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Current Challenges and Potential Strategies in CAR-T Cell Therapy

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Abstract. CAR-T therapy, involves modifying the patient's immune T cells in a laboratory setting to recognize antigens on tumor cells. Significant progress has been made in the treatment of hematologic malignancies, thanks to advancements in cancer treatment, namely the discovery of immunotherapies. Nevertheless, the therapy encounters obstacles such as antigen evasion, effects that target both tumor and non-tumor cells, restricted penetration into tumors, a tumor microenvironment that suppresses immune response, and adverse effects such as CRS and neurotoxicity. This paper analyzes the complexities of CAR-T treatment, its fundamental principles, and potential uses for many forms of cancer, including solid tumors. The primary objective is to improve the effectiveness and safety of the therapy by the manipulation of CAR-T cells, alteration of CAR structures, and investigation of combined treatment approaches. Although CAR-T therapy shows promise in transforming cancer treatment, additional refinement is necessary to optimize its therapeutic efficacy and decrease undesirable side effects. This will facilitate the development of more efficient and individualized cancer treatments.

Keywords: CAR-T therapy, challenges, potential strategies.

1. Introduction

Nowadays, with the popularization of cancer screening, the emphasis on early diagnosis, and the advancement of medical treatment, the overall survival rate of cancer patients around the world has shown a continuous upward trend. However, malignant neoplasms remain the most serious public health problem worldwide. Immunotherapy is a cutting-edge area of cancer treatment that is significantly changing how the disease is managed. Approved antibodies that regulate the immune system's checkpoints and adoptive cell therapy, such as chimeric antigen receptor (CAR-T) therapy. CAR-T treatment provides the benefit of effectively targeting a diverse array of cancer types and possessing potent capacities to proliferate and eradicate malignancies. However, there are significant challenges such as CRS and on-target off-tumor effects, as well as antigen loss. To address these challenges, efforts are being made to increase antigen specificity, use dual CAR or safety switches, and explore combination therapies. Understanding the various facets of CAR therapy is crucial in order to comprehend its efficacy and potential shortcomings. A primary area of current research is the development of CAR-T cell therapy to tackle these obstacles and enhance efficacy while prioritizing patient safety [1]. This review systematically introduces CAR structure and discusses current challenges and potential strategies in CAR-T therapy.

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2. CAR structure

CARs are protein fusions where the extracellular domain that binds to antigens comes from antibodies and is physically connected to the intracellular domain that signals T cells, containing the costimulatory and CD3-zeta domains. One common approach involves utilizing the scFv derived from an anti-CD19 antibody. This format is being refined to enhance clinical efficacy by adjusting the peptide linker composition and length, as well as selecting the VH and VL sequences [2].

The hinge domain is positioned between the scFv and CAR transmembrane sections, and it serves a vital function in enhancing the binding forces of a scFv-CAR construct. Initially, the hinge region from IgG1 was commonly used, but recent research has focused on altering the hinge length by considering alternatives such as using hinge sequences from other isotypes like IgG2 and modifying potentially flexible areas. It is hypothesized that a longer hinge could improve antigen binding by providing more freedom and flexibility to the scFv-CAR module, leading to more efficient recognition and interaction with target antigens [2-4].

The transmembrane region is essential to ensure that chimeric antigen receptors can tether signals to the intracellular domain and transduce them to activate antitumor immune responses. For CAR engineered cytotoxic T-cells, three transmembrane domains are most commonly used: CD3ζ, CD4, and CD8α. CD3ζ is derived from the TCR complex protein CD3ζ, which allows CAR function, proliferation, and cytokine production. Its side chain has intrinsic activities, whereas CD4 and CD8α are co-receptors of TCR. T-cells require these molecules in the processes of targeting and recognizing pMHC on target cells, delivering activating signals, and deferring inhibitory signals. The CAR-T cells engineered with CD4 or CD8α protein neither produce secretory cytokines nor show proliferation. Therefore, CD3ζ has a more superior transmembrane domain compared to CD4 and CD8α [2-4].

