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Current Challenges and Future Prospects of TCR-T Cell Therapy in Cancer Treatment

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Abstract: T cell receptor-engineered T cell (TCR-T) therapy has emerged as a promising approach in cancer immunotherapy, with applications in both hematologic malignancies and solid tumors, by targeting tumor-specific antigens. However, several challenges hinder its widespread practical application. First, the complexity and specificity of tumor antigen selection limit treatment efficacy. Second, challenges in optimizing TCR affinity and overcoming tumor-induced immunosuppression limit the further refinement of therapeutic strategies. Third, the clinical application of current TCR-T products is constrained by limited patient response rates, potential safety concerns, and high manufacturing costs. Recent research progress includes advancements in high-throughput antigen screening technologies, such as single-cell RNA sequencing and peptide-MHC multimer platforms, which have improved the identification of neoantigens and TCRs with high affinity and specificity. Additionally, innovative strategies like combination therapies with immune checkpoint inhibitors (e.g., PD-1 blockers) and STING agonists have shown potential to enhance TCR-T cell efficacy and persistence in the tumor microenvironment. Future breakthroughs may involve the development of universal TCR-T platforms and gene-editing technologies (e.g., CRISPR/Cas9) to reduce costs and improve scalability. In conclusion, while TCR-T therapy faces significant challenges, its potential to revolutionize cancer treatment is undeniable. Addressing issues such as tumor heterogeneity, immune evasion, and manufacturing efficiency will be critical for its broader clinical adoption.

Keywords: TCR-T cell therapy, tumor target antigens, killing mechanism, clinical application, drug approval

1. Introduction

TCR-T cell therapy, has shown promising potential in treating malignant tumors. This approach involves genetically engineering T cells to express specific T cell receptors (TCRs) that recognize tumor antigen peptides presented by major histocompatibility complex (MHC) molecules, activating an anti-tumor immune response [1]. Advances in immunology and genetic engineering have driven its development from targeting shared tumor antigens to neoantigens, which arise from tumor-specific mutations. Given their high individual specificity, TCR-T cell therapies targeting neoantigens offer a more precise and effective treatment option [2]. Unlike chemotherapy and radiotherapy, this therapy enhances immune targeting, minimizing damage to normal cells and reducing side effects [3]. Additionally, it can recognize various tumor-specific and tumor-associated antigens, making it applicable to multiple malignancies [4]. However, several challenges hinder its

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widespread clinical use. The process of screening and cloning TCRs for neoantigens is complex and time-consuming. The tumor microenvironment, characterized by immunosuppressive factors like TGF-β and PD-L1, weakens TCR-T efficiency [5]. Moreover, optimizing TCR specificity and affinity is crucial, as inappropriate TCRs may cause off-target effects and severe side effects [3].

This study aims to address several key research questions in TCR-T therapy, including how tumor microenvironmental factors suppress TCR-T function, efficient methods for improving neoantigen screening to enhance specificity and coverage of tumor heterogeneity, strategies for balancing TCR affinity and specificity to reduce off-target toxicity, and clinical translation barriers related to reducing preparation time and costs for broader adoption. This study has significant theoretical and practical implications. Elucidating the mechanisms of TCR-T cell interactions with the tumor microenvironment can provide new targets for optimizing T cell function [3, 5]. Clinically, advancements in high-throughput antigen screening platforms and universal TCR-T technologies can improve response rates for solid tumor treatments and extend patient survival [1, 6]. Industrially, establishing standardized production systems with combination therapy strategies can accelerate the translation from the laboratory to the clinic application [2, 4].

2. Challenges and key technologies of TCR-T therapy

2.1. Clinical challenges and key constraints of TCR-T cell therapy

TCR-T cell therapy has shown great potential in targeting specific tumor antigens with high selectivity. However, it still faces many challenges in clinical application that affect its efficacy and widespread adoption. The tumor microenvironment (TME) is a critical determinant of TCR-T cell therapy's efficacy, persistence, and functional stability. The TME, which consists of tumor cells, stromal cells, immune cells, and extracellular matrix, creates an immunosuppressive milieu that hampers the function and persistence of TCR-T cells. Studies have shown that high levels of inhibitory cytokines (e.g., TGF-β and IL-10) suppress T cell activation and proliferation, indirectly impairing TCR-T cell function [7]. Hypoxia in the TME induces metabolic reprogramming in T cells, shifting them from oxidative phosphorylation to glycolysis, which suppresses their proliferation and cytotoxicity [8]. This metabolic shift is mediated by hypoxia-inducible factors (HIFs), which upregulate PD-L1 expression on tumor cells and further inhibit T cell activity. Moreover, tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) further inhibit the proliferation and effector functions of TCR-T cells by secreting various growth factors and cytokines [6]. Additionally, the hypoxic environment and acidic pH in the TME negatively impact the survival and effector functions of TCR-T cells. Hypoxia induces metabolic reprogramming in T cells, suppressing their proliferation and cytotoxicity [8]. To enhance the efficacy of TCR-T cells, combination with immune checkpoint inhibitors or TME-modulating drugs has become an important intervention strategy.

