

Application of CAR-T cell therapy in the treatment of liver cancer

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Abstract. Liver cancer is a frequent malignant tumor with a high fatality rate. However, due to the challenges of early detection and the potentially significant dangers and side effects connected with conventional cancer therapy approaches. Immunotherapy has been a well-liked cancer treatment in recent years due to its efficacy and safety. One of these is chimeric antigen receptor T cells (CAR-T), genetically modified T lymphocytes that recognize and activate T lymphocytes in response to antigens specific to tumors. They aid in the elimination of tumors. Its therapeutic efficacy in the treatment of solid malignancies is currently the subject of extensive research. Despite reaching its fifth generation, CAR-T cell therapy still faces a number of challenges when treating solid malignancies like liver cancer. As a result, this article begins with describing the creation and workings of CAR-T cells. The function it plays in treating liver cancer, including pursuing several ethical goals, was then highlighted. Future treatments for liver cancer and potentially other solid tumors can use this as a benchmark.

Keywords: Liver cancer, CAR-T, immunotherapy, targeted therapy.

1. Introduction

Liver cancer is a frequent malignant tumor with a high fatality rate. Traditional treatment methods have serious drawbacks. Only one area of the body at a time may be surgically removed from the tumor, but tumor cells can spread to other areas of the patient's body via the blood and lymphatic system, which can have unforeseen effects such as tumor recurrence. Chemotherapy and radiotherapy stop the development of cancer cells by destroying them within the body, but they also cause harm to healthy cells that cannot be disregarded. The majority of patients are detected in the medium to late stages of the disease since it is challenging to make an early diagnosis of liver cancer. The success of conventional treatment approaches is limited, and patients' 5-year survival rates cannot be guaranteed.

Due to its effective and long-lasting anti-tumor effects on the body, immunotherapy has recently attracted a lot of attention. Examples include immune checkpoint inhibitors, cancer vaccines, and immune cell therapies. One of these, CAR-T, has attracted considerable interest as an immune cell therapy because to its extraordinary safety and effectiveness. It has been used to treat hematological cancers with effectiveness. As research into CAR-T cell therapy has advanced, interest in its application to the treatment of solid tumors has also increased. This article examines the use of CAR-T cell therapy for the treatment of liver cancer in light of how it functions.

2. Mechanism and Development of CAR-T Cell Therapy

2.1. Mechanism

Clinically, most CAR-T cell treatments go as follows: leukapheresis, the selective removal of blood cells from patients, engineering and CAR implantation of T cells, growth, and re-infusion of CAR-T cells into the patients. After activation, CAR-T cells are designed to attack cancer cells in one of three ways: 1) by releasing a significant amount of perforin granase to disrupt the plasma membrane of the cancer cells, 2) by triggering the FAS-FASL pathway to induce spontaneous programmatic death, or 3) by releasing a variety of cytokines to either directly or indirectly subdue

tumor cells and create a TME that inhibits tumor growth [1]. The structure of CAR-T cells is shown in Figure 1 [2].

One may argue that CAR-T cell therapy has benefits over several common medical procedures. Because scFv may induce MHC-independent T-cell activation, showing that antigen targets can be more diverse, tumor cells lacking MHC can be aggressively targeted and destroyed. The intracellular domain of CAR-T cells also contains a costimulatory signaling region that promotes stronger, faster immune responses as well as a longer lifespan. Additionally, CAR-T cells are far stronger in homing and tissue penetration than antibody-based medications. Not to add, CAR-T cells have the capacity to grow continuously and transform into memory T cells in vivo [1]. All of the aforementioned traits help explain why CAR-T cell therapies are so highly successful.

