Role of CAR-T-Based Immunotherapy in the Treatment of Hepatocellular Carcinoma

Yinuo Liang *

University of Nottingham, Nottingham, United Kingdom

* Corresponding Author Email: Yinuo.Liang@nottingham.ac.uk.

Abstract. Liver cancer is one of the most frequent malignant tumors worldwide, with a high rate of morbidity and death. The intricacy and variety of liver cancer mean that while there are an increasing number of therapeutic options—such as surgery, radiation, chemotherapy, immunotherapy, etc.—the effectiveness of these approaches is not always optimal. This is despite advances in medical technology. A low long-term survival rate and the possibility of metastasis and recurrence persist for many individuals following therapy. Chimeric antigen receptor T-cell (CAR-T) immunotherapy has great potential for application in liver cancer treatment due to its highly targeted nature, potent and long-lasting anti-tumor effects, and high degree of personalization. In this paper, the basic structure and mechanism of action of CAR-T are firstly introduced. Then, it summarizes the application of CAR-T therapy in liver cancer by targeting different targets in liver cancer, such as Glypican-3 (GPC3), Recombinant Mucin 1 (MUC1), and NKG2D, respectively. Finally, the limitations of current CAR-T therapies and future research directions are discussed.

Keywords: CAR-T, Immunotherapy, liver cancer.

1. Introduction

Because of the variety of its histological forms, primary hepatocellular carcinoma, a prevalent category of malignant tumors, presents a significant treatment challenge. Treatment decisions must be made with more precision and individualization due to the existence of distinct forms of cholangiocarcinoma, hepatocellular carcinoma (HCC), and hepatic cholangiocarcinoma (HCC-C) [1, 2]. Liver cancer is a significant global health concern due to its high morbidity, mortality, and unfavorable prognosis. It is particularly prevalent in developing nations like China, where it is the primary cause of cancer-related fatalities [3].

Traditional tumor excision and liver transplantation are excellent therapeutic options for liver cancer, but their applicability is restricted by a number of criteria, including the patient's liver function and the location and size of the tumor. As a result, patients with liver cancer now have more treatment choices because to advancements in medical technology, including transarterial chemoembolization (TACE), transarterial radioembolization (TARE), and percutaneous radiofrequency ablation (RFA) [4, 5]. These methods are particularly suitable for patients with early-stage liver cancer or poor liver function, and create conditions for subsequent complete resection by reducing tumor size.

However, the patient's body will unavoidably suffer some degree of harm from therapies like chemotherapy and tumor removal. Thus, the present focus of research is on finding a low-risk, highly effective therapeutic strategy. CAR-T cell therapy is a new immunotherapy with significant potential for treating cancer. It modifies tumor cells to increase T cell recognition and killing capabilities.

This article mainly aims to the application of CAR-T cells in the treatment of liver cancer. It collects articles related to CAR-T cells targeting GPC3, MUC1, and NKG2D, and proposes promising research directions and guiding roles for future liver cancer treatment.

2. CAR-T cells immunotherapy

2.1. The structure of CAR-T cells

A novel approach to cancer immunotherapy, CAR-T cell therapy makes use of intricate cell engineering and molecular biology methods. We can see from the information you have given us that

CAR-T cell structure and design are continuously being refined and enhanced to increase therapeutic effectiveness and safety [6].

Extracellular and antigen recognition structural domains, extracellular hinge areas, transmembrane structural domains, and intracellular signaling structural domains are the essential components that make up CAR-T cells. Each component has a distinct purpose, and when combined, they guarantee that CAR-T cells can detect and destroy tumor cells with accuracy.

The primary mechanism underlying the specificity of CAR-T cells is the preferential binding to tumor-associated antigens (TAAs) on the surface of tumor cells by single-chain variable fragments (scFv) mediated by the extracellular and antigen recognition structural domains. In contrast, the extracellular hinge region facilitates the creation of flexible interfaces between CAR-T cells and target cells, an essential function for the release of cytokines, cell proliferation, and memory establishment.

CAR is anchored to the cell membrane by the transmembrane structural domain, which also affects receptor aggregation and promotes steady CAR expression. On the other hand, when an antigen binds to intracellular signaling structure domains, T cell activation occurs, leading to immunological responses that are unique to that antigen.