Secondly, tumor cells evade immune surveillance through various mechanisms, directly affecting the efficacy of TCR-T cell therapy. First, tumor cells can downregulate the expression of major MHC Class I molecules, weakening the recognition ability of TCR-T cells [3]. Secondly, tumor cells can secrete immunosuppressive factors (e.g., PD-L1), which bind to PD-1 on T cells, inhibiting the activation of TCR-T cells and leading to functional loss [1]. Moreover, tumor heterogeneity exacerbates immune evasion. Tumor cells may undergo mutations, epigenetic modications, or antigenic variation, leading to the loss or downregulation of specific antigens and enabling escape from TCR-T cell recognition and attack [4]. Current research directions include developing TCR-T cells targeting multiple antigens and combining them with other immunotherapies to overcome immune evasion mechanisms.

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Thirdly, the process of manufacturing TCR-T cells remains complex and time-consuming, limiting its widespread clinical application. Personalized therapy requires isolating patient-derived T cells, expanding them in vitro, and genetically engineering them before reinfusion, a process that typically takes weeks to months [2]. During this period, tumor evolution may lead to antigen loss, microenvironmental changes, or therapy resistance, reducing treatment efficacy. Screening for the specificity and affinity of TCRs is crucial to avoid off-target toxicity to normal cells. However, current technologies still face challenges in screening effective TCRs, especially for neoantigens [5]. Moreover, quality control and standardization in the production process are also difficulties in clinical promotion; any inconsistency in batches may affect the safety and efficacy of treatment. Finally, the high cost of personalized treatment limits the application of TCR-T cell therapy. Future research can explore more efficient automated production platforms and synthetic biology methods to reduce costs and improve production efficiency [6].

2.2. Tumor target antigen screening in TCR-T cell therapy

Tumor antigens are proteins or molecules that are preferentially or uniquely expressed on tumor cells and can trigger an immune response. Based on their origin and expression characteristics, tumor antigens are classified into three main types: tumor-specific antigens (TSAs), tumor-associated antigens (TAAs), and neoantigens. TSAs are exclusively expressed on tumor cells and not on normal cells, usually generated by tumor-specific mutations. TAAs, such as carcinoembryonic antigen (CEA) and MUC1, are present on both normal and tumor cells but are overexpressed in malignancies. Neoantigens, generated by tumor-specific mutations, are recognized as foreign by the immune system and presented to T cells via major histocompatibility complex (MHC) molecules, thereby activating specific immune responses [1]. The precise selection of tumor antigens is crucial for enhancing the effectiveness of TCR-T cell therapy. Recent advancements in antigen screening have significantly improved TCR-T therapy development. Traditional approaches rely on genomic sequencing and proteomics to compare gene expression profiles between tumor and normal cells. High-throughput sequencing technologies now allow for rapid mutation identification and neoantigen prediction. Moreover, synthetic biology and immune repertoire analysis enable the screening of T cell receptors (TCRs) with high affinity and specificity for clinical application [2]. For example, single-cell RNA sequencing provides in-depth insights into tumor-infiltrating T cells, facilitating the identification of TCRs with superior tumor antigen recognition capabilities [3]. These innovations enhance the potential of personalized TCR-T cell therapy.

Despite these advances, several challenges remain. First, tumor heterogeneity complicates the identification of universal antigen targets, especially in solid tumors where mutation profiles vary significantly among patients [4]. Second, the immunosuppressive tumor microenvironment can also affect the efficacy of TCR-T cells, inhibiting their recognition and attack of tumor antigens [5]. The affinity and specificity of TCRs are also key factors affecting treatment efficacy. Low-affinity TCRs may fail to effectively recognize tumor cells, while high-affinity TCRs may cause non-specific toxic reactions [9]. Finally, the time-sensitive nature of personalized treatment necessitates further optimization of antigen screening technologies to improve efficiency and accuracy [10].

