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Advances in CAR-T cell therapy in HCC

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ABSTRACT
Because of the limited efficacy of conventional therapies, Hepatocellular carcinoma (HCC) is a global health concern. A new therapeutic strategy, chimeric antigen receptor T-cells (CAR-T), has demonstrated considerable potential in treating HCC. This paper reviews the structure and mechanism and clinical application of CAR-T cells to treat HCC, with a particular focus on experimental research targeting different antigens and the combination of CAR-T CAR-T and other therapies.

KEYWORDS
HCC; CAR-T cell; Immunotherapy; Treatment

1 Introduction

Hepatocellular carcinoma (HCC) is the most frequently seen primary liver cancer, accounting for over 90% of cases, and the third leading cause of cancer-related deaths[1]. Approximately 70% of the cases are diagnosed in intermediate to late stages and require systemic treatment[3].

Early-stage HCC treatments, including liver transplantation and surgical resection, are not feasible for the vast majority of patients due to high costs, liver insufficiency, and a high risk of recurrence. Therefore, most patients are treated by non-surgical methods such as TACE, RFA, and PEI.

Recently, systemic molecular therapy has become prevalent for advanced HCC, with first-line drugs (e.g. multi-tyrosine kinase inhibitors) and second-line drugs (e.g. anti-angiogenic drugs)[4].In addition, the development of immunologic and targeted drug therapies has brought CAR-T therapies to the forefront of cancer treatment, offering significant benefits to HCC patients. However, there are several problems with CAR-T cells, including the immune microenvironment and side effects, such as CRS and hemophagocytic lymphohistiocytosis[5].

This article reviews the structure and mechanism of CAR-T cells and their clinical progress in treating HCC, emphasizing the latest experimental research on combining different target CAR-T cells with other therapeutic strategies, and providing a reference for future research and clinical applications.

2 CAR-T

2.1 Cellular Composition

CAR-T therapy represents a promising approach to anti-tumor. Genetic modification of T lymphocytes for tumor killing is its main feature. CAR-T cells consist of three major functional regions: the extracellular region (ECD), the transmembrane region (TMD), and the intracellular region (ICD). The ECD is responsible for recognizing and binding tumor-specific antigens, the TMD is responsible for signaling, and the ICD is responsible for enhancing activated signals[6].

Five generations of CARs have been developed for T cell therapy, each improving the areas of intracellular signals needed for T cell activation. The first generation is made up of a single bond structural domain (scFv) and a CD3ζ signaling structural domain. In the second and third generations designed afterward, co-stimulatory structural domains (e.g. 4-1 BB) were added to improve activation, proliferation, and immune tolerance. The fourth and fifth generations further integrate additional cytokines or chemokines (e.g. interleukin-12) to regulate the tumor microenvironment, enhance T-cell function, and recruit other immune cells[7].

2.2 CAR-T cell mechanism

CAR-T cells enhance the response of T cells to cells expressing specific antigens. After preparation and injection into the human body, CAR-T cells activate signaling domains (e.g. CD3ζ) within the CAR by specifically identifying and binding to target antigens on the surface of cancer cells through scFv, thereby triggering an intracellular signaling cascade. After being activated, CAR-T cells will kill and injure tumor cells by directly releasing cytotoxins (e.g. perforin and granzyme), secreting cytokines (e.g. IFNγ, TNF-α), and activating and attracting other immune cells (e.g. macrophages) to target tumor cells expressing the antigens[8].

3 CAR-T Application for HCC

Recent advancements in CAR-T therapy for malignant hematological diseases have highlighted its potential in treating hepatocellular carcinoma. CAR-T therapy has become a new therapeutic tool in HCC, with potential targets including Glypican-3 (GPC3), CD133 and AFP. While CAR-T cells can specifically target HCC positive for these antigens, there is a risk of toxicity to normal cells expressing the target antigen[9]. For solid tumors like HCC, specific antigens are rare and lack specificity. Consequently, the extracellular structure of CAR-T cells is being optimized to enhance specificity, combat antigen escape, and mitigate toxicity. Research on co-targeted CAR-T cells, which can specifically kill two target antigen-positive tumor cells, is ongoing, with clinical results showing superiority. Among them, Dual CAR-T involves co-expressing multiple CARs in one single T cell, while tandem CAR-T involves tandem expression of multiple scFv in a single CAR[6].