From the first generation to the fifth generation, CAR-T cell development has gone through various stages, with each generation having unique traits and advancements. The first generation CAR-T cells consisted only of a single CD3ζ intracellular structural domain and exhibited limited T cell proliferation and activation potential. In contrast, second-generation CARs enhanced T cell persistence and anti-tumor efficacy by introducing additional co-stimulatory signaling structural domains, such as CD28 or 4-1BB. The subsequent third, fourth and fifth generations have built on this foundation with further optimizations and improvements, as is shown in Figure 1.

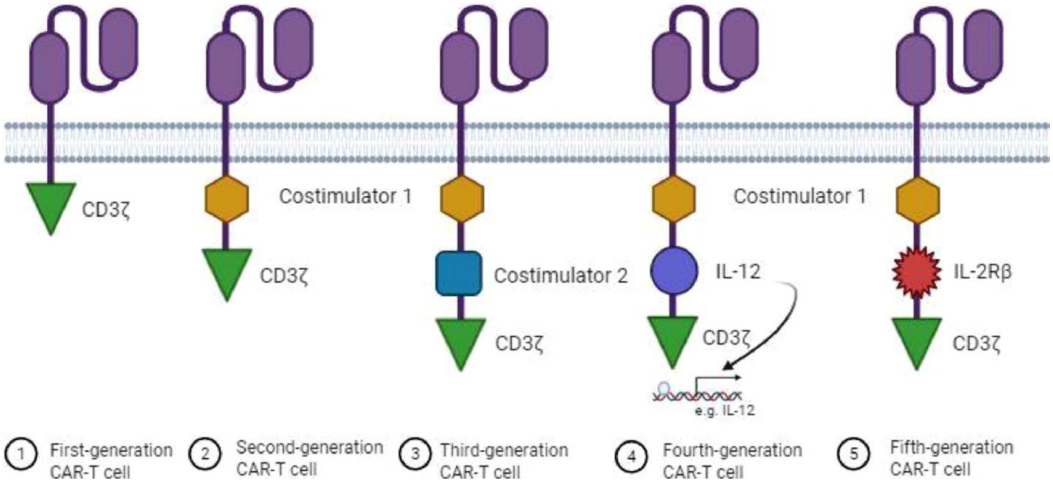

Fig 1. Five generations of CAR-T cells [6].

2.2. The mechanism of action of CAR-T cells

The use of chimeric antigen receptors (CARs) to alter T cells so they can accurately identify and combat tumor cells is the basis of the CAR-T cell therapy process. First, the patient's immune system is used to remove T cells. These cells are grown, isolated in vitro, and genetically modified to carry a CAR, which serves as a targeted navigation tool to direct T lymphocytes to identify certain tumor cells within the body. The patient receives a second infusion of the CAR-T cells, or altered T cells. The CAR molecule experiences aggregation and stability upon recognition and binding by the scFv, which then starts a signaling cascade. An important stage in this process is the phosphorylation of the ITAM motif on the CD3ζ chain, which, as Figure 2 [7] illustrates, starts signaling via the 70 kDa ζ-associated protein (ZAP70), a signaling pathway similar to the T-cell receptor (TCR). T cell effector responses result from this, and these include cytokine release, metabolic changes, proliferation, and cytotoxic consequences. Although granzymes and perforins are the major ways that CAR-T cells cause tissue damage, there is evidence that they also use death receptors and BH3-interacting

structural domain death agonists (BIDs) as downstream molecular activation pathways. Granzyme and perforin are the primary cytotoxic agents secreted by CAR-T cells. These agents have the power to damage tumor cells' cell membranes and ultimately cause tumor cell death. Furthermore, CAR-T cells have the ability to alter the proteins at the tumor site, which eliminates or lessens the ability of cancer cells to cause harm. This treatment not only successfully eradicates tumor cells but also increases the patients' time to survival. In CAR-T cell treatment, the co-stimulatory structural domains are also crucial. These structural features can improve T cell activation, proliferation, and persistence, which will increase CAR-T cell killing of tumor cells.

