12]. At the same time, Chamberlain et al. showed that CRISPR-Cas9 technology is seamlessly and successfully incorporated into TIL-based ACT protocols. By employing a simple editing strategy, they achieved substantial PD-1 knockout in the final product without observable off-target effects, facilitating the adoption of techniques to improve TIL-based ACT [8].

5.3. Current challenges and future prospects

Due to the current insufficient number of clinical samples, off-target effects remain a potential risk, and existing detection methods still have limitations. Although clinical human samples have been utilized in certain trials, individual variations in gene editing efficiency persist. While experiments with animal models have demonstrated the technology's feasibility, its application in humans remains an unresolved challenge. Delivery methods like electroporation are affected by individual differences in survival rates, limiting the technology's current use to small-scale trials rather than large-scale clinical applications. In terms of manufacturing processes, reducing costs and simplifying operations to enable clinical adoption remain critical issues to address. Current research indicates that existing clinical treatments predominantly employ combination therapies with demonstrated efficacy improvements. The combination of gene editing and immune checkpoint inhibitors further enhances tumor immune responses by relieving inhibitory signals and blocking immune evasion, providing valuable research directions for clinical development. CRISPR/Cas9-engineered PD-1 T cells are poised to become a novel strategy in tumor immunotherapy.

6. Conclusion

This study integrates the latest clinical trial data and conclusions, focusing on clinical trials and gene editing techniques. Through a systematic review of the mechanism of action of PD-1/PD-L1 and CRISPR/Cas9 in the anti-tumor process of T cells, combined with recent clinical research analysis, it is confirmed that this technology has significant therapeutic effects and does not cause serious adverse reactions, showing good clinical application prospects. The study verifies the feasibility of this strategy and provides research directions for clinical treatment options. As an innovative strategy to engineer immune cells to combat tumor immunosuppression using gene editing technology, CRISPR/Cas9-mediated PD-1 engineered T cells combine precision gene editing precision with the potent cytotoxic potential of adoptive cell therapy. This method opens up a new way to break through the existing immunotherapy bottleneck and overcome the treatment problems of solid malignancies.

Existing studies and clinical trial results show that most mouse experiments are based on specific conditions (such as relatively loose tumor tissue) and fail to solve the challenges faced by multi-tumor therapy, and the sample size of clinical studies is still insufficient. At the same time, in human research, the editing efficiency and standardization problems caused by individual differences need to be solved urgently. However, with the advancement of technology, the deepening of academic awareness, and the accumulation of clinical experience, this strategy is expected to become a key component of future tumor immunotherapy, bringing hope to patients.

This paper outlines future research directions, focusing on the development of safer, more efficient, and intelligent multigene editing strategies. It is recommended to explore synergies with other treatment modalities to ultimately achieve precise, durable, and controlled anti-tumor immunotherapy. Current clinical research should prioritize solving individual differences in sample collection, simplifying operation processes, eliminating cost barriers, and maximizing the transformation of experimental results into clinical applications.

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