CAR-T cell therapy in treating advanced prostate cancer

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Abstract. Prostate cancer (PCa), a common cancer among older men, poses unique issues due to its slow progression and limited therapeutic choices for advanced stages. Since standard medicines such as chemotherapy and radiation have limited efficacy, it is in urgent need to develop novel therapies. CAR-T cell treatment modifies patients' T cells to produce chimeric antigen receptors that target certain antigens expressed on cancer cells, thereby enhancing the immune system's ability to identify and eliminate malignant cells. Targets for CAR-T cell therapy in prostate cancer include PSCA, PSMA, and EpCAM. Numerous studies have been conducted on PSMA, which is overexpressed in PCa cells. CAR-T cells that target PSMA have shown encouraging results in preclinical and clinical trials. Similarly, PSCA and EpCAM are promising targets for CAR-T cell therapy, with continuing research focused on improving their efficacy and safety profiles. The evolution of CAR-T cell therapy, spanning multiple generations, reflects continual efforts to enhance therapeutic outcomes. From first-generation CAR-T cells lacking co-stimulatory signals to advanced fourth and fifth-generation CAR-T cells equipped with additional functionalities like cytokine secretion, each iteration represents a progression towards improved efficacy and safety. However, optimizing CAR-T cell design, managing side effects, and identifying appropriate antigen targets still need to be addressed to realize the full promise of CAR-T cell therapy in PCa treatment. This review introduces the mechanisms, structures, evolution, and uses of CAR-T cell therapy in the treatment of PCa and discusses its great potential as a transformative cancer treatment strategy.

Keywords: Prostate cancer; CAR-T therapy; immunotherapy.

1. Introduction

Prostate cancer (PCa) develops in the prostate gland, known as the prostate, is in charge of producing seminal fluid, which feeds and helps move sperm, a part of the male reproduction system. One of the most prevalent cancers, prostate cancer primarily affects elderly men. The average age at the time of diagnosis is 66 years. The stages of prostate cancer include stages 1 to 4. Stage one means that the cancer is still limited, hasn't spread yet and the PSA levels are low. Stage 4, means cancer has already spread to distant lymph nodes, bones or other parts of the body. Prostate cancer is considered a frequently slow-growing and slow-spreading cancer, for this reason, it has higher survival rates compared with other types of cancer. The five-year relative survival rate is around 98% and the fifteen-year relative survival rate is also a high 99% [1].

In the past few years, a lot of different therapeutic methods have been approved for treating advanced prostate cancer including cryotherapy, chemotherapy, radiation therapy, ect. These therapies often have drawbacks including side effects such as urinary and sexual dysfunction, limited efficacy for certain cancer stages, and potential long-term complications like secondary cancers or damage to surrounding tissues.

Immune checkpoint inhibitors (ICIs) are a class of immunotherapy medication that inhibits T-cell activities from being inactivated by preventing checkpoint proteins from engaging with their partner proteins. These antibodies target signaling molecules that are either co-inhibitory or regulatory, such as CTLA-4, PD-1, and PD-L1 [2].

A major breakthrough in the field of cancer research would be the development of genetically modified T cells with the ability to overcome immunological tolerance to malignancy. Adoptive cell transfer is another term for the treatment of CAR-T cells. In this treatment, a patient's blood is used to modify T-cells in a lab. This adds a lab-made gene for a receptor known as CAR, which helps the patient's T cells find and eliminate cancer cells more effectively. This review introduces the function

mechanism of CAR-T therapy and structure of CAR, as well as discusses application and challenges of CAR-T therapy in PCa.

2. Function mechanism of CAR-T cell therapy

T cells are a type of white blood cell in human body which called lymphocytes. T-cells have surface proteins called receptors that recognize abnormal antigens, acting as a surveillance system for abnormal cells. However, sometimes they fail to accurately detect cancerous cells. CAR T-cells are modified T-cells that recognize specific antigens on cancer cell surfaces. The process of apheresis is when the medical team takes a sample of patients' T cells from their bloodstream. The T cells are taken to a lab where they edit the cells, the process is called genetically engineering the T cells. When the edited T cells grow and multiply into the right amount of cells, scientists then drop the lab-made gene back into patients' bloodstream. CAR T-cell therapy is a "living drug" because, once modified T cells are in the bloodstream, CAR T-cell receptors identify and eliminate malignant cells. The cells then proliferate, producing a steady supply of CAR T-cells [3].

3. Structure of CAR-T

The structure of CAR-T is composed of four regions or domains including an antigen recognition domain, a transmembrane domain, a hinge region and an intracellular T cell signaling domain.

