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Chimeric Antigen Receptor (CAR) T-cell Immunotherapy for Hepatocellular Carcinoma: A Review of Current Progress and Challenges

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Abstract: Hepatocellular carcinoma (HCC) remains a formidable global health challenge, representing a leading cause of cancer-related mortality with limited effective therapeutic options for advanced stages. While conventional treatments have shown some efficacy, the inherent aggressive nature and complex tumor microenvironment of HCC necessitate innovative approaches. Chimeric Antigen Receptor (CAR) T-cell therapy has revolutionized the treatment landscape for hematological malignancies, demonstrating profound and durable responses. However, its translation to solid tumors, including HCC, has encountered significant hurdles, such as the lack of specific tumor antigens, an immunosuppressive tumor microenvironment, and the risk of on-target, off-tumor toxicities. This review provides a comprehensive overview of the recent advancements in CAR T-cell therapy specifically tailored for HCC, highlighting promising target antigens identified to date, discussing the design strategies employed to enhance their efficacy, and outlining the persistent challenges that require innovative solutions. Understanding the current progress and remaining obstacles is crucial for guiding future research and realizing the full therapeutic potential of CAR T cells in the fight against HCC.

Key words: Hepatocellular Carcinoma, CAR T-cell therapy, Immunotherapy, Solid Tumors, Tumor Antigens, Adoptive Cell Therapy.

INTRODUCTION

Hepatocellular Carcinoma, CAR T-cell therapy, Immunotherapy, SHepatocellular carcinoma (HCC) stands as a major global health concern, being the most common primary liver cancer and a leading cause of cancer-related deaths worldwide [1, 2]. Its incidence continues to rise globally, particularly in Asian countries, underscoring its significant epidemiological and economic burden [3, 4, 5]. HCC frequently develops in the context of chronic liver diseases, such as viral hepatitis (Hepatitis B and C), alcohol-related liver disease, and non-alcoholic fatty liver disease, leading to a complex disease landscape [1]. Despite advancements in diagnostic modalities and therapeutic strategies, the prognosis for patients with advanced HCC remains poor, with limited effective treatment options [1, 6]. Traditional treatments like surgical resection, liver transplantation, locoregional therapies, and systemic chemotherapy offer varying degrees of success, but often face limitations due to advanced disease presentation, tumor recurrence, or drug resistance [6].

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The last decade has witnessed a paradigm shift in cancer treatment with the advent of immunotherapy, harnessing the body's own immune system to combat malignancies [7]. Adoptive Cell Therapy (ACT), a subset of immunotherapy, involves the ex vivo expansion and reinfusion of immune cells with enhanced anti-tumor capabilities [9]. Among ACT approaches, T-cell receptor (TCR)-based therapies and Chimeric Antigen Receptor (CAR) T-cell therapies have emerged as highly promising strategies for cancer treatment [8, 10, 11].

CAR T-cell therapy involves genetically engineering a patient's own T cells to express a CAR, which is a synthetic receptor designed to recognize a specific antigen on the surface of cancer cells [8, 11]. A typical CAR construct comprises an extracellular single-chain variable fragment (scFv) derived from an antibody, a hinge region, a transmembrane domain, and an intracellular signaling domain, most commonly the CD3ζ chain, coupled with one or more co-stimulatory domains (e.g., CD28, 4-1BB) to enhance T-cell activation and proliferation [21, 30]. This modular design enables CAR T cells to bypass the conventional major histocompatibility complex (MHC)-restricted antigen presentation, allowing them to directly recognize tumor-associated antigens (TAAs) [17, 18].

The clinical success of CAR T-cell therapy in hematological malignancies, particularly B-cell leukemias and lymphomas, has been nothing short of revolutionary, leading to remarkable response rates and durable remissions [16, 17]. This success has fueled intense interest in extending this therapeutic modality to solid tumors, including HCC [12, 13, 15]. However, the application of CAR T cells in solid tumors faces unique and significant challenges that differ from those encountered in liquid cancers [14, 23, 24, 27]. These challenges include the heterogeneous nature of solid tumor antigens, the presence of an immunosuppressive tumor microenvironment (TME), the limited trafficking and persistence of CAR T cells within the tumor, and the risk of severe on-target, off-tumor toxicities [12, 13, 14, 23, 24, 27].

Given the urgent unmet need for effective treatments in advanced HCC, CAR T-cell therapy presents a compelling avenue for investigation. This review aims to comprehensively summarize the recent advances in CAR T-cell therapy for HCC, focusing on novel target antigens, innovative CAR designs, and strategies to overcome the formidable challenges inherent in solid tumor immunotherapy.