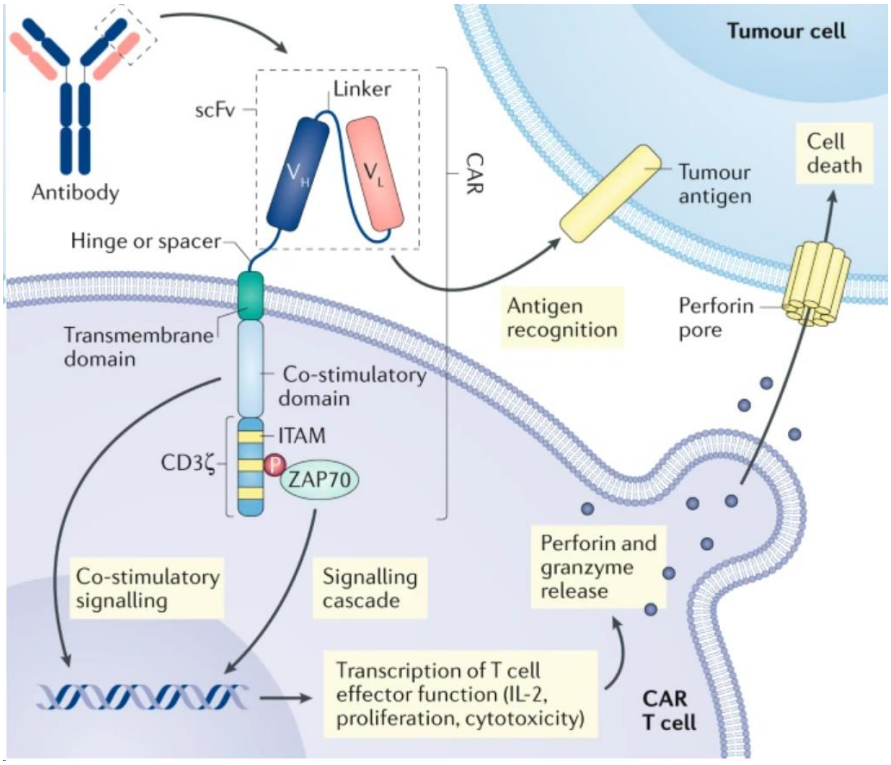

Fig 2. The mechanism of CAR-T cell therapy [7].

3. Literature References

3.1. GPC3

Because of its tumor selectivity, GPC3 (phosphatidylinositol proteoglycan 3) is seen as a very promising target for tumor immunotherapy. GPC3 expression is often elevated in hepatocellular carcinoma (HCC) and is linked to a poor prognosis. GPC3 is therefore a prime candidate for CAR-T cell treatment.

Researchers created chimeric anti-GPC3 scFv-CD3ε modified T cells (also referred to as CT0180) in preclinical experiments by fusing a fusion protein of anti-GPC3 single-chain variable fragment (scFv) with CD3ε to change T cells. This altered T cell was able to identify and attach to GPC3 on the surface of hepatocellular carcinoma cells, which caused the T cell to go into death mode and deal a targeted blow to the cancerous cells [8].

The results showed that CT0180 displayed competitive anti-tumor activity compared with 28ζ or BBζ chimeric antigen receptor T cells and exhibited superior performance in some aspects, such as lower cytokine release. This means that CT0180 may trigger fewer side effects during treatment.

Furthermore, several research groups have created allogeneic anti-GPC3 CAR-T cells by gene editing using CRISPR/Cas9 technology. These cells use a variety of gene editing techniques to improve anti-tumor efficaciousness and decrease graft-versus-host disease and host rejection. These allogeneic anti-GPC3 CAR-T cells demonstrated notable cytotoxicity against GPC3-expressing hepatocellular carcinoma cell lines both in vitro and in vivo, according to experimental data [9].

3.2. MUC1

Preclinical studies of MUC1-targeted CAR-T cells have also shown potential and promise in the field of hepatocellular carcinoma. Hepatocellular carcinoma and other malignancies are tightly linked to the abnormal expression of MUC1, a transmembrane glycoprotein, at the initiation and development of these diseases. MUC1 is a possible target for CAR-T cell treatment in hepatocellular carcinoma since it frequently corresponds with tumor aggressiveness, invasiveness, and propensity to spread.

