

CAR-T Cell Therapy in Solid Tumor

Mingzheng Lyu

The High School Affiliated to Renmin University of China, Beijing, China

lvzmzh@jxqcglass.cn

Abstract. Chimeric antigen receptor (CAR) T cell immunotherapy has gained significant popularity in recent years as a promising approach for cancer treatment. This form of immunotherapy involves the genetic modification of T cells to enable them to recognize and attack cancer cells within the body. Despite the advancements in CAR-T cell therapy and related technologies, several challenges persist in the field of solid tumor research. These challenges include antigen escape, off-target effects, and the complex immune microenvironment. This study aims to evaluate the advantages and drawbacks of CAR-T cell therapy specifically in the context of solid tumors, shedding light on potential avenues for further development. Furthermore, the application of CAR-T cell treatment in solid tumors, such as breast cancer, is discussed. The study also addresses various limitations associated with CAR-T cell therapy, including the influence of the immune microenvironment and the potential for antigen evasion. In addition, the study explores future directions for CAR-T cell therapy, highlighting the potential role of synthetic biology. By examining these aspects, this research aims to contribute to the understanding of CAR-T cell treatment and provide valuable insights to combat solid tumors effectively.

Keywords: CAR-T; solid tumor; immunity.

1. Introduction

CAR-T cell therapy is one of the most promising tumor therapies in clinical practice because genetic engineering technology is used to create patients' T lymphocytes to express chimeric antigen receptors (CAR) [1]. At present, researchers secrete interferon- γ based on the type and quantity of intracellular costimulatory sequences and whether they are modified by specific genes such as interleukin (IL)-17, IL-12, and IL-18. IFN- γ), tumor necrosis factor α , or scFv that expresses anti-programmed cell death-ligand 1(PD-L1) monoclonal antibodies divide the CA-R-T cells into four generations [1]. A number of problems have made it difficult to use CAR-T cell therapy on solid tumors, which has led to less-than-stellar clinical trial outcomes. Further refinement of CAR-T cell design is essential to improve tumor recognition accuracy and cytotoxicity in order to tackle these issues. Solid tumors, unlike blood tumors, are encircled by a complex microenvironment of fibrous matrices and immunosuppressive cells, which form physical and immunological barriers that guard the tumor and counter immune cell assaults. Scientists are actively investigating various approaches to surmount these difficulties. A malignant tumor's high rate of morbidity and mortality is proof of the serious threat it poses to human health. The leading cause of mortality in China, malignant tumors are responsible for 23.91% of all fatalities, according to the most recent statistics provided by the National Cancer Center of China. Based on data from the International Center for Research on Cancer, a division of the World Health Organization, it was reported that China had 4.56 million newly diagnosed cancer cases in 2020 [2]. Currently, extensive pre-clinical and clinical investigations have been conducted on CAR-T cell therapy for various solid tumors, including glioma, neuroblastoma, gastric cancer, liver cancer, and prostate cancer, yielding promising initial outcomes [3]. The field of CAR T cells has seen a great progression, with nanotechnology and synthetic biology playing a major role. This discussion will examine a case study of CAR-T cell therapy's use in treating solid tumors as well as its potential future.

2. CAR-T cell therapy's application to the treatment of solid cancers

Research on CAR-T cell therapy focuses on a wide range of targets. These targets include classic tumor markers, newly discovered tumor-associated antigens, and molecules that are also highly expressed in normal tissue cells, such as clusters of differentiation antigens [1]. To exemplify, Breast cancer is very common in women. CAR T cell therapy for breast cancer has been paid more and more attention. HER2-positive JIMT-1 breast tumors have developed resistance to the FDA-approved anti-breast cancer drug trastuzumab [4]. Gábor Tóth and associates used targeted CAR-T cells in xenograft research on SCID mice to overcome this problem. The findings showed that even a modest number of HER2-specific CAR-T cells with their activity focused through heterologous T-cell responses could elicit a potent anti-tumor response against antibody-resistant xenografts [4]. These results have significant implications for choosing the optimal CAR-T cell therapy dosage for treating solid malignancies. A recent clinical trial (NCT0102138) involving CAR-T cells targeting HER2-negative tumors after neoadjuvant chemotherapy showed a trend towards improved survival, confirming the safety of this treatment method and making breakthrough progress in combating solid tumors [5]. Glioblastoma multiforme (GBM), a common primary brain tumor, may be treated using CAR-T cell therapy. With regard to this solid tumor, CAR-T cell therapy is largely used for patients who have previously had unsuccessful or limited treatments, with an emphasis on interleukin-13 receptor alpha-2 (IL-13R2) targeting for research purposes [4]. By focusing on this antigen, tumor recurrence is prevented in addition to overt illness. This is due to the fact that IL-13R2, a possible target for the therapy of glioblastoma, is only minimally expressed in healthy brain tissue, minimizing any potential negative consequences. Including cancer stem cells and adult tumor cells, it is overexpressed in more than 50% of glioblastoma cells, as demonstrated by [4].

