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Enhancing CAR-T responses against solid tumors: Impact of reduced CARs affinity and addition of PD-1 complexes

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Abstract. Cancer's rising prevalence demands novel treatments. Chimeric Antigen Receptor T-cell (CAR-T) therapy emerges as a transformative option. Conventional cancer treatments often lack efficacy and carry severe side effects. This study focuses on enhancing CAR-T therapy against solid tumors. Strategies include lowering CAR affinity for antigens and integrating PD-1&CD-28 chimeric antigen complexes. While CAR-T therapy excels against hematological malignancies, challenges remain, such as immune checkpoint upregulation and potential toxicity. The research aims to optimize CAR-T therapy's efficacy through innovative modifications. Alternative strategies like multi-targeted CARs and combinations with immune checkpoint inhibitors are explored. Overall, this study advances cancer therapy by addressing CAR-T therapy's limitations and proposing complementary strategies for improved personalized treatments.

Keywords: CAR-T, PD-1, Immunotherapy Advancements, Cancer, Affinity

1. Introduction

Cancer was one of the most feared diseases of the 20th century. In the 21st century, it will spread further, with the incidence rate continuing to rise. In 2020, there were a total of 18,094,716 million cancer cases reported worldwide. The age-standardized rate for all types of cancer (excluding non-melanoma skin cancer) was 190 per 100,000 individuals when considering both men and women. Fortunately, cancer can be cured with timely detection and treatment by the following means: 1) surgical removal of cancer; 2) utilizing chemotherapy or specific drugs such as hormonal therapy; 3) applying radiation therapy [1].

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However, conventional cancer treatments have limited efficacy, and the associated side effects are usually severe. With the rapid development of medical science in the past few decades, immunotherapy, which enhances the immune system's ability to recognize, target, and eliminate cancer cells, shows superiority to traditional therapies. Immunotherapy includes two main types: T cell receptor-engineered T cell (TCR-T cell) therapy and Chimeric antigen receptor (CAR) T-cell (CAR-T cell) therapy. This article mainly focuses on CAR-T cell therapy. Chimeric Antigen Receptor T-cell (CAR-T) therapy is a form of immunotherapy that employs genetically engineered T cells to target specific cancer antigens. The process involves modifying a patient's T cells to express a chimeric antigen receptor (CAR) that recognizes a specific antigen on cancer cells. These modified T cells, when reintroduced into the patient, bind to the target antigen, triggering an immune response that destroys the cancer cells.

CAR-T cell therapy has shown exciting clinical efficacy against hematological malignancies, but applying CAR-T cell therapy for solid tumors remains challenging. Traditional CAR-T therapy faces several drawbacks, one of which is the potential upregulation of immune checkpoint molecules, for example, Programmed death-ligand 1 (PD-L1), on tumor cells and immune cells. PD-L1 initiates an immune inhibitory signal when it binds to PD-1 receptors, suppressing T cell activation and functionality, thereby preventing T cells from attacking tumor cells. Tumor cells exploit this mechanism to evade host immune attack, making it challenging for the immune system to recognize and eliminate tumor cells effectively. Moreover, CAR-T cells can also bind with normal cells because of the presence of antigens not only in the tumor cells but also in the normal cells. Then CAR-T cells attack the normal cells and cause symptoms, which are called toxicity.

There are many studies working on finding ways to improve the efficacy. In this article, we will introduce a modification on CAR-T therapy which was primarily intrigued by the following two studies.

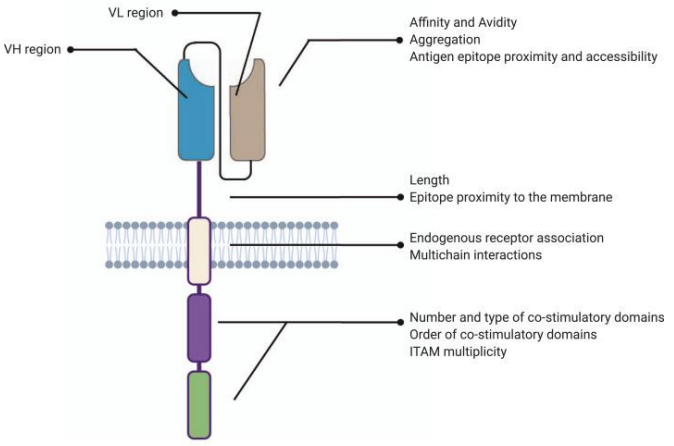

Figure 1. Structure of CAR [2]