3. Killing mechanism of TCR-T cells

The killing mechanism of TCR-T cells depends on antigen recognition, T cell activation, proliferation, and cytokine-medicated tumor elimination. TCRs play a central role in recognizing tumor antigens presented by MHC molecules on tumor cells. The specificity and affinity of TCRs determine their ability to distinguish between malignant and normal cells. Genetic engineering techniques have been employed to optimize TCR affinity, thereby enhancing tumor recognition and improving therapeutic

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outcomes [3]. However, achieving an optimal TCR affinity range is essential, as overly high-affinity TCRs may lead to off-target effects and autoimmunity [1]. Current TCR-T cell therapies still face challenges in selecting appropriate tumor antigens and TCRs, especially in the treatment of solid tumors [2]. T cell activation is a complex process involving multiple signaling pathways. The core steps are as follows: First, the binding of TCR to the antigen-MHC complex is the initial signal for T cell activation. This binding not only activates the TCR but also triggers a series of downstream signaling pathways, including the MAPK and NF-κB signaling pathways, which are crucial for T cell proliferation and differentiation [5]. After T cell activation, the cells begin to proliferate, producing a large number of effector T cells to enhance the attack on tumor cells. Additionally, cytokines play an important role in this process; for example, IL-2 is a key factor in promoting T cell proliferation [4]. However, in the tumor microenvironment, the overexpression of inhibitory factors and cytokines can lead to T cell functional suppression, thereby affecting their proliferative capacity [3].

Cytokines play a key role in the anti-tumor effects of TCR-T cells by regulating T cell activity and promoting tumor cell apoptosis. The following are the functions of several major cytokines: Cytokines such as IFN-γ and TNF-α can enhance the cytotoxicity of T cells and promote tumor cell apoptosis [9]. Moreover, cytokines can also regulate the tumor microenvironment to enhance T cell infiltration and activity, thereby improving anti-tumor efficacy [11]. However, the abnormal expression of immunosuppressive factors and cytokines in the tumor microenvironment can lead to decreased T cell function, affecting treatment efficacy [12]. Therefore, understanding the role of cytokines in the killing mechanism of TCR-T cells is important for optimizing T cell therapy.

4. Clinical application and future development of TCR-T therapy

4.1. Approved TCR-T drugs

The approval process for TCR-T therapies is subject to strict regulatory oversight to ensure both safety and efficacy. During the preclinical research stage, researchers assess the safety, biological activity, tumor specificity, and off-target toxicity of TCR-T cells through in vitro experiments and animal models. Tumor specificity is evaluated by testing TCR-T cell reactivity against a panel of normal tissues to ensure minimal cross-reactivity. Off-target toxicity is assessed using computational predictions (e.g., homology modeling) and functional assays (e.g., cytokine release assays) to identify potential adverse interactions with non-tumor cells. During the preclinical research stage, researchers assess the safety, biological activity, tumor specificity, and off-target toxicity of TCR-T cells through in vitro experiments and animal models. Subsequently, clinical trials are conducted in three phases: Phase I focuses on safety and dose escalation, Phase II evaluates preliminary efficacy and optimal dosing, and Phase III validates efficacy, safety, and long-term clinical benefits in a larger patient population [1]. In terms of regulation, the U.S. Food and Drug Administration (FDA) requires sufficient clinical evidence demonstrating safety and efficacy for TCR-T therapies. For serious or life-threatening conditions, expedited pathways such as Breakthrough Therapy Designation, Fast Track, and Accelerated Approval are available to facilitate early patient access [2]. Similarly, the European Medicines Agency (EMA) adheres to a rigorous approval process, emphasizing that clinical trial designs must meet ethical and scientific standards to ensure patient rights [3].

Currently, several TCR-T drugs have been approved by the FDA and EMA for the treatment of cancers such as melanoma, leukemia, and lymphoma. These therapies, designed based on patient-specific tumor antigens, have shown high treatment specificity and efficacy, particularly in patients with relapsed and refractory tumors [4]. Some clinical trial results have shown that certain TCR-T therapies can significantly reduce tumor volume and extend patient survival [13]. However, the efficacy of TCR-T therapy in solid tumors remains limited due to factors such as the immunosuppressive effects of the tumor microenvironment and the persistence of T cells, which

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require further optimization [5]. In terms of safety, TCR-T therapy may cause adverse reactions such as cytokine release syndrome (CRS), neurotoxicity, and infections [6]. In clinical trials, CRS is the most common side effect, typically presenting as fever, fatigue, and muscle pain, and can lead to multi-organ dysfunction in severe cases [11]. Moreover, due to the highly personalized nature of TCR-T therapy, individual differences among patients may lead to different adverse reactions. Therefore, clinicians need to closely monitor patient responses and take timely intervention measures [9]. Although TCR-T therapy shows great promise in cancer treatment, its safety still needs to be further verified in larger clinical trials to ensure long-term patient safety and efficacy [14].