When the receptor interacts with the target antigen and is exposed to the external environment of the cell, the antigen recognition site in CARs will identify antigens in the receptor's ectoderm. The single chain of variable fragments (ScFv), a chimeric protein comprising light and heavy chains of immunoglobins, is the primary way that this domain is connected [4]. Other approaches, such as TNF receptors, structure protein, and cytokines also play a role.

The transmembrane domain has received fewer studies, but it has been demonstrated to influence surface expression levels and membrane stability of expressed CARs [4]. In addition, it regulates clustering, activation, and other critical reactions in CAR assembly. A substantial amount of unspecific toxicity may result from excessive cytokine release. The majority of CARs have a transmembrane sequence that is derived from the same protein, but the CD3ζ, CD4, CD8α, or CD28 domains provide CAR-T cells distinct capabilities. Whereas domains derived from CD8α or CD28 improve membrane stability, domains derived from CD3ζ cause enhanced T-cell activation and lower complex stability [5]. It requires more investigation to fully comprehend their impact on CAR-T effectiveness.

The hinge region of CARs stretches from the transmembrane domain to the antigen-binding domain, giving the scFv flexibility and improving target epitope recognition. The hinge's composition and length affect activation outputs, signaling, and CAR expression. While short hinges are more beneficial for membrane-distal epitopes, long spacers are better suited for glycosylated antigens or membrane-proximal epitopes [5]. Long spacers have the potential to cause activation-induced cell death in addition to robust in vivo responses. Following engineering, CAR-positive T cells are additionally counted and purified using hindered areas.

The most common component of the endodomain, which is the functional end of the receptor, is CD3 ζ. It has three tyrosine-based activation motifs for immunoreceptors [5]. Its function is to send a signal to the T cell. For CAR-T cells to respond effectively, co-stimulatory signals are essential. The majority of FDA-approved CAR-T cells include a primary signaling domain derived from CD3ζ, while co-stimulatory domains come from CD28 and 4-1BB (CD137). The primary intracellular signaling domain of CD-3 Zeta is its cytoplasmic domain, and co-stimulatory domains or molecules are also necessary for CAR T cell activation. Co-stimulatory domains regulate apoptosis, activation-induced cell death, metabolic cycles, and T cell differentiation pathways. Ensuring the success of CAR-T cell therapy calls for enhancing the design strategy of these domains.

4. Evolution of CAR-T therapy

Based on the composition and structure of the endodomain, the evolution of CARs during the past thirty years can be loosely divided into five generations (Figure 1).

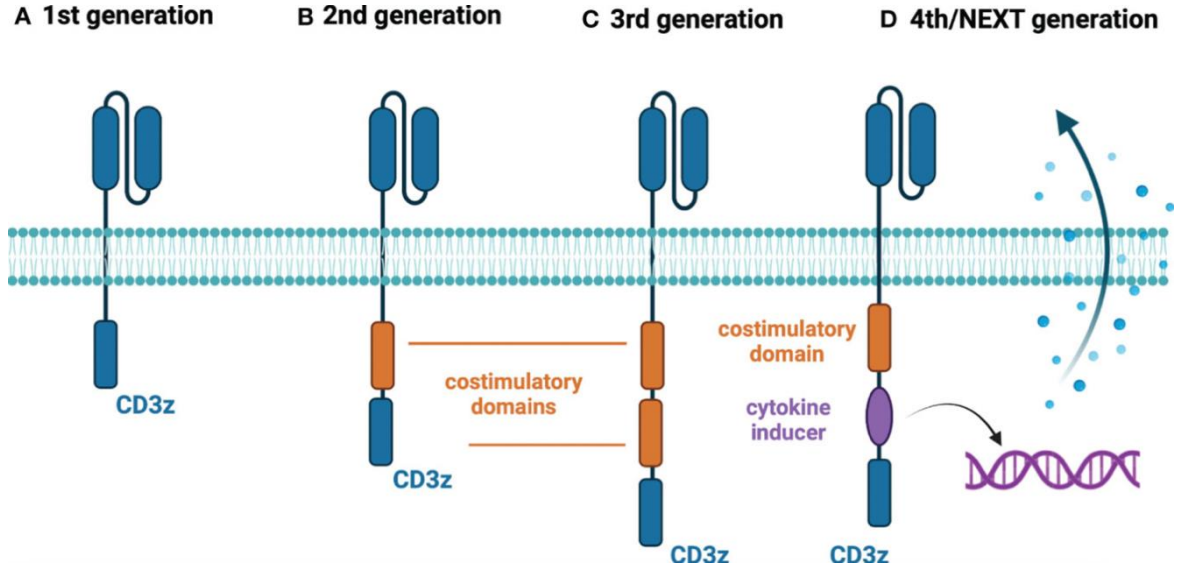


Fig 1. Generation of CAR-T cells [9].