3. The difficulties faced by CAR-T cell therapy in the treatment of solid tumors

Blood arteries, stromal cells, immune cells, extracellular matrix, and cytokines are among the elements that make up the tumor microenvironment, a complicated and constantly changing entity. The complex tumor immune suppressive milieu that characterizes solid tumors primarily serves to suppress immune cell function and promote tumor dissemination and progression. Tryptophan is degraded together with metabolic blockage in T cells and poor function of effector T cells, which are primarily caused by the hypoxic state of the tumor microenvironment and its deficiency in nutrients [6]. IDO (indoleamine 2,3-dioxygenase) release from tumor cells and MDSCs (myeloid-derived suppressor cells) from bone marrow both speed up this process. Additionally, tryptophan deficiency impairs CAR-T cell growth and cytotoxicity [6]. The tumor microenvironment (TME) refers to the local environment surrounding the tumor, where the expression of PD-L1, a programmed death ligand found on tumor cells, leads to an increase in inhibitory receptors on the cell surface. This, in turn, triggers an internal negative regulatory mechanism that diminishes the activity of CAR-T cells, ultimately resulting in immune tolerance [7]. Furthermore, soluble inhibitors such as prostaglandin E2, transforming growth factor- β , interleukin-6, and IL-10 can impede CAR-T cell function by inhibiting tumor cell apoptosis. Additionally, various chemicals can facilitate the development of suppressor cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which contribute to an immunosuppressive environment. These cells produce reactive oxygen species (ROS) and arginase-1 (Arg-1), collectively establishing an immunosuppressive microenvironment. Tregs play a crucial role in immunological responses, with type I and CD4+CD25+ Tregs being two of the most important subgroups, as seen in Figure 2. Tregs can prevent the production of sufficient effector cells by directly eradicating surplus T cells through IL-2, making it impossible to fight off malignant tumors [8]. CAR-T cells are ultimately rendered useless by the tumor microenvironment, which is characterized by acidic, nutrient-deficient, and hypoxic circumstances [7].

A significant obstacle is the absence of chemokine receptor expression. White blood cells are drawn to infection sites by chemokines, which also help immune cells reach the tumor microenvironment and help T cells affect tumor growth. In contrast, chemokines have the ability to

bind to their respective chemokine receptors, attracting immune cells into the tumor microenvironment (TME). This process can have either stimulatory or inhibitory effects on tumor growth [7]. Chemokines such as chemokine 15 (CXCL5) and chemokine 12 (CXCL12) can be secreted by specific solid tumors [8]. Unfortunately, T cells frequently lack the chemokine receptors necessary to recognize the chemokines released by solid tumors. It is disappointing that T cells only express low levels of chemokine receptors that are specific to CCL2, including CCR2b and CCR4, despite solid tumors possessing high levels of chemokines like CCL2 [8]. As a result, T cells' capacity to homing is decreased as a direct result of this.

Migration and infiltration are also significant issues. Due to the intricate pathophysiology of solid tumors, there can be a local deficiency of T cells when specific antigen-targeting CAR-T cells are administered to the body. Nevertheless, it is essential to achieve sufficient infiltration of CAR-T cells into tumor tissue for the successful treatment of malignant cancers. Tumor infiltration is achieved through adhesion, rolling, chemotaxis, and extravasation, which are all dependent on specific recognition, as demonstrated in [6]. Therefore, current research suggests that local treatment is more favorable for CAR-T cell infiltration than systemic treatment. However, on one hand, the local infusion of CAR-T cells is limited to single tumor nodules or oligometastatic tumors, and for multiple metastatic tumors, CAR-T cells still need to migrate to tumor sites based on their own characteristics to exert antitumor effects [6]. This issue has been partially addressed, as will be discussed in the following sections.

The lack of specific antigens and off-target effects are also significant issues. The components crucial to CAR-T cells include the transmembrane region, intracellular signaling domain, hinge region, and extracellular antigen recognition domain [7]. The effectiveness of CAR-T treatment is greatly influenced by the expression of the target antigen. Many tumor-associated antigens (TAAs) can be expressed in normal cells, which presents a challenge because this would not be a problem if the majority of TAAs were expressed only in cancer cells. Hematological cancers frequently target CD19, CD22, and BCMA, which are particular TAAs. When CAR-T cells attack normal cells, they could be able to have off-target effects in the absence of TAAs. As an example, since HER2 is expressed in breast cancer cells, a patient with colon cancer underwent a high-dose treatment of HER2 CAR-T cells. Unfortunately, cardiac and pulmonary tissues also expressed HER2. The patient developed significant respiratory distress within 15 minutes and passed away from the illness 5 days after receiving the infusion [7].