METHOD

2.1. Challenges of CAR T-cell Therapy in Hepatocellular Carcinoma

Despite the groundbreaking success in hematological cancers, the translation of CAR T-cell therapy to solid tumors, including HCC, has been fraught with difficulties [12, 13, 14]. Several major obstacles contribute to the attenuated efficacy observed in the solid tumor setting:

- Immunosuppressive Tumor Microenvironment (TME): The HCC TME is notoriously hostile to immune cells, characterized by a dense extracellular matrix, hypoxia, low pH, nutrient deprivation, and the presence of immunosuppressive cells (e.g., myeloid-derived suppressor cells, regulatory T cells) and soluble factors (e.g., TGF-β, IL-10) [14, 32]. This environment impedes CAR T-cell infiltration, persistence, and effector function within the tumor parenchyma [33].
- Lack of Specific Tumor Antigens: Identifying ideal target antigens for HCC is critical but challenging. An ideal antigen should be highly and homogeneously

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expressed on tumor cells, with minimal or no expression on healthy tissues, to avoid on-target, off-tumor toxicity [25, 26]. The heterogeneous nature of HCC, both inter- and intra-tumorally, can lead to antigen escape, where tumor cells lose or downregulate the target antigen, resulting in relapse [31].

- On-target, Off-tumor Toxicity: Even if an antigen is predominantly expressed on tumor cells, its low-level expression on vital healthy organs can lead to severe and potentially fatal adverse events upon CAR T-cell activation [28, 29]. This was notably observed in early trials targeting HER2 or carbonic anhydrase IX (CAIX), which led to pulmonary and renal toxicity, respectively [28, 29].

- Poor CAR T-cell Trafficking and Persistence: Unlike hematological malignancies where tumor cells are readily accessible in the bloodstream or bone marrow, CAR T cells often struggle to infiltrate and persist within dense solid tumors. The physical barriers posed by the stromal components and the harsh TME contribute to their limited presence and longevity at the tumor site [14].

- Manufacturing and Cost: The current manufacturing process for autologous CAR T cells is complex, time-consuming, and expensive, requiring specialized facilities and highly trained personnel. This limits widespread accessibility and scalability [19, 20].

2.2. Promising Target Antigens for CAR T-cell Therapy in HCC

Overcoming the antigen challenge is paramount for the success of CAR T-cell therapy in HCC. Researchers have identified and investigated several promising tumor-associated antigens (TAAs) for targeting HCC [44]:

- Alpha-Fetoprotein (AFP): AFP is a well-established serum biomarker for HCC, often elevated in patients with the disease [40, 41]. While primarily an intracellular protein, fragments of AFP can be presented on the cell surface via MHC class I molecules. CAR T cells targeting the AFP-MHC complex have shown preclinical efficacy against liver cancer [42]. However, the variability in AFP expression and presentation on the cell surface poses challenges.

- Glypican-3 (GPC3): GPC3 is a heparin sulfate proteoglycan that is highly expressed in the majority of HCC tissues but minimally or negligibly expressed in normal adult tissues, making it an attractive target [43, 44, 45, 46]. GPC3 plays a role in promoting HCC growth and Wnt signaling [47]. Preclinical studies have demonstrated that GPC3-targeted CAR T cells exhibit significant anti-tumor activity against HCC in vitro and in patient-derived xenograft models [48, 49]. Strategies like split CARs targeting GPC3 have been explored to potentially reduce cytokine release syndrome while maintaining efficacy [50].

- CD147 (Basigin/EMMPRIN): CD147 is a transmembrane glycoprotein overexpressed in HCC and plays a crucial role in tumor progression, invasion, and metastasis by stimulating matrix metalloproteinases [51, 52]. CAR T cells engineered to target CD147 have demonstrated potent anti-tumor activity against HCC cell lines and xenografts in preclinical studies [53, 54].

- Mucin 1 (MUC1): MUC1 is a transmembrane glycoprotein aberrantly overexpressed and glycosylated in many carcinomas, including HCC [55]. Its overexpression correlates with tumor progression and resistance to therapy. Preclinical studies have shown that MUC1-specific CAR T cells can exert cytotoxic effects against HCC cells [56].

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- Epithelial Cell Adhesion Molecule (EpCAM): EpCAM is a cell surface glycoprotein often overexpressed in various carcinomas, including HCC, and is considered a cancer stem cell marker [57]. CAR T cells targeting EpCAM have shown anti-tumor effects in ovarian cancer models [59] and HCC-relevant studies, suggesting its potential for targeting tumor cells with stem-like properties [58].