4.1. First-generation CAR-T cells

When CAR-T cells were first manufactured in the late 1980s, they only possessed one intracellular CD3η domain. Because first-generation CAR T cells lacked co-stimulatory and cytokine signaling, early tests utilizing these cells showed low cytotoxicity and proliferation [6]. Because CAR T cells carry a single-chain variable fragment (scFv) unique to a cancer antigen, they activate the T cell receptor (TCR) signaling pathway when they bind to a cancer cell. Nevertheless, the effectiveness of first-generation CAR T cells is limited by their inability to survive in the body and pierce solid tumors [7].

4.2. Second generation CAR-T cells

Second-generation CAR T cells were created in the late 1990s [7]. Second-generation CAR-T cells include extracellular antigen recognition domains and intracellular signal transduction domains. Their main components are the external antigen recognition domain and the intracellular signal transduction domain. The extracellular domain consists of a single-stranded variable fragment (scFv) that binds to a specific antigen produced on the surface of cancer cells [7]. The CD3ζ domain (a component of the intracellular domain) and one or more costimulatory domains transmit activation signals. Second-generation CAR-T cells contain scFv, which can recognize and bind to cancer cells or target antigens. This binding activates CAR-T cells, which then release cytotoxic chemicals that kill cancer cells. However, the use of scFv alone is not sufficient to fully activate T cells and provide long-term antitumor activity [8].

4.3. Third generation CAR-T cells

The third generation of CAR-T cells aims to improve efficiency by combining CD28 and 4-1BB costimulatory domains. This complements each other, especially in low-burden diseases with limited tumour cell signal. Additional co-stimulation may benefit T cell expansion and elimination, while 4-1BB prolongs CAR-T cell persistence [9]. According to a clinical trial including 16 patients with non-Hodgkin's lymphoma, they found that the limitations of each separate costimulatory domain may be overcome by merging CD28 and 4-1BB signaling in a third-generation (3G) CAR. Patients with minimal disease load may benefit most from this combination.

4.4. Fourth and Fifth-generation CAR-T cells

Fourth-generation CAR-T cells integrate antigen detection and activation functions with the potential to release therapeutic chemicals such as cytokines or antibodies [7]. Like the second-generation CAR-T cells, the fourth-generation CAR-T cells are engineered to express a CAR with two signaling domains. The two signaling domains are the activation domain, which activates T cells upon detecting the tumor antigen, and the costimulatory domain. Furthermore, upon recognizing an antigen, fourth-generation CAR-T cells have the ability to initiate an inducible gene expression mechanism [10].

The fifth generation of CAR T-cell therapy was created to address the drawbacks of earlier iterations of the treatment. The fifth generation of CAR-T cells include an extra intracellular domain. Cytokine receptor antigens (CARs) are made up of shorter intracellular domains of cytokine receptors, including the IL-2R chain segment, that have a pattern that permits them to bind transcription factors such as STAT-3/5. As a result, the emitted signal reactivates and enhances the immune system, while also driving CAR-T cells to remain active and produce memory T cells [11].

5. Application of CAR-Ts in PCa

Identifying prostate tumour-associated antigen (TAAs) is the first step towards developing an effective CAR-T cell therapy. Ideal antigens for cancer cells should be constitutive and expressed specifically. Targeted immunotherapy has emerged as a promising approach, focusing on specific antigens expressed in prostate cancer cells.

5.1. PSMA

Glutamate carboxypeptidase is present in PSMA [12]. Target antigen PSMA is expressed in all stages of prostate cancer and becomes more prevalent in tumors with a higher grade. It may play a part in tumor angiogenesis and metastatic processes because it is also elevated in other tissues, including the kidneys, salivary glands, small intestine, and ovarian tissue. A crucial protein in PCa cells called PSMA is overexpressed and rises in tumor aggressiveness, especially in CRPC. Research indicates that PCa can be treated and resistant to by CAR-Ts that target PSMA. Multiple co-stimulatory chemicals have been demonstrated to offer potential for second-generation CAR-Ts. PSMA Positron Emission Tomography/Computed Tomography (PET/CT) has revolutionized the imaging of prostate cancer in the last five years [13]. PSMA has been employed in theranostics and diagnostic imaging. This imaging technique is better than traditional medical imaging modalities because it binds to the PSMA receptor on prostate cancer cells with a tiny chemical. The safety and feasibility of PSMA-targeting TGFβ-insensitive armoured CAR-Ts have been verified in a clinical experiment. The efficacy of PSMA-targeted radioligands in PCa diagnosis and clinical applications has also been validated by recent clinical trials.