The main focus of CAR engineering has been to optimize CAR constructs, especially the Intracellular signaling domain, in order to enhance effectiveness. The first-generation CARs, featured a CD3ζ or FcRγ signaling domain. Nevertheless, these domains in isolation proved insufficient in generating efficacious and enduring T cell responses, as evidenced by clinical investigations. Subsequent studies have highlighted the significance of co-stimulation in maintaining the presence of CAR-T cells, specifically in CD-19-targeted CAR-T cells. These cells exhibited enhanced production of IL-2 and proliferation when co-stimulatory domains were introduced. As a result, second-generation CARs were created, incorporating a single costimulatory domain in addition to the CD3ζ signaling domain. Examples of these costimulatory domains are CD28 and CD137. It has been demonstrated in various hematological malignancies and is currently being investigated for their efficiency in treating solid tumors [4].

3. Limitations of CAR-T cell therapy

3.1. Antigen escape

The issue of antigen escape is a significant obstacle in the domain of CAR-T treatment. It can give rise to significant obstacles leading to treatment failure, thereby undermining the efficacy of this innovative therapeutic approach. Antigen escape manifests itself through diverse mechanisms, encompassing the downregulation, masking, or shedding of target antigens, in addition to the splicing variation in the target antigen gene. Such intricate tactics employed by cancer cells enable them to evade recognition and subsequent eradication by CAR-T cells, ultimately facilitating the resurgence of the disease. In addition, the intricacy associated with CAR-T cell therapy is exacerbated by the noticeable variability observed in the quantities of antigens expressed within tumors [5].

3.2. On-target off-tumor effects

The precise targeting of CAR-T cells to their intended site, while minimizing any unintended effects on healthy tissues, is a significant safety problem. The activation of CAR-T cells through antigen-specific stimulation leads to the secretion of many cytokines, which results in the destruction of both tumor and non-tumor tissues expressing the specific tumor-associated antigens (TAAs). Additionally, the release

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of inflammatory cytokines and chemokines by the activated CAR-T cells contributes to the effectiveness of tumor elimination. Antigens specific to CAR-T cell designs, which are used as first therapy options for hematologic malignancies, are expected to be expressed in normal tissues as well, leading to off-target damage.

At now, the assurance of safety regarding effects that occur on the intended target but not on healthy tissue depends greatly on the specific design of the chimeric antigen receptor (CAR), which includes the outer binding region and co-stimulation. Targeting off-tumors possessing no distinct phenotypic expression is likely to be reduced. Nude mice, gene knockout, small molecule inhibitors, and suicide genes are also being employed to alleviate on-target off-tumor effects. However, these strategies also have limitations despite current preclinical or clinical evaluation [6].

3.3. CAR-T cell trafficking and tumor infiltration

The effectiveness of CAR-T cell therapy is directly related to the efficient ability and rapidity of T cell trafficking and migration to, inside, and ultimately infiltrating the tumor. There is a correlation between a low level of tumor infiltration by given T cells and a negative outcome. An noted drawback of CAR-T cell therapy, in comparison to allogeneic HSCT-based therapy, is that even with local tumor injection, CAR-T cells often exhibit limited or insufficient penetration into tumors over a lengthy period of time. Therefore, the local expansion, longevity, and effectiveness of the CAR-T cells are consequently impacted. The CAR alone may not direct the cells to the solid tumor, limit immune cytotoxicity, or decrease gene delivery at the tumor masses. Modifying the system which can achieve better T cell trafficking and activation is required [7].

3.4. Immunosuppressive microenvironment

TME is a complex ecology consisting of several types of immune cells. Within the group of immune cells, there exist regulatory T cells, also referred to as Tregs, that have the function of inhibiting immunological responses. MDSCs are a specific group of immune cells present in the tumor microenvironment that also play a role in immune suppression in this setting. Tumor-associated macrophages, or TAMs, are a type of immune cell that can further exacerbate the suppressive nature of the microenvironment.

In addition to these immune cells, there are immunosuppressive cytokines and metabolites that add another layer of complexity to the TME. These chemicals possess the capacity to impede the action of immune cells, so further obstructing their regular functioning. The immune suppression occurring inside the tumor microenvironment is a substantial obstacle to the efficacy of CAR-T cell treatment [8].