3.1 GPC3

GPC-3 is an acetyl heparin sulfate proteoglycan, and most HCC patients have significantly elevated sGPC3N levels. Due to its highly specific and sensitive properties, GPC3 has become a prominent target[10].

Shi et al.[11] successfully constructed the first CAR-GPC3T cells for treating advanced HCC, demonstrating survival rates of 50%, 42%, and 10% at 6 months, 1 year, and 3 years, respectively, in 13 patients. Most patients tolerated the treatment well, with two achieving partial remission and one maintaining stable disease for over 44.2 months. GPC3 is more highly expressed in HCC. In another clinical trial, Pang et al. [12] co-transfected CAR vectors with 7×19 lentivirus to T cells, resulting in complete tumor disappearance in one patient after 30 days of anti-GPC3-7×19 CAR-T treatment.

To address the limitations of single antigen targeting, in one clinical trial[13], researchers constructed GPC3-BiTE CAR-T cells, which demonstrated stronger cytotoxicity and effectively overcame immune escape compared with single-target CAR-T. In another phase I clinical trial[14], among six patients treated with CT017CAR-T cells, expressing both CAR-GPC3 and RUNX3, one achieved PR and two had stable disease, with progression-free survival, median survival, with PFS, MST and OS of 3.5, 3.2, and 7.9 months, respectively. This again demonstrated the benefits of dual-target CAR-Ts, providing preliminary evidence for a safe alternative treatment option for patients with advanced HCC, further improving the effectiveness and safety of tumor cell killing.

3.2 CD133

CD133 is a transmembrane glycoprotein associated with cancer-related signaling pathways and cell proliferation, linked to patient survival and post-treatment relapse. CD133 is a promising target highly expressed in most HCCs, allowing for the elimination of cancer stem cells with low associated toxicity[15].

In a clinical trial[16], CAR-T-133 cell therapy was administered to 21 patients. The results testified a median overall survival (OS) of 12 months and a median progression-free survival (PFS) of 6.8 months. CAR-T-133 cells reached their highest frequency between 7 and 21 days after infusion. Of these patients, 14 patients among them had stable disease after the first cell infusion. This testified promising anti-tumor activity. Some side effects were within manageable limits and testified to a manageable safety profile.

Another study[17] generated bispecific CAR-T (CD133 and GPC3) cells by transfecting mcDNA vectors into T cells via an electroporation system. The bispecific CAR-T cells testified a stronger capacity to eliminate GPC3 and CD133 double-positive HCC cells. No serious "off-target" effects on major organs were detected.

3.3 AFP

AFP is a secreted glycoprotein highly expressed in hepatocellular carcinoma.

Liu et al.[18] constructed an entirely human chimeric antigen receptor ET1402L1-CAR (AFP-CAR), demonstrating that AFP-CAR-T cells selectively bind the associated tumor surface antigen in vivo while avoiding damage to negative cells of multiple tissue types, exhibiting potent antitumor activity, providing a new avenue for HCC immunotherapy.

In one phase I clinical trial[19] of 15 HCC patients, AFP-derived peptides (AFP357 and AFP403) were administered subcutaneously, with one patient achieving complete remission, eight achieving stable disease, and six experiencing progression. No significant toxic side effects were observed, demonstrating safety and immunogenicity in HCC patients. However, the impact of AFP-derived peptides may be limited to serum AFP-positive patients, and further investigation is needed due to the small sample size.

3.4 c-MET

Aberrant activation of c-Met promotes the development and progression of many cancers, including HCC. CAR-T therapies targeting c-Met hold substantial promise for the treatment of HCC and other malignancies[20].

Huang et al.[21] constructed 2G and 3G c-Met CAR-T cells. It was demonstrated that c-Met CAR-T cells successfully targeted and eliminated c-Met+ HCC cells, showing high targeting and tumor-killing effects. Moreover, 3G CAR-T cells exhibited greater specificity and efficiency. It is expected that this will broaden the pathway for targeted therapy of c-Met+ hepatocellular carcinoma.

Further research on MET-specific CAR-T cells[22] was conducted by constructing CARs containing either CD28ζ or 4-1BBζ co-stimulatory domains. Both types of T cells demonstrated specific cytotoxicity against c-Met+ HCC cells. However, MET-CAR.CD28ζ T cells, while showing stronger anti-tumor effects, also exhibited higher T cell exhaustion, highlighting the need for strategies to mitigate T cell depletion and enhance their therapeutic efficacy in vivo.

3.5 Other targets

Several other transmembrane glycoproteins have been identified as promising therapeutic targets for HCC.