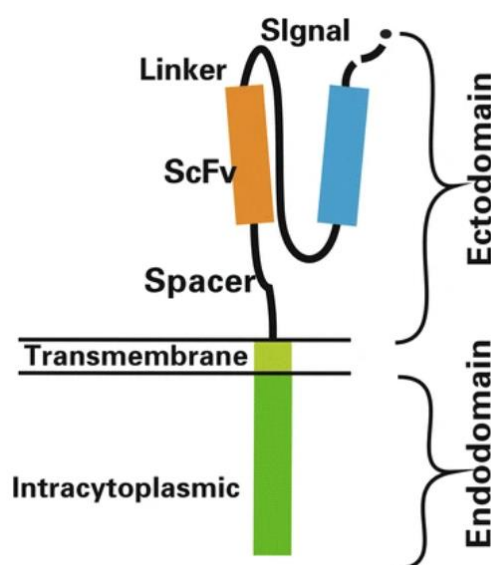


Fig. 1 The structure of CAR-T cells [1].

2.2. Development

The intracellular domain of CAR-T cells underwent a lot of modifications and iterations to obtain high efficacy. The intracellular domain for the initial generation only contains immunoreceptor tyrosine-based activation patterns that stimulate CAR-T cells. The costimulatory molecule CM1 is introduced for the second generation. The intracellular domain of the second generation's second generation receives one extra CM for the third generation. The second generation's intracellular domain of the fourth generation, also known as TRUCKs, has an inducer of a constitutively or reducibly produced chemokine added to it in order to provide universal cytokine-mediated death. The second generation's intracellular domain has been supplemented with an intracellular cytokines-receptor in the fifth generation. Iteration of the intracellular domain is only a fraction of the whole development of CAR-T cell therapy. Other domains, like the antigen binding domain, are also carefully tailored by scientists to heighten efficacy. The development and treatment principles of CAR-T cells are shown in Figure 2 [3].

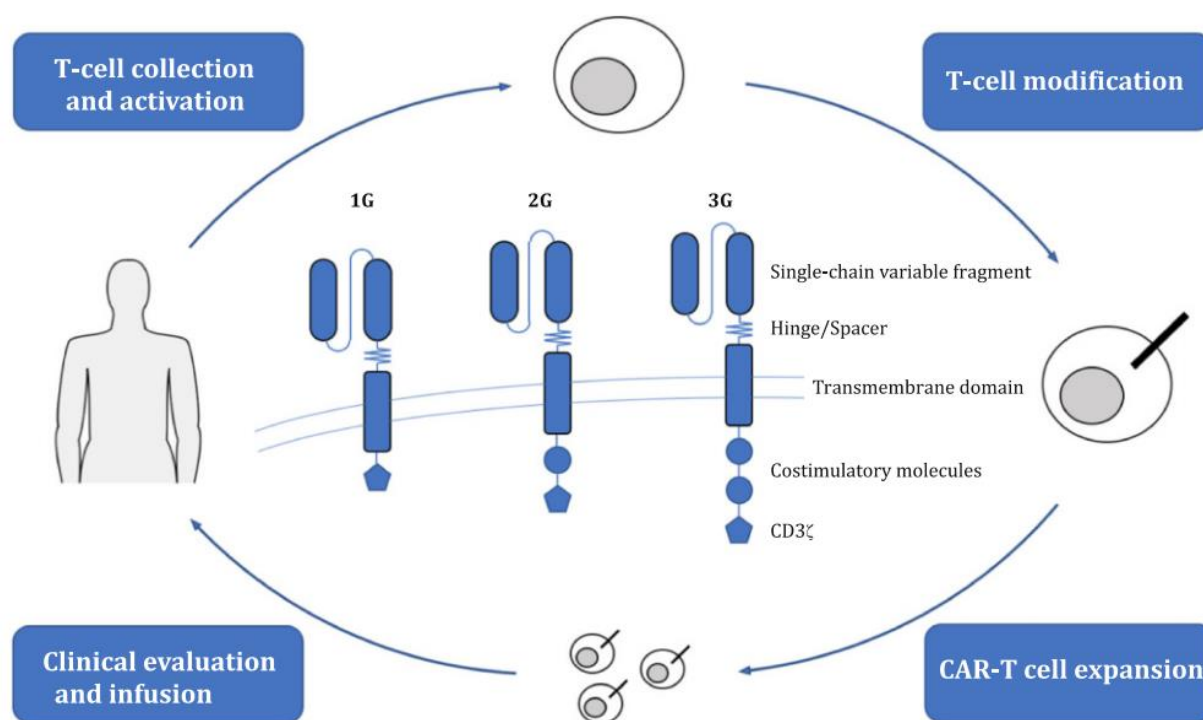


Fig. 2 development and therapeutic principles of CAR-T cells [3].