2. Summary on existing literatures

In 2015, Liu et al. reported that the level of affinity of scFv (the antigen-binding domain of CAR-T cells) has a crucial role in discriminating between tumor cells and normal cells. Tumor cells carry specific antigens, including ErbB2 and EGFR, which are also presented on normal cells but with a fewer amount. The lower-affinity scFv (containing CAT scFv) can only bind to cells with a high level of antigen expression, while high-affinity scFv (containing FMC63 scFv) targets any cells with antigen presence, causing immunological responses with different intensities. High-affinity scFv CAR-T cells can kill both normal and tumor cells without discrimination. In contrast, lower-affinity scFv CAR-T cells, achieved through site-directed mutagenesis on amino acids, maintain essential antitumor efficacy while minimizing the risk of "on target, off-tumor toxicity" (unintended attacks against normal cells). The application of lower-affinity scFv on patients can be a potential strategy for treating solid tumors since tumor cells commonly overexpress the antigens [3].

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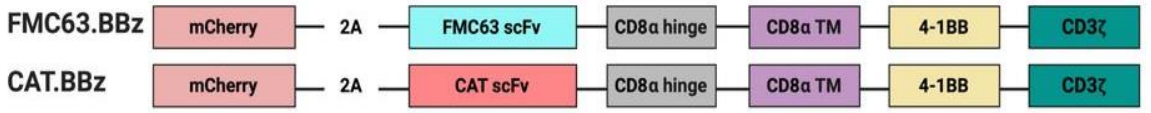

Figure 2. Structures (components) of FMC63 and CAT CAR-T cells [4]

In 2016, Liu et al.explored ways to enhance the efficacy of second-generation CAR-T cell reactivity against late-stage tumors. A major cause is the hypofunction of tumor-induced TIL due to the upregulation of IR, inhibiting the receptors' activity of T cells. Moreover, the presentation of immunosuppressive factors such as PD1 in the tumor microenvironment hinders the function of CAR-T cell functions. In order to address this, researchers found that blocking the PD 1 pathways by introducing a chimeric switch receptor called PD1CD28 complex will enhance CAR-T cells' capacities to kill tumor cells and lead to better outcomes. In the experiment, modification of PD1CD28 CAR-T cells demonstrated significant tumor control in two different established tumor models: EMMESO and PC3-PSCA. The mechanisms were investigated by researchers who contribute to enhancing CAR-T cell therapy, including increased TIL efficiency, cytokine secretion, and reduced other inhibitor receptors such as LAG3 and TIM3. In addition, this provides a potential way to deliver second-generation CAR-T cells and specifically switch on more third-generation activation. In the end, one question discussed in the article was how can the PD1CD28 chimeric switch receptors enhance CAR-T cell therapy and be further optimized and translated into efficient therapeutic treatments for human patients with solid tumors [5].

3. Hypothesis

Since CAR-T therapy may be susceptible to T cell exhaustion during treatment, CAR-T cells can exhibit diminished function after prolonged or continuous stimulation, characterized by reduced cytokine production, impaired proliferation, and decreased cytotoxic activity. This exhaustion is often associated with the lack of co-stimulatory signals such as CD28 or the failure to target immune checkpoint molecules like PD-L1 in CAR-T cells. Without these crucial signals, CAR-T cells may have difficulty effectively recognizing and attacking cancer cells, leading to a reduction in the overall effectiveness of CAR-T therapy. To overcome this limitation, researchers continuously explore methods to mitigate T cell exhaustion and improve the long-term functionality of CAR-T cells. Based on the summary of the above articles, these two emerging approaches can improve the outcome of treatment with CAR-T cell therapy. Therefore, we hypothesized that the targeted immune response (antitumor response) of CAR-T cells to solid tumors could be enhanced by reducing the affinity of CARs for cancer cell surface antigens and adding a PD-1 chimeric antigen complex.