Among them, MUC1, a transmembrane glycoprotein implicated in HCC invasion and metastasis, is overexpressed in abnormally glycosylated forms in many tumor tissues. This makes MUC1 a potential molecular target for therapy[20]. Ma et al.[23] constructed targeting glycosylated MUC1 CAR-T cells. Their results revealed that both CAR-T generations specifically killed MUC1-overexpressing HCC cells, with 3G CAR-T cells demonstrating superior proliferation rates, IL-2 secretion, and cytotoxic efficiency compared to 1G CAR-T cells. However, as this research was primarily conducted at the cellular level, further validation through clinical trials is required (NCT02587689).

Another potential target is NKG2D[24], a transmembrane glycoprotein whose ligand (NKG2DL) is overexpressed in tumor cells. A novel CAR-T design incorporating NKG2D-BBζ was shown to eliminate HCC cells with high NKG2DL expression effectively while sparing HCC cells with silenced or deleted NKG2DL genes. This suggests that NKG2D-based CAR-T therapy is both safe and reliable, providing an alternative option for patients with NKG2DL-positive HCC.

EpCAM, a highly expressed transmembrane glycoprotein in HCC, also presents a potential therapeutic target. Currently, multiple trials (NCT02729493, NCT03013712, NCT05028933) are underway to evaluate the efficacy and safety of EpCAM CAR-T cells in HCC patients[20].

DLK1, another transmembrane protein with upregulated expression in HCC, has also been studied as a therapeutic target. Zhai et al.[25] conducted an ex vivo trial evaluating DLK1-targeted CAR-T cells, demonstrating potent cytotoxic activity on DLK1-positive HCC cells, which effectively inhibited the growth of the HepG2 hepatic carcinoma cell line.

4 CAR-T combination therapy

There are still a lot of problems in the treatment of CAR-T cells in HCC, particularly those associated with the tumor microenvironment (TME)[26]. TME factors lead to limited CAR-T therapy for HCC, which are mainly manifested in the inhibition of CAR-T cell infiltration, inhibition of the function and activity of CAR-T cells, antigen escape, heterogeneity of tumor antigen expression, and susceptibility to off-target effects[6]. In addition, this treatment induces multiple side effects, such as CRS, hemophagocytic lymphohistiocytosis, and CAR-T cell-associated encephalopathy syndrome[26]. To overcome these obstacles, innovative strategies are being actively investigated, including co-targeting CAR-T designs as mentioned in the above-related experiments, blocking immune checkpoint pathways, upregulating T-bet expression, and combining with other treatment modalities[6].

4.1 Blocking the PD-1/PD-L1 pathway

PD-1/PD-L1 inhibits T-lymphocyte activation and is involved in immune escape from solid tumors. The PD-1/PD-L1 signaling pathway is critical for TME-mediated immunosuppression.

A study[27] developed GPC3-28Z-sPD1 CAR-T cells. The study demonstrated that the anti-tumor activity of GPC3-specific CAR-T cells could be enhanced by inhibiting the PD-1/PD-L1 pathway. These modified CAR-T cells provide a novel strategy to enhance the efficacy of CAR-T cell therapy by blocking the PD-1/PD-L1 pathway to overcome T-cell depletion. Similarly, another preclinical study[28] engineered dual-targeted CAR-T cells containing anti-PD-1 and anti-GPC3 scFvs. These cells showed manifested stronger tumor-killing effects and longer survival in the HCC model. However, this study had the limitation of not considering other immune cells in the TME.

4.2 Tyrosine kinase inhibitors or CKIs

It has been shown to increase therapeutic efficacy by combining CAR-T with a tyrosine kinase inhibitor (TKI) or checkpoint inhibitor (CKI).

Sorafenib is an effective first-line therapeutic agent for advanced HCC, Sun et al[29] reported a case of Asian HCC with

recurrent and pulmonary metastasis following resection of liver cancer, followed by CAR-GPC3 T-cells (CT011) combined with sorafenib. It was subsequently found that the patient achieved PR at month 3 and CR at month 12 after the first infusion, enhancing the local immune response and the tumor-killing effects of the GPC3 CAR-T cells. This therapy is expected to be a key treatment for GPC3 + HCC. Other TKIs (e. g. sunitinib) and CKIs (e. g. nivolumab, pembrolizumab, tremelimumab) have also been suggested to be combined with CAR-T therapy. However, individualized treatment approaches are necessary, taking into account patients ' immune profiles[30].