For financial considerations, the initial clinical trial with CAR-T cells that target PSMA was stopped. While the disease progressed in the remaining patients, two of them had declining PSA values of 50% and 70% [13]. It was discovered that the first-generation CAR-T construct had poorer T-cell growth and recruitment, which resulted in less-than-ideal levels of IL-2. In patients with prostate cancer, PSMA expression may also be a predictor of disease recurrence. PSMA expression was found via immunostaining with the mAb 7E11 in a recent series of tumors evaluated by Ross and colleagues1. This expression is linked with tumor grade, pathologic stage, P SA, and aneuploidy. Notably, PSMA expression independently predicted the chance of biochemical recurrence in a multivariate analysis.

5.2. PSCA

PSCA is one of the most common targeted antigens for CAR-T cell therapy for prostate cancer [13]. According to reports, PSCA shows up to ten times more staining when compared to tumor stage,

Gleason score, universal expression in bone metastases, and trend toward androgen independence [13].

Because of its prostate-specific characteristics, PSCA, a protein that is overexpressed in the majority of prostate cancers, is essential to PCa immunotherapy. According to studies, it might have distinct physiological functions in various tissues, serving as a tumor suppressor or carcinogen. When PSMA and PSCA are combined in CAR-T cells, the accuracy of mCRPC targeting can be improved, and the aggressiveness of tissues that express a single antigen can be decreased. Recently, a PSCA-CAR-T related clinical investigation was carried out.

This work examines PSCA protein expression in clinical prostate cancer specimens using five monoclonal antibodies. The results show that PSCA expression is found in 94% of primary tumors and 100% of bone metastases, and that it increases with Gleason score, tumor stage, and androgen-independence progression. This shows that in cases of advanced prostate cancer, PSCA may be a helpful molecular target.

5.3. EpCAM

Transmembrane glycoprotein EpCAM, also referred to as CD236 is expressed in prostate cancer [13]. The EpCAM, a protein present in epithelial cells that facilitates cell attachment, is produced by the EPCAM gene. A single cut in the protein releases the intracellular domain (EpICD), which communicates with the nucleus. Because EpCAM expression inhibits cell-cell adhesion and promotes cell motility, proliferation, and differentiation, it is essential for the metastatic spread of tumors. Consequently, it is not specific to the prostate but rather is widely overexpressed in solid tumors. As a result, EpCAM is being studied in a number of solid tumors, including intraperitoneal carcinomatosis and carcinomas of the breast, stomach, colon, ovary, and nasopharyngeal [14]. Together with other proteins, EpICD creates a complex that controls gene activity related to cell activities like as growth, division, differentiation, and migration, all of which are essential for the development of cells and tissues. In 85% of adenocarcinomas and 72% of squamous cell carcinomas, EpCAM expression is detected. Prostate cancer hormone-refractory tumor tissue exhibits higher expression, which decreases with the Gleason score [15]. Results have also shown that better patient prognosis is correlated with higher EpCAM expression in the pT2 lung cancer subgroup.

According to the studys, prostate cancer tissues and cell lines exhibit overexpression of EpCAM, with altered expression starting early in the prostate carcinogenesis process. Epithelial-to-mesenchymal transition induction resulted in the repression of EpCAM; however, chemoresistant cells exhibited re-induction of EpCAM via miRNAs miR-200c and miR-205. Prostate cancer cells cleave EpCAM, although this process has no discernible effect on the cells' proliferative potential. Therefore, EpCAM overexpression is a crucial early stage in prostate cancer progression, involving the dynamic expression of the epithelial cell adhesion molecule and processes of EMT and MET.

6. Conclusions

CAR-T cell therapy is a possible way to revolutionize cancer treatment, notably in PCa. This innovative therapy harnesses the immune system's potential by genetically altering T cells to express CARs, allowing them to better target and destroy cells that are cancerous. CAR-T treatment has tremendous potential for improving therapeutic outcomes in PCa patients by targeting particular antigens such as PSMA, PSCA, and EpCAM.

CAR-T therapy has evolved from first-generation to advanced fourth and fifth-generation cells, reflecting ongoing attempts to improve efficacy and safety. Each iteration advances the design and functionality of CAR-T cells, overcoming challenges such limited persistence and tumor penetration. Furthermore, continuing research focuses on refining CAR-T cell design, controlling side effects, and identifying optimal antigen targets in order to optimize the therapeutic potential of this novel method. Looking ahead, CAR-T therapy in PCa will require further improving antigen selection and CAR design to improve specificity and reduce off-target effects. Furthermore, combination therapies that

combine CAR-T cells with different therapy modalities like as immune checkpoint inhibitors and targeted therapies may provide synergistic advantages by overcoming tumor heterogeneity and resistance mechanisms. In addition, advances in manufacturing techniques and delivery technologies seek to simplify the production and administration of CAR-T cells, making this therapy more accessible and scalable for widespread clinical application.

Future CAR-T therapy holds great promise for changing the face of cancer care and increasing outcomes for patients all over the world as research delves further into the intricacies of the immune system and tumor microenvironment.

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