4.2. Future research directions

With the development of TCR-T therapy, innovation in antigen screening technology has become a research focus. Tumor antigen heterogeneity and immune evasion mechanisms pose significant challenges. Tumor cells often downregulate antigen presentation (e.g., MHC class I molecules) or undergo antigen loss mutations to evade immune detection. Additionally, the immunosuppressive TME further limits TCR-T cell efficacy. To address these issues, researchers are exploring high-throughput screening platforms and combination therapies (e.g., with immune checkpoint inhibitors) to enhance antigen recognition and T cell function. New screening technologies, such as high-throughput screening platforms using peptide-MHC multimers and antigen-TCR pairing screening based on single-cell transcriptomics and microfluidic technology, have been proposed and preliminarily applied in clinical research. These technologies can identify TCRs with high affinity and specificity in a short time, thereby improving the targeting and efficacy of treatment [1, 2]. With the advancement of genomics and single-cell transcriptomics, researchers can rapidly identify tumor neoantigens and optimize TCR specificity and affinity using gene-editing technologies such as CRISPR/Cas9 to achieve personalized treatment goals [3, 4]. To enhance the anti-tumor efficacy of TCR-T cells, researchers have proposed several strategies. Several strategies, including small-molecule drugs, cytokine combination therapies, and gene-editing technologies, have shown promising prospects. For example, Cbl-b inhibitors can enhance the proliferation and cytokine secretion of TCR-T cells, thereby improving their anti-tumor activity [15, 16]. Additionally, combining STING agonists with TCR-T cell therapy can significantly enhance antigen presentation by tumor cells, thereby improving the recognition and killing effects of TCR-T cells [17, 18]. The successful implementation of these strategies not only enhances the function of TCR-T cells but also improves their persistence and efficacy in the tumor microenvironment.

Moreover, the standalone application of TCR-T cell therapy may be limited in some cases, making combination with other treatments a research hotspot. Recent studies have shown that combining TCR-T cells with immune checkpoint inhibitors, chemotherapy drugs, and other immunotherapies can significantly improve treatment outcomes. For example, the combination of TCR-T cells with PD-1 inhibitors not only enhances T cell activity but also overcomes the immunosuppressive effects of the tumor microenvironment, thereby increasing patient survival rates [5, 19]. Additionally, researchers are exploring the combination of TCR-T cells with CAR-T cells, hoping to further enhance anti-tumor effects through synergistic mechanisms [20, 21]. This multimodal treatment strategy may become the mainstream direction for tumor immunotherapy in the future. With continuous advancements in TCR engineering, gene editing, and combination immunotherapy strategies, TCR-T cell therapy is expected to play a greater role in cancer treatment, improving patient prognosis and quality of life.

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5. Conclusion

TCR-T therapy is gaining increasing importance in cancer immunotherapy. By genetically engineering patients' own T cells to recognize and attack tumor cells, this therapy has shown great clinical potential. However, its clinical application still faces challenges in terms of safety, efficacy, and personalized treatment. Although the anti-tumor mechanism of TCR-T therapy has been elucidated, there are still differences in the understanding of its mode of action among different studies. Factors such as TCR affinity, signaling pathways, and the tumor microenvironment may all affect treatment efficacy. Therefore, future research needs to integrate a multidisciplinary perspective to comprehensively analyze the mechanisms of TCR-T therapy and optimize treatment strategies. While some TCR-T therapies have shown certain efficacy in clinical trials, how to improve the persistence of TCR-T cells in the tumor microenvironment while reducing side effects remains a key issue. This requires not only in-depth basic research exploration but also continuous optimization of clinical trials to improve the safety and adaptability of the therapy. In the future, the development of TCR-T therapy will focus more on personalized treatment strategies and explore its combination with other immunotherapies (such as immune checkpoint inhibitors and vaccine therapies). Multidisciplinary research is expected to further enhance the clinical value of TCR-T therapy and provide more effective solutions for cancer treatment.

In summary, as an emerging immunotherapy strategy, TCR-T cell therapy, despite facing many challenges, has significant clinical potential that cannot be ignored. By analyzing approved drugs and combining the latest scientific findings, we can not only summarize the current state of this field but also provide important guidance for future research. Only by deeply understanding its mechanisms can we promote the further development of TCR-T cell therapy, improve its clinical application efficacy, and bring hope to more patients.

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