4.3 T-box (T-bet)

T-bet, a transcription factor critical for Th1 differentiation, is a major regulator of anti-tumor activity mediated by CD4+ T helper cells. A study testified that constructed B7H6-specific CARs increased T-bet expression and secreted more pro-inflammatory and Th1 cytokines while decreasing Th2 cytokine secretion by fusing with T-bet or its variants. Therefore, it can be hypothesized that upregulation of T-bet expression can improve the immune function of CAR-T cells[31].

4.4 Bionanotechnology

Combining the benefits of nanocarriers with cellular immunotherapy is suitable for targeted drug delivery with enhanced efficacy and safety.

Tang et al..[32] designed protein nanogels (NGs) to deliver higher doses of cytokines without inducing toxicity. These nanogels enabled selective drug release and enhanced CAR-T therapeutic efficacy. Ma and colleagues[33] prepared a novel photothermal therapeutic agent by coating constructed GPC3 CAR-T cells with IR780-loaded mesoporous silica nanoparticles. This approach demonstrated superior targeting and anti-tumor effects, suggesting its potential in advancing CAR-T-based cancer therapy.

4.5 Radiotherapy and chemotherapy

Combining CAR-T therapy with chemotherapy or radiotherapy has also been explored as a strategy to improve clinical outcomes. Chemotherapy and radiotherapy can modulate TME, and disrupt tumor stroma, potentially enhancing CAR-T efficacy. However, they may also reduce CAR-T cell activity, underscoring the need for careful optimization of such combination therapies[7].

5 Conclusion

CAR-T cell therapy is one of the most promising treatments in the international arena. There are still severe challenges for CAR-T therapy for HCC and other solid tumors, toward impeded tumor cell infiltration, immunosuppression, immune escape, and low specificity, as well as toxicities and side effects such as CRS. Innovative strategies, such as optimizing CAR-T cell design, employing multi-targeted approaches, and integrating other therapeutic modalities, have made significant progress but remain insufficient to address all obstacles. Therefore, further research is still needed to solve these problems. Looking forward to the future application of CAR-T cells in the treatment of HCC by further exploring TME, continuing the experiments and research on CAR-T cell therapy and the strategy of combining it with multiple treatments, and focusing on the improvement of the safety and effectiveness of CAR-T cell therapy.

References

[1] Rumgay, H. et al. Global burden of primary liver cancer in 2020 and predictions to 2040. J. Hepatol. 77, 1598–1606 (2022).

[2] Oura K., Morishita A., Tani J. & Masaki T. Tumor immune microenvironment and immunosuppressive therapy in hepatocellular carcinoma: A review. Int. J. Mol. Sci. 22, 5801 (2021).

[3] Crocetti L., Bargellini I. & Cioni R. Loco-regional treatment of HCC: Current status. Clin. Radiol. 72, 626–635 (2017).

[4] Llovet, J. M. et al. Hepatocellular carcinoma. Nat. Rev. Dis. Primer 7, 6 (2021).

[5] Zhao M., Huang H., He F. & Fu, X. Current insights into the hepatic microenvironment and advances in immunotherapy for hepatocellular carcinoma. Front. Immunol. 14, 1188277 (2023).

[6] Shen, J., Yang, D. & Ding, Y. Advances in promoting the efficacy of chimeric antigen receptor T cells in the treatment of hepatocellular carcinoma. Cancers 14, 5018 (2022).

[7] Ma, H. Y. et al. Advances in CAR T cell therapy for non-small cell lung cancer. Curr. Issues Mol. Biol. 45, 9019–9038 (2023).

[8] Benmebarek, M.-R. et al. Killing mechanisms of chimeric antigen receptor (CAR) T cells. Int. J. Mol. Sci. 20, 1283 (2019).

[9] Khan, A. A., Liu, Z.-K. & Xu, X. Recent advances in immunotherapy for hepatocellular carcinoma. Hepatobiliary Pancreat. Dis. Int. HBPD INT 20, 511–520 (2021).