3. The role of CAR-T cell therapy in the treatment of liver cancer

3.1. Glypican-3 (GPC3)

One of the most critical targets of CAR-T cell therapy for HCC is GPC3. Heparan sulfate proteoglycan with a 580-AA size. GPC3 is overexpressed in several malignant cancers, including hepatocellular carcinoma and melanoma. 53% of HCC patients had substantially higher GPC3 serum levels, according to Capurro et al. [4]. Additionally, it has been hypothesized that GPC3 functions through activating the Wnt signaling pathway to support the growth of HCC. It is possible to identify HCC by looking for GPC3 overexpression. Morgan et al. discovered that HCC cells' capacities to proliferate and invade were hampered when GPC3 expression was inhibited, indicating that GPC3 expression on HCC cells was involved in these activities [5]. Therefore, GPC3 is used as a target in the diagnosis and therapy because of its high sensitivity and excellent specificity.

3.2. Alpha-fetoprotein (AFP)

A glycoprotein that is secreted, AFP has a molecular weight of about 70 KD, 591 amino acids, and 4% carbohydrate residues. The physiological functions of AFP include transport, immunosuppression, and activation of apoptosis. There is hepatic inflammation and expression in 60% to 80% of HCC cases. AFP is expressed in MHC class I molecules in addition to intracellularly (where it is transformed into peptides) and on the cell surface. Biology-wise, AFP promotes tumor growth, and the body's extremely high levels of AFP expression portend poor prognosis. However, they were treated as CAR T cells due to problems with AFP localization. Designing therapeutic targets remains difficult.

Regarding clinical studies, AFP-CAR-T (NCT03349255), which was administered through intravenous injection and intrahepatic artery therapy for AFP-positive HCC, was stopped and no results have yet been published. AFP objectives, though, seem to still have a lot of unrealized potential. There are several intriguing therapeutic applications if the localization problem can be resolved [6].

3.3. Mucin 1 (MUC1)

Immunohistochemistry study has identified MUC1, a membrane-bound glycosylated phosphoprotein that is overexpressed in HCC, as a potential HCC target. 67.7% of patients who tested positive for MUC1 had metastases within three years, compared to 31% of those who did not express MUC1. MUC1 is hence a possible target for CART liver cancer [7].

3.4. Natural-killer group 2 member D (NKG2D)

Immunotherapeutic drugs may employ NKG2DL to treat HCC since it is overexpressed in the illness. SMMC-7721 and MHCC97H cell lines showed a special cytotoxicity in third generation NKG2DL-CAR-T in vitro experiments. MICA and ULBP2 cells were resistant to cytotoxicity with NKG2DL-dependent NKG2DL. With high NKG2DL, imaging is imaging susceptibility for HEP3B. In contrast to the NKG2DLs-negative cell line, NKG2D-BBz CART cells lysed HCC cells with high expression of NKG2DLs, according to a study by Sun et al. [8].

4. Limitations and strategies of CAR-T cell therapy

According to previous studies, scientists have disclosed four categorized limitations: antigen escape, immune-suppressive TME, on-target off-tumour effect, and poor trafficking and infiltration ability.

4.1. Antigen escape

Antigen Escape is one of the most formidable difficulties that challenge traditional CAR-T cell therapy. Studies show that refractory Acute Lymphocytic Leukemia (ALL) patients may possess downregulation of CD19, while refractory myeloma patients may possess downregulation of B-cell maturation antigen (BCMA). What's more, the downregulation of IL13Ra2 in refractory glioblastoma patients indicates that solid tumour is capable of developing antigen escape as well. Antigen escape exhibits a severe decrease in efficacy in CAR-T cells since the crucial process of specific binding is disabled.