3.5. CAR-T cell-associated toxicities

CRS is an intricate syndrome resulting from substantial variations in the levels of abundant proinflammatory cytokines. Cytokines are swiftly discharged by transplanted CAR-T cells and other types of effector immune cells, which persistently assault tumor targets. CRS typically manifests between four to fourteen days after CAR-T cell infusion. It is characterized by a range of symptoms such as cytopenia (reduced blood cell counts), coagulation disorders, cyanosis (bluish discoloration of the skin), dyspnea (difficulty breathing), and hypoxia (low oxygen levels in tissues). In severe cases, CRS can escalate to Grade 4 or 5, posing a significant risk of mortality.

In addition to CRS, another complexity linked to CAR-T cell therapy is neurotoxicity. Neurotoxicity is a result of systemic inflammation triggered by the excessive secretion of cytokines, which can potentially cause damage to the brain. Interestingly, neurologic deficits may manifest earlier than CRS and may even occur with a modest increase in cytokine levels. The symptoms of ICANS encompass a broad range of neurological impairments, including delirium, confusion, seizures, aphasia (difficulty speaking or understanding language), obtundation (altered level of consciousness), and encephalopathy (brain dysfunction). These manifestations require close monitoring and timely intervention to ensure the best possible clinical outcomes [9].

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4. Strategies to ameliorate toxicities in CAR-T cell therapy

4.1. Engineering CAR-T cells to ameliorate toxicity

Various strategies have been tested to provide T-cells with the capacity to ameliorate toxicity. Such strategies include the use of cell-intrinsic suicide genes, such as inducible caspase 9 or the fusion of human thymidylate kinase in combination with an anti-herpes drug, sensitizing CAR T-cells to apoptosis. In addition to suicide switches, the activation of negative regulatory receptors like PD-1 or CTLA-4 could be used as potential methods to manage unregulated T-cell responses. Furthermore, besides modifying the functional activity of T-cells in their original location, the harmful effects can be reduced by improving the uncontrolled release of cytokines, particularly when combined with mechanisms that provide negative feedback control. Finally, the use of CRISPR/Cas9 engineering may serve as an efficient and relatively unbiased approach to overcome potential negative immune responses.

While contemporary genetic engineering methods have provided greater adaptability in altering the structure of CAR-T cell products, it is crucial to tackle several concerns in order to optimize the effectiveness and safety of CAR-T cell therapy. Significant endeavors have been focused on enhancing the efficiency of the constituents of CAR molecules. The integration of these alterations can produce novel generation Chimeric Antigen Receptor (CAR) constructs that enhance the longevity and efficacy of CAR-T cells [10].

4.2. Altering CAR structure to ameliorate toxicity

CAR structures derived from mice can cause toxicity in humans, but using human or humanized scFvs in CAR-T therapy may reduce immunogenic reactions. Studies on humanized CD19 CAR-Ts showed superior tumor cell lysis, decreased immunogenicity, and increased persistence compared to CAR-Ts with murine scFvs. Moreover, the selection of the hinge region and transmembrane domain in CAR architectures can impact the way they are expressed and stable. Clinical studies have shown that humanized CAR-Ts can reduce cytokine levels and neurotoxicity. Additionally, the choice of costimulatory domains, can impact the differentiation of CAR-Ts. The CD28 domain may lead to a rapid initial response but subsequent exhaustion, while the 4-1BB domain may provide longer persistence and lower toxicity. The selection of the optimal CAR structure depends on the patient's sensitivity and ability to tolerate cytotoxicity [11].

CAR immunogenicity refers to the capacity of a chimeric antigen receptor (CAR) to elicit an immunological response in patients, potentially leading to an immune reaction against the patients themselves. This immune response is triggered by the presence of a significant amount of the CAR obtained from human sources, along with different vector elements. On one hand, CAR immunogenicity both challenges viral vector-mediated CAR-T cell stable expression as well as threatens their efficacy. On the other hand, CAR immune responses can be beneficial, as clinical experience suggests that CAR immunogenicity usually predicts an early and strong therapeutic efficacy in patients. Additionally, CAR immune responses can be deleterious, reducing transgene expression, leading to rapid clearance of CAR-T cells, and subsequent cancers. Potential strategies that can avoid immune responses include using murine or humanized CARs, frequency diminished expression, etc.