4. Potential future advancements of CAR-T cell therapy in the realm of solid tumors

The innovative company YF has proposed a strategy called "Dimensionality Reduction Strike" that offers a potentially effective approach to treating solid tumors from a clinical application perspective. The core of this method is to "transform solid tumors into hematologic malignancies." The process involves addressing visible solid tumors through conventional surgery or intervention, utilizing cellular therapy to eliminate tiny lesions and circulating tumor cells (CTCs), thereby achieving a clinical effect where $1+1>2$ [9]. Solid tumors are like fortified strongholds, and the dimensionality reduction strategy relies on interventions or surgeries to breach these strongholds and fundamentally overcome them with the use of drugs. With this strategy, it is possible to find the appropriate dosage range of CAR-T drugs directly, ensuring safety (avoiding off-target tumor toxicity) while addressing the issues of patient relapse and metastasis. Therefore, there is no need to rely on a single drug or extremely high doses to address all solid tumors [9]. This method can to some extent address the occult residual tumor metastases or in situ lesions that are not visible on imaging, thereby improving the therapeutic outcomes after surgery or intervention. The Peri Cruiser® technology platform developed by YF aims to expand the application of CAR-T cell therapy in solid tumors while enhancing safety by addressing the toxicity associated with conventional tumor-associated antigens (TAAs). Through the utilization of various shRNA strategies, this approach enables control

over the distribution of CAR-T cells in the body, reducing toxicity and minimizing damage to normal tissue cells, thereby improving overall safety. Additionally, YF has introduced platforms such as T-Booster to enhance the proliferation and invasion capabilities of CAR-T cells, as well as the SNR CAR-T platform to target tumor heterogeneity [9].

Synthetic biology employs engineering principles to artificially synthesize modules, biological components, gene circuits, and other technologies to modify and design cells. The synNotch gene circuit system is a type of gene circuit that has been successfully expressed on the cell surface by researchers such as KOLE and HYRENIUS-WITTSTEN [10]. They have established CAR-T cells embedded with synNotch receptors, which can simultaneously bind to and be activated by antigen A and antigen B. In contrast to conventional dual-target CAR-T cells, the synNotch receptor has the ability to protect normal cells that only express antigens A or B from being attacked by CAR-T cells. This is because when it binds to antigen A on the tumor's surface, transcription factors are activated, activating T cells to create a CAR that targets antigen B, the second antigen. This merits attention. This allows the T cells to kill the tumor [10]. Recently, ALLEN et al. [11] successfully combined the synNotch gene circuit with an IL-2 induction circuit. This upgraded CAR-T cell can effectively control the activation time as it initiates cytokine secretion upon reaching tumor cells. This advancement enables efficient tumor clearance and holds significant potential for future applications. The upgraded IL-2-inducing circuit bypasses the limitations of CAR or TCR activation and is directly controlled through the synNotch receptor system. This leads to a notable improvement in the elimination of immunosuppressive solid tumors and the penetration of CAR-T cells, while simultaneously preventing systemic IL-2 production and its detrimental consequences. This is due to the fact that in this gene circuit, these CAR-T cells only produce IL-2 when they come into direct touch with tumor cells [11]. In solid tumors, a challenge arises as the target antigen is only present on a subset of tumor cells within the tissue. This poses difficulties for CAR-T cells in eliminating tumor cells lacking the target antigen, potentially resulting in tumor recurrence [10]. To address this challenge, researchers have utilized synthetic biology to design multi-targeted CAR-T cells. These modified CAR-T cells can recognize multiple target molecules present on the surface of tumor cells. In situations where a tumor cell lacks or has minimal expression of a specific target molecule, other target molecules can be identified by the corresponding CAR-T cells [10]. For example, Yang Meijia et al. [12] developed a bispecific tandem CAR-T cell that simultaneously targets CD70 and B7-H3. This innovative design combines the CD70 single-chain variable fragment (scFv) and the B7-H3 scFv using a linker, allowing a single CAR molecule to target both tumor antigens. This bispecific tandem approach effectively addresses the issues of mutational and antigenic heterogeneity in solid tumors, as it achieves control of melanoma xenografts and lung cancer at low doses. There are three others, Conjugated CAR cells involve introducing two CAR molecules into a single T cell. CAR-T cells, linked to small molecules, can identify various small molecule adapters with a shared marker, thus enabling them to target distinct tumor antigens [2]. A recent study conducted by a research group [13] has introduced a novel approach to CAR-T cell therapy based on in situ antigen modification, particularly targeting highly heterogeneous tumors or those lacking suitable antigens. Building upon advancements in synthetic biology, this innovative strategy involves fusing the EGFRvIII antigen onto the surface of tumor cells that do not naturally express it, alongside EGFRvIII-targeting CAR-T cells. By employing this approach, the limitations posed by low positive rates and the extensive heterogeneity of tumor antigens observed in artificial modification techniques can be overcome. Consequently, CAR-T cells become capable of efficiently eradicating tumor cells that lack sufficient native targets [10]. This significant contribution has propelled the advancement of CAR-T cell research and provides promising experimental support for the development of universal CAR-T cells.

5. Conclusion

Despite the remarkable progress made in CAR-T cell therapy for solid tumors, there are still numerous unresolved challenges and difficulties that need to be addressed. This article presents

various instances of CAR-T cell therapy, serving as compelling evidence to support the feasibility and ongoing development of this innovative treatment modality. Also, it introduces in detail its limitations and shows the problems that need to be faced. These last two directions are very promising. It is to be hoped that, with the collective exertion of all, CAR T-cell therapy will progress and make a significant stride in the realm of human wellbeing.

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