4. Materials and Methods

In this study, we will use human breast cell lines (SK-BR-3 or BT-474) for cell culture experiments and highly immunodeficient NSG mice for in vivo testing. Our research will focus on three experimental approaches: targeted therapies on cultured breast cancer cells, assessing treatments in NSG mice with human breast cancer xenografts, and investigating combination therapies in both models. These approaches aim to provide valuable insights into potential therapeutic strategies against breast cancer.

The first experiment serves as our control group, where no treatment is administered to the NSG mice bearing tumor xenografts. Through careful monitoring and data collection, we will observe the natural progression of tumor growth and overall health without any intervention. Following the control experiment, we will move on to the next phase of our study, where we aim to investigate the potential benefits of using lower-affinity scFv (EGFR/ErbB2) in CAR-T cell therapy against solid tumors. We drew blood from the patient and then purified and isolated T-cells. The T-cells were then transduced with lentiviral vectors containing FMC63 scFv (High Affinity) instead of CAT scFv (Low Affinity). The modified T-cells and tumor cells are then injected into NSG (Highly immunodeficient) mice. Measurements of the size of the tumor cells were taken over time. The mice receiving lower-affinity

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Car-T cell treatment showed a dramatic increase in survival rate, therefore showing that lower-affinity Car-T cells can drastically increase the efficacy of the treatment.

Moving on to our third experimental approach, we will investigate the impact of incorporating PD1CD28 chimeric antigen complexes into CAR-T cells. Through gene editing technology, the extracellular domain of PD-1 is combined with the transmembrane and intracellular domains of CD-28, forming a new receptor complex that simultaneously possesses the main functions of both PD-1 and CD-28. Due to the incomplete structure, cancer cells cannot transmit inhibitory signals to T cells through the extracellular domain of PD-1. However, most of the functions of CD-28 are retained, allowing the modified CAR-T cells to enhance T cell activation while preventing cancer cell inhibition. In a mouse model, mice treated with CD-28&PD-1 CAR-T therapy showed significant tumor regression, and the survival rate increased compared to traditional CAR-T therapy. The simultaneous action of both protein receptors led to a slight increase in the survival rate compared to Experimental Group 1 [6].

One of the drawbacks of CAR-T therapy is that CAR-T cells induce “on-target off-tumor toxicity, which is an unwanted attack against the normal cells. To overcome this limitation, we can use ErbB2 and EGFR CARs with lower affinity.

The therapeutic index is an indicator that shows the relationship between effect and toxicity of treatment. It indicates a greater margin of safety between therapeutic and toxic doses, implying the treatment is safer to use. The therapeutic index of CAR-T therapy indicates the relationship between the number of CAR-T cells needed for effective treatment and the level of toxicity. If the therapeutic index of CAR-T therapy is high, this treatment can produce effective results without toxicity and be considered safer.

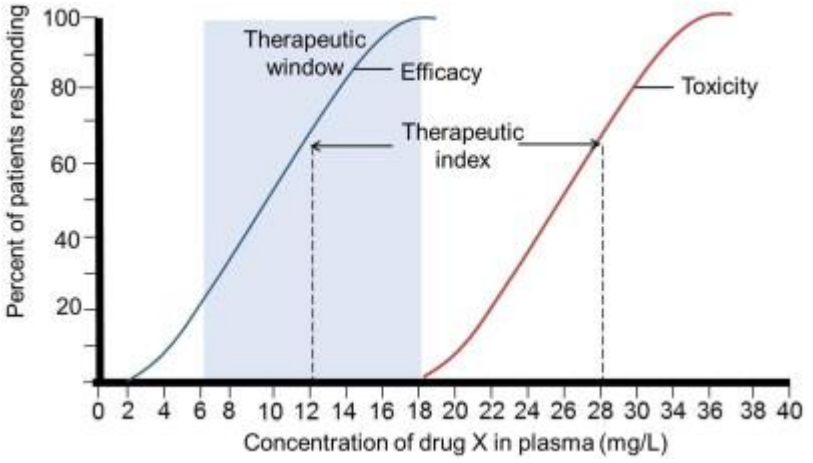

Figure 3. Therapeutic index [7]

Finally, we will explore the potential benefits of combination therapy by integrating the previous two experimental approaches. In this fourth and crucial experiment, we aim to harness the advantages of the lowered affinity scFv CAR-T cells and the PD1CD28 chimeric antigen complexes. This study mainly focuses on combining low-affinity therapy and CD-28 & PD-1 therapy. In low-affinity therapy, the expression level of the antigen may be influenced by the inflammatory response, affecting the targeting ability of CAR-T cells to cancer cells, which could be related to the overactivation of T cells.