CAR constructs have components that can trigger anti-CAR immune responses, but there are several approaches to make them less immunogenic. Humanizing tumor-reactive single-chain variable fragments (scFvs) can prevent immune responses against chimeric antigen receptor (CAR) therapies that use scFvs produced from mice. This humanization process reduces the immunogenicity of the scFvs and may be more suitable for individuals experiencing disease relapse. An alternative approach involves substituting conventional single-chain variable fragments (scFvs), which have demonstrated efficacy in preclinical models. Additionally, researchers are investigating other approaches, such as replacing scFvs with different tumor-specific domains and modifying CAR spacers to inhibit activation of innate immune cells. It is recommended to conduct head-to-head clinical trials to further investigate these approaches by comparing mouse-derived and humanized scFvs [12].

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5. Conclusion

CAR-T cell therapy, has the ability to totally eradicate tumor cells and is regarded as one of the most promising approaches to cancer treatment. However, when compared to hematologic malignancies, CAR-T cell immunotherapy encounters greater obstacles and problems when used for solid tumors. The specificity, effectiveness, and safety of CAR-T cell immunotherapy for solid tumors require additional enhancement and validation. Above, this review introduces strategies for enhancing the effectiveness and implementation of CAR-T therapy. With the support of genetic engineering technology, CAR-T cell technology is gradually becoming mature and popular, and innovative upgrading strategies will also help overcome the bottleneck faced by CAR-T cells in the treatment of solid tumors. Several preclinical studies are currently being conducted in several sectors to investigate the fundamental conditions and combination tactics for optimizing CAR-T cells. Although certain key questions remain unanswered, the enhanced effectiveness and safety of CAR-T cell treatment continue to be highly promising. The field is always evolving and will undoubtedly yield positive results gradually.

References

[1] H. Dana, G. M. Chalbatani, S. A. Jalali, H. R. Mirzaei, et al., "CAR-T cells: Early successes in blood cancer and challenges in solid tumors," Acta Pharmaceutica Sinica B, vol. 2021, Elsevier.

[2] Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol. 2020 Mar;17(3):147-167.

[3] Chang, ZeNan L, and Yvonne Y Chen. “CARs: Synthetic Immunoreceptors for Cancer Therapy and Beyond.” Trends in molecular medicine vol. 23,5 (2017): 430-450.

[4] Sterner, Robert C, and Rosalie M Sterner. “CAR-T cell therapy: current limitations and potential strategies.” Blood cancer journal vol. 11,4 69. 6 Apr. 2021,

[5] H. Lin, X. Yang, S. Ye, L. Huang et al., "Antigen escape in CAR-T cell therapy: Mechanisms and overcoming strategies," Biomedicine & Pharmacotherapy, 2024.

[6] Flugel, Christian L et al. “Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours.” Nature reviews. Clinical oncology vol. 20,1 (2023): 49-62.

[7] Liu, Guangna et al. “Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment.” Cellular & molecular immunology vol. 18,5 (2021): 1085-1095.

[8] Nakamura, Kyohei, and Mark J Smyth. “Myeloid immunosuppression and immune checkpoints in the tumor microenvironment.” Cellular & molecular immunology vol. 17,1 (2020): 1-12.

[9] Xiao X, Huang S, Chen S, Wang Y, Sun Q, Xu X, Li Y. Mechanisms of cytokine release syndrome and neurotoxicity of CAR T-cell therapy and associated prevention and management strategies. J Exp Clin Cancer Res. 2021 Nov 18;40(1):367.

[10] Hazafa, Abu et al. “CRISPR/Cas9: A powerful genome editing technique for the treatment of cancer cells with present challenges and future directions.” Life sciences vol. 263 (2020): 118525.

[11] Wang, Haobing et al. “Strategies for Reducing Toxicity and Enhancing Efficacy of Chimeric Antigen Receptor T Cell Therapy in Hematological Malignancies.” International journal of molecular sciences vol. 24,11 9115. 23 May. 2023,

[12] Wagner, Dimitrios L et al. “Immunogenicity of CAR T cells in cancer therapy.” Nature reviews. Clinical oncology vol. 18,6 (2021): 379-393.