It is possible to deploy tandem or double-targeted CAR-T cells since malignant cells have a propensity to develop antigen escape of a single targeted CAR-T cell. The longevity and rate of lasting remission of these CAR-T cells are significantly increased, which also greatly reduces the impact of antigen escape. These CAR-T cells are functioning properly if the target antigen is found. In a trial of six patients with B cell malignancies, the use of bispecific CAR-T cells that target both CD19 and CD22 demonstrated encouraging results [9].

4.2. Immune-suppressive TME

Although the avoidance of the MHC pathway will tremendously facilitate antitumor efficacy, there are still other factors that may cause immunosuppressive TME, including but not limited to immunosuppressive cells.

A trial using CD19 targeted CAR-T cell therapy combined with pembrolizumab to treat 14 children with B-cell malignant tumors showed extraordinary safety and efficacy. It eliminates TGF in CAR-T cells through CRISPR-CAS9- β Genes, making them more resistant to immunosuppressive signals, will help rebalance immunosuppressive TME.

4.3. On-target off-tumor effect

The antigen produced in tumor cells can occasionally also be expressed in normal cells, especially in solid tumors. This can lead to excessive CAR-T cell activation and simultaneous death of normal tissues. On-target off-tumor impacts is the name given to this phenomenon.

Current strategies to prevent on-target off-tumor effects focus on selecting antigens that are effective against cancers while also minimizing on-target off-tumor effects. Another strategy would be to design CARs that can activate CAR-T cells by binding to both of the targeted antigens. For

instance, the on-target off-tumor impact of synNotch CAR-T cells is highly constrained since they need to recognize both the priming antigen EGFRvIII and the death antigen EphA2 or IL13R2 in order to activate [10].

4.4. Poor trafficking and infiltration ability

The difficulty in treating solid tumors is also due to the CAR-T cells' poor penetration and trafficking into tumor lesions. Unlike haematological tumours, CAR-T cells intended to treat solid tumours must get past physical obstacles including tumour stroma and immunosuppressive TME. As a result, their mobility and penetration rate are significantly reduced.

The direct delivery of CAR-T cells into tumor lesions will reduce trafficking distance and on-target off-tumor toxicity. However, this approach is only effective in oligometastatic cancers. Since CAR-T cells would be drawn to chemokines produced by tumors, giving CAR-T cells a tumour-derived chemokine receptor also dramatically improves trafficking. Last but not least, the inclusion of heparanase, a decomposer of heparin sulfate proteoglycan, a key component of tumor stroma that inhibits infiltration, permits the destruction of the physical barrier. Heparanase is a decomposer of heparin sulfate proteoglycan.

To encapsulate, various methods to overcome the four chief limitations are under active development. Nonetheless, there is still a long way to go before the industrialization of numerous promising CAR-T cell therapies. Apart from efficacy and safety, cost and feasibility in the industrial aspect still need more importance attached.

5. Summary

Although CAR-T immunotherapy has showed promise in the treatment of solid malignant tumors like liver cancer, extensive clinical research is still being conducted on it. The lack of precise antigen targets, tumor microenvironment suppression, and inadequate CAR-T infiltration in liver cancer tissue are the main obstacles to employing CAR-T immunotherapy for the treatment of liver cancer. Although preliminary evidence from a number of solid tumors indicate that CAR-T treatment is typically safe and that its toxicity and side effects are controllable, these factors are nevertheless crucial to take into account. Immune/immune combination therapy using immunological checkpoint inhibitors has produced encouraging clinical outcomes for patients, but it still faces problems with primary and secondary drug resistance. It is a common direction that new tumor immunotherapies are always being explored. Population adaptability, cell dosage, infusion methods, and the best combination treatment regimens are further issues that require research. I believe that with the continuing development of CAR-T immunotherapy, improved treatment results for liver cancer and even other tumors may be achieved.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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