Through genetic engineering, researchers have created MUC1-targeted CAR-T cells in preclinical experiments by adding chimeric antigen receptors (CARs) that are particularly able to identify MUC1 into T cells. For therapeutic purposes, these altered T cells were able to identify and target MUC1-expressing liver cancer cells with precision. The results showed that MUC1-targeted CAR-T cells demonstrated potent killing ability against liver cancer cells in in vitro experiments. They were able to specifically bind to MUC1 on the surface of hepatocellular carcinoma cells and activate the killing mechanism of T cells, leading to the death of hepatocellular carcinoma cells. In addition, these CAR-T cells have shown a better safety profile with relatively few side effects [10].

However, it should be noted that preclinical studies on MUC1-targeted CAR-T cells in liver cancer are still in their infancy. Despite the encouraging preliminary results, further clinical trials are needed to validate its efficacy and safety. In addition, the heterogeneity of hepatocellular carcinoma is a factor to be considered; hepatocellular carcinoma cells from different patients may have different antigen expression profiles, and thus CAR-T cell therapy targeting MUC1 may not be applicable to all hepatocellular carcinoma patients.

3.3. NKG2D

Preclinical research on liver cancer has demonstrated the promise and effectiveness of NKG2D-targeted CAR-T cell treatments. Natural killer cells (NK cells) and certain T-cell subsets express NKG2D (Natural Killer Cell Receptor Group D), an activating receptor that can detect a variety of ligands on the surface of tumor and virus-infected cells.

In preclinical models, researchers used genetic engineering techniques to construct CAR-T cells expressing the NKG2D receptor so that they could specifically recognize and attack liver cancer cells expressing the NKG2D ligand. The main advantage of this therapy is that the NKG2D ligand is widely expressed on the surface of a wide range of tumor cells, including liver cancer cells, making it more broadly applicable. The results of the study showed that NKG2D-CAR-T cells demonstrated the ability to kill liver cancer cells in both in vitro and in vivo experiments. They were able to effectively recognize and remove hepatocellular carcinoma cells, thereby inhibiting tumor growth and spread. In addition, NKG2D-CAR-T cells may have lower off-target effects and fewer side effects than conventional CAR-T cell therapies because they work by recognizing natural ligands on tumor cells [11].

Nevertheless, more research and clinical trials are required to confirm the safety and effectiveness of NKG2D-CAR-T cell treatments, which are now in the preclinical research stage. It is especially necessary to assess its effectiveness in terms of anti-tumor activity, durability, and potential adverse effects in people.

4. Conclusion

CAR-T cell therapy is a novel therapeutic approach for the treatment of liver cancer, a serious medical condition. This article describes the use of CAR-T cells in the treatment of liver cancer and addresses their possible importance as well as future approaches.

Not every patient with liver cancer responds well to CAR-T treatment, though. This is primarily because liver cancer is complicated and heterogeneous, making it challenging for CAR-T cells to eradicate all liver cancer cells. The cost of CAR-T cell therapy preparation is rather costly because each patient's treatment plan must be unique. Furthermore, there are some hazards connected to liver

cancer treated with CAR-T cells. Patients may suffer from severe responses, including neurotoxicity and cytokine release syndrome (CRS), following the reinfusion of CAR-T cells. The patient's health may be impacted by these bad effects, and they could even be fatal. Above all, it is unclear if CAR-T will be effective in treating liver cancer in the long run. While there may be some short-term therapeutic benefits associated with CAR-T cell therapy for liver cancer, further clinical research is necessary to demonstrate the treatment's long-term success.

Precise design for CAR-T cells is an important research direction in the future. With the continuous progress of gene editing technology, researchers can design CAR-T cells more precisely so that they can recognize and attack liver cancer cells more accurately. This can not only improve the therapeutic effect, but also reduce the occurrence of adverse reactions. However, one of the avenues for future research and development is the combined therapy program of CAR-T therapy for liver cancer. Enhancing the therapeutic impact can be achieved by combining CAR-T therapy with other therapies (e.g., radiation, chemotherapy, surgery, etc.) that work in concert. Future goals should also include lowering the price and expanding access to CAR-T treatment. It is anticipated that more liver cancer patients will have access to CAR-T therapy as a therapeutic option because to improvements in the manufacture of CAR-T cells, lower production costs, and more governmental support. It is anticipated that CAR-T therapy will become more crucial in the treatment of liver cancer as a result of ongoing research efforts and technological advancements.

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