- c-Met: The hepatocyte growth factor (HGF)/c-Met axis is frequently activated in HCC and plays a role in tumor growth, invasion, and metastasis [60]. Bispecific CAR T cells targeting c-Met alongside PD-L1 have been explored to enhance therapeutic effects on HCC, highlighting strategies to overcome TME-mediated immunosuppression [61].

- NKG2D Ligands: Natural Killer Group 2 Member D (NKG2D) is an activating receptor expressed on NK cells and subsets of T cells. Its ligands (e.g., MICA/B, ULBPs) are stress-induced proteins often overexpressed on tumor cells, including HCC, but are minimally expressed on healthy cells [62, 63, 64]. NKG2D-based CAR T cells have shown promising results in eradicating HCC in preclinical models [65].

- CD133: CD133 is a cancer stem cell marker implicated in the initiation, progression, and recurrence of HCC [66, 67]. Targeting CD133 with CAR T cells aims to eliminate the cancer stem cell population, which is often resistant to conventional therapies and contributes to relapse. Early phase clinical trials investigating CD133-directed CAR T cells for advanced metastatic malignancies, including HCC, have been initiated, showing some preliminary efficacy [68, 69].

RESULTS

The burgeoning body of research into CAR T-cell therapy for HCC has yielded promising preclinical results, primarily demonstrating the feasibility and potential efficacy of targeting various tumor-associated antigens.

3.1. Preclinical Efficacy Across Diverse Targets: Numerous in vitro and in vivo studies using HCC cell lines and patient-derived xenograft models have consistently shown that CAR T cells engineered to target specific HCC antigens can effectively recognize and lyse tumor cells. CAR T cells directed against AFP-MHC complexes, GPC3, CD147, MUC1, EpCAM, c-Met, NKG2D ligands, and CD133 have all demonstrated significant anti-tumor activity, leading to reduced tumor growth and improved survival in animal models [42, 48, 49, 53, 54, 56, 58, 61, 65]. These studies provide strong foundational evidence for the therapeutic potential of CAR T cells in HCC. Notably, novel CAR designs, such as split CARs (e.g., GPC3-targeted [50]) and bispecific CARs (e.g., c-Met/PD-L1 [61]), have shown promise in enhancing efficacy and potentially mitigating some toxicities or overcoming TME challenges.

3.2. Emerging Clinical Trial Data: While the majority of solid tumor CAR T-cell trials are in early phases, some specific trials for HCC have started to emerge [44]. Initial reports from phase I/II clinical trials, particularly those targeting CD133 in advanced metastatic malignancies including HCC, have provided preliminary insights into safety and efficacy [68, 69]. These early results, while limited, indicate the feasibility of administering CAR T cells to HCC patients and provide valuable safety data regarding potential toxicities. However, comprehensive efficacy data and long-term outcomes for HCC-specific CAR T trials are still maturing and require further investigation [27].

3.3. Identification of Persistent Challenges: Despite the preclinical success, the

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translation to robust clinical efficacy has highlighted the persistent challenges in solid tumor CAR T therapy. Research consistently identifies the immunosuppressive nature of the HCC TME as a major barrier to CAR T-cell infiltration, expansion, and sustained anti-tumor activity [14, 32]. Antigen heterogeneity and the risk of antigen escape remain significant concerns, as evidenced by preclinical observations and the need for new CAR designs to address this [31]. Furthermore, the risk of on-target, off-tumor toxicities, though less reported in HCC-specific trials compared to initial solid tumor CAR T efforts [28, 29], continues to be a crucial consideration in antigen selection and CAR design. The logistical and economic complexities of CAR T-cell manufacturing also remain a barrier to broad accessibility [19, 20].

In summary, the results from preclinical and early clinical studies demonstrate the strong scientific rationale and initial promise of CAR T-cell therapy for HCC, while simultaneously underscoring the necessity for continued innovation to overcome the specific hurdles presented by this challenging malignancy.

DISCUSSION

The landscape of HCC treatment is undergoing a significant transformation, with immunotherapy playing an increasingly important role [39]. CAR T-cell therapy, having demonstrated remarkable success in hematological cancers, represents a highly promising, albeit challenging, frontier for solid tumors like HCC. Our review of recent advances underscores the immense potential but also the formidable obstacles that must be overcome to fully realize this therapy's benefits for HCC patients.