On the other hand, in CD-28 & PD-1 therapy, the non-complete functionality of the PD-1 pathway and the enhanced activation of T cells by CD-28 could lead to T cell overactivation and immune toxicity. In this context, we propose combining these two treatment approaches. By reducing the TCR’s affinity for the antigen, we aim to prevent cytotoxicity caused by T cell overactivation resulting from CD-28&PD-1 stimulation. In mouse experiments, we anticipate that the combined therapy will yield significantly better treatment outcomes than individual treatment methods. If our hypothesis holds true,

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we expect a substantial increase in mouse survival rates, leading to a more gradual slope in the survival curve [8,9].

We will employ histology and flow cytometry analyses to complement these experiments to compare tumor and normal cell interactions. By examining tumor tissues and assessing immune cell infiltration, we can gain deeper insights into the mechanisms driving the response to CAR-T cell therapy. Additionally, flow cytometry will allow us to quantify specific immune cell populations, providing valuable data on the immune response in the tumor microenvironment.

5. Alternative Approaches

We discover several possible strategies as we look for different ways to improve CAR-T cell therapy for solid tumors. Firstly, we could use multi-targeted CARs that can recognize multiple tumor antigens simultaneously, making them more specific and reducing harm to healthy cells. Secondly, we can explore the potential of combining the PD1CD28 switch receptor with other immune checkpoint inhibitors to boost CAR-T cell function and overcome resistance from the immune system within the tumor. Moreover, new gene-editing techniques like CRISPR/Cas9 could help us create more powerful and long-lasting CAR-T cells. Additionally, we could investigate the effects of combining CAR-T cell therapy with radiation or immune-stimulating substances, which might lead to more robust anti-tumor responses. By conducting thorough preclinical studies, we can ensure that these findings are effectively translated into safe and effective treatments for patients with solid tumors [10,11].

6. Discussion

The combined treatment of CD-28&PD-1 in CAR-T therapy shows great promise in enhancing the immune response against cancer. However, it also has certain limitations that need to be addressed for optimal effectiveness.

First, the extensive gene editing required to modify the retroviruses and introduce multiple foreign genes may result in a loss of viral activity. Striking a balance between gene editing and maintaining viral efficacy is crucial to ensure the success of the combined therapy.

Second, the complex nature of CD-28 & PD-1 therapy involving gene editing and cell engineering demands specialized Equipment, expertise, and experience. These processes are typically conducted in specialized laboratories or manufacturing facilities, which may limit their accessibility and widespread implementation.

Third, individual variations in patients' immune systems can influence the response to CD-28 & PD-1 therapy. Factors such as age, gender, genetic inheritance, and immune history need to be considered when tailoring the treatment to individual patients.

To further enhance the combined therapy, several strategies can be explored. This includes investigating different CAR-T cell affinities and testing various chimeric antigen complexes that target different immune checkpoints or costimulatory molecules. In vivo imaging techniques, such as bioluminescence or positron emission tomography, can provide valuable insights into the treatment's effectiveness and response [12].

Moreover, combining CD-28 & PD-1 therapy with immunomodulatory drugs, such as checkpoint inhibitors or cytokine therapies, could create a more robust and synergistic anti-cancer immune response.

In conclusion, while the combined therapy of CD-28&PD-1 holds immense potential, addressing its limitations and exploring complementary strategies will pave the way for more effective and personalized cancer treatments [13].

7. Conclusion

Potential experimental approaches to enhance the immune response of CAR-T therapy were designed by comprehensively combining affinity tuning therapy with PD-1&CD-28. The proposed modification is expected to show enhanced T cell targeting and activity by reducing the affinity of scFv and inhibit-

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ing tumor-induced inhibitory signals. This work provides a promising prospect for applying CAR-T therapy in solid tumors with high efficacy.

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