[10] Shen, K.-Y., Zhu, Y., Xie, S.-Z. & Qin, L.-X. Immunosuppressive tumor microenvironment and immunotherapy of hepatocellular carcinoma: Current status and prospectives. J. Hematol. Oncol.J Hematol Oncol 17, 25 (2024).
[11] Shi, D. et al. Chimeric antigen receptor-glypican-3 T-cell therapy for advanced hepatocellular carcinoma: Results of phase I trials. Clin. Cancer Res. 26, 3979–3989 (2020).
[12] Pang, N. et al. IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. J. Hematol. Oncol.J Hematol Oncol 14, 118 (2021).
[13] Cao, G. et al. GPC3-targeted CAR-T cells secreting B7H3-targeted BiTE exhibit potent cytotoxicity activity against hepatocellular carcinoma cell in the in vitro assay. Biochem. Biophys. Rep. 31, 101324 (2022).
[14] Fu, Q. et al. RUNX-3-expressing CAR T cells targeting glypican-3 in patients with heavily pretreated advanced hepatocellular carcinoma: A phase I trial. eClinicalMedicine 63, 102175 (2023).
[15] Schmohl, J. & Vallera, D. CD133, selectively targeting the root of cancer. Toxins 8, 165 (2016).
[16] Dai, H. et al. Efficacy and biomarker analysis of CD133-directed CAR T cells in advanced hepatocellular carcinoma: A single-arm, open-label, phase II trial. Oncoimmunology 9, 1846926 (2020).
[17] Yang, C. et al. Targeted delivery of a PD-1-blocking scFv by CD133-specific CAR-T cells using nonviral sleeping beauty transposition shows enhanced antitumour efficacy for advanced hepatocellular carcinoma. BMC Med. 21, 327 (2023).
[18] Liu, H. et al. Targeting Alpha-Fetoprotein (AFP)–MHC Complex with CAR T-Cell Therapy for Liver Cancer. Clin. Cancer Res. 23, 478–488 (2017).
[19] Nakagawa, H. et al. Association Between High-Avidity T-Cell Receptors, Induced by α-Fetoprotein-Derived Peptides, and Anti-Tumor Effects in Patients With Hepatocellular Carcinoma. Gastroenterology 152, 1395-1406.e10 (2017).
[20] Aggeletopoulou I., Kalafateli, M. & Triantos, C. Chimeric antigen receptor T cell therapy for hepatocellular carcinoma: Where do we stand? Int. J. Mol. Sci. 25, 2631 (2024).
[21] Huang, X. et al. c-met-targeted chimeric antigen receptor T cells inhibit hepatocellular carcinoma cells in vitro and in vivo. J. Biomed. Res. 36, 10–21 (2021).
[22] Qin, A. et al. Tyrosine kinase signaling-independent MET-targeting with CAR-T cells. J. Transl. Med. 21, 682 (2023).
[23] Ma, Y. et al. Specific cytotoxicity of MUC1 chimeric antigen receptor-engineered jurkat T cells against hepatocellular carcinoma. Acad. J. Second Mil. Med. Univ. 35, 1177 (2014).
[24] Sun, B. et al. Eradication of hepatocellular carcinoma by NKG2D-based CAR-T cells. Cancer Immunol. Res. 7, 1813–1823 (2019).
[25] Zhai, Y. et al. DLK1-directed chimeric antigen receptor T-cell therapy for hepatocellular carcinoma. Liver Int. Off. J. Int. Assoc. Study Liver 42, 2524–2537 (2022).
[26] Javan H., Dayyani F. & Abi-Jaoudeh N. Therapy in advanced hepatocellular carcinoma. Semin. Interv. Radiol. 37, 466–474 (2020).
[27] Pan, Z. et al. Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1-CH3 fusion protein. Cancer Immunol. Immunother. CII 67, 1621–1634 (2018).
[28] Li, D. et al. Bispecific GPC3/PD-1 CAR-T cells for the treatment of HCC. Int. J. Oncol. 62, 53 (2023).
[29] Sun, H. et al. Long term complete response of advanced hepatocellular carcinoma to glypican-3 specific chimeric antigen receptor T-cells plus sorafenib, a case report. Front. Immunol. 13, 963031 (2022).
[30] Hendrickson, P. G. et al. The promise of adoptive cellular immunotherapies in hepatocellular carcinoma. Oncoimmunology 9, 1673129 (2020).
[31] Gacerez A. T. & Sentman C. L. T-bet promotes potent antitumor activity of CD4+ CAR T cells. Cancer Gene Ther. 25, 117–128 (2018).
[32] Tang, L. et al. Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. Nat. Biotechnol. 36, 707–716 (2018).
[33] Ma, W. et al. Coating biomimetic nanoparticles with chimeric antigen receptor T cell-membrane provides high specificity for hepatocellular carcinoma photothermal therapy treatment. Theranostics 10, 1281–1295 (2020).