The identification of highly expressed and relatively specific tumor antigens, such as GPC3, AFP, and CD147, is a crucial step forward. GPC3, in particular, stands out due to its high expression in HCC and minimal expression in normal tissues, making it an attractive candidate for targeted therapy [43, 44, 45]. Preclinical data for GPC3-targeted CAR T cells are compelling, showing robust anti-tumor activity [48, 49]. However, the heterogeneity of antigen expression within and between HCC tumors remains a concern, potentially leading to antigen escape and recurrence [31]. This necessitates strategies such as targeting multiple antigens simultaneously using bispecific CARs (e.g., c-Met/PD-L1 [61]) or pooled CAR T-cell products, or exploring novel antigens (e.g., NKG2D ligands [65]) that may be more universally expressed or less prone to escape.

The immunosuppressive nature of the HCC tumor microenvironment poses a significant hurdle to CAR T-cell efficacy [14, 32]. Traditional CAR T cells may fail to infiltrate the dense tumor stroma or succumb to inhibitory signals and factors within the TME, leading to poor persistence and limited anti-tumor activity [33]. Future CAR T-cell designs must incorporate features that enable them to overcome these barriers. This could involve engineering CAR T cells to secrete pro-inflammatory cytokines, express resistance to immunosuppressive factors (e.g., dominant-negative TGF-β receptors), or enhance their metabolic fitness within the harsh TME [14]. Furthermore, localized delivery strategies, such as regional infusions (e.g., hepatic artery infusion), might improve CAR T-cell trafficking and concentration at the tumor site, minimizing systemic toxicities [34].

On-target, off-tumor toxicity remains a critical safety concern, particularly for HCC given the vital functions of the liver. While early trials with targets like HER2 caused significant adverse events [28], careful

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antigen selection with stringent specificity to tumor cells, combined with advanced CAR designs, can mitigate this risk. Novel CAR architectures, such as inducible ON/OFF switches, synNotch CARs, or split/dual CAR systems [30, 50], offer promising avenues for greater control over CAR T-cell activity, allowing for activation only in the presence of specific conditions or antigens, thereby improving the therapeutic index. The ongoing clinical trials targeting various antigens, including CD133, will provide invaluable data on the safety profile of these therapies in HCC patients [68, 69].

Beyond optimizing CAR design, combining CAR T-cell therapy with other treatment modalities holds immense promise. Integrating CAR T cells with checkpoint inhibitors could synergistically enhance anti-tumor responses by relieving T-cell exhaustion and promoting a more permissive TME. Similarly, combining CAR T cells with conventional therapies like radiation or chemotherapy might improve tumor antigen presentation or reduce tumor burden, creating a more favorable environment for CAR T-cell activity. Emerging strategies also include combining CAR T cells with oncolytic viruses or small molecule inhibitors that remodel the TME.

Finally, the manufacturing complexity and high cost of autologous CAR T cells present significant logistical and economic challenges for broad clinical implementation [20]. Research into allogeneic "off-the-shelf" CAR T-cell products and non-viral gene transfer methods offers potential solutions to improve accessibility and reduce costs, thereby making this transformative therapy available to a wider patient population [19].

In conclusion, CAR T-cell therapy holds tremendous potential for transforming the treatment paradigm for HCC. While significant progress has been made in identifying promising targets and improving CAR designs, the inherent challenges of the HCC tumor microenvironment and antigen heterogeneity necessitate continuous innovation. Future research will focus on developing more sophisticated CAR constructs, optimizing delivery strategies, combining CAR T cells with complementary therapies, and scaling up manufacturing processes to finally bring this powerful immunotherapy to routine clinical practice for HCC patients. The journey is challenging, but the sustained effort promises to bring hope to patients with this aggressive cancer.

CONCLUSION

Hepatocellular carcinoma remains a malignancy with urgent unmet needs, driving the exploration of novel therapeutic avenues. Chimeric Antigen Receptor (CAR) T-cell therapy, having demonstrated remarkable success in hematological cancers, represents a cutting-edge approach with significant potential for HCC. Recent advances have identified several promising tumor-associated antigens, including GPC3, AFP-MHC, CD147, MUC1, EpCAM, c-Met, NKG2D ligands, and CD133, which serve as viable targets for CAR T-cell engagement. Preclinical studies have largely validated the efficacy of CAR T cells against HCC in various models. However, the translation of this promise into widespread clinical success for HCC is still hampered by the complex and immunosuppressive tumor microenvironment, the challenge of antigen heterogeneity and escape, and the persistent risk of on-target, off-tumor toxicities. Continued innovation in CAR design, strategic combinatorial approaches with existing therapies, and advancements in manufacturing processes are crucial to overcome these formidable barriers. While the journey is long, sustained research and development efforts hold the key to unlocking the full therapeutic power of CAR

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T-cell therapy and revolutionizing the treatment of hepatocellular carcinoma.

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