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The Application of CAR-T Cell Therapy

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Abstract. Cancer is undoubtedly the number one killer in today's society. Traditional surgical treatment methods have their own limitations, especially in the treatment of end-stage or hematological tumors. Emerging immunotherapy is an unprecedented breakthrough technology in cancer treatment. For example, PD-1/PD-L1 and CAR-T immunotherapy. CAR-T has been proposed for many years, it is only in the last few years that it has been improved and applied in the clinic. Its effectiveness in the treatment of cancer in hematology is encouraging. For example, it has a great curative impact in the acute leukemia, in the field of non-Hodgkin lymphoma ,it is also recognized as one of the most prospective and developed tumor treatments. Clinical application of CAR-T has attracted extensive attention. This therapy is characterized by the ability to detect tumor-associated antigens preferentially detected by T cells, which has a significant anti-tumor effect. This article will focus on HCC, list the existing HCC CAR-T treatment targets and briefly describe associated clinical research.

Keywords: CAR-T Cell Therapy, Hepatocellular, Carcinoma

1. Introduction

Over the last two years, immunotherapy has taken the limelight. Particularly in cancer therapy, immunotherapy has become an unprecedented breakthrough technology. As an example, PD-1/PD-L1 immunotherapy drugs. However, this immune drug is only one type of tumor immunotherapy. In addition to using drugs to relieve the inhibition of immune cells by tumor cells, another idea is to directly use the patient's existing T cells, take them out of the body for transformation, replicate them in large quantities, and then infuse them back into the patient's body. This is another aspect of immunotherapy type - immune cell therapy.

It is widely known that there are many types of immune cells in the human body. Therefore, immune cell therapy also includes different types, mainly in two categories: one is the non-specific old technology represented by CIK, DC-CIK, NK, etc.; the other is CAR-T, TCR-T as a representative new technology that requires specificity of in vitro genetic modification, and this is the most commonly therapy right now [1].

CAR-T therapy is chimeric antigen receptor T cell therapy. The costimulatory molecules, after being in vitro expansion, are re-infused back into patients, where they attack their own tumor cells after recognition [2]. CAR consists of 4 parts:

Among them, the Binding domain for extracellular target antigen; hinge region; transmembrane binding domain; intracellular signaling domain [2]. According to their different structures, CAR was four generations:

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The first-generation CAR contains the ITAM region of the CD3 molecule ζ chain only. which cannot effectively activate T cells, and has effective or even no clinical efficacy.

The second-generation CAR builds on the first-generation CAR by including an ITAM region derived from the CD28 or CD137 (4-1BB) in the cell [3]. T cells can obtain the same time after binding to the target antigen, and the binding point is in the extracellular antigen recognition region. Signals for antibody stimulation and costimulation This makes the second-generation CAR's activation ability far superior to that of the first-generation CAR, and it also has a good therapeutic effect in clinical treatment.

According to common consensus, CD28 transmits a stronger activation signal than CD137, so that T cells can develop high killing activity quickly and that the activation signal sent by CD137 will endure for a longer period. Because of retroviruses make up the majority of the transfection viral vectors utilized in second-generation CAR-T cells, which can accommodate and carry limited gene fragments, it is challenging to simultaneously transfect CD28 and CD137's ITAM regions, two costimulatory signal molecules, into T lymphocytes [4]. Therefore, People must decide between receiving CAR-T cells with a better activation power and a longer activation durability.

Although the third generation CAR uses lentivirus as the transfection vector, it shares much of its basic structure with the second-generation CAR. The lentiviral vector is less likely to cause carcinogenesis than the retroviral vector and is therefore safer for use in therapeutic settings. Additionally, because it can deliver larger gene fragments to T cells, its CAR intracellular portion frequently has two or more ITAM domains for costimulatory signaling [5]. Theoretically, third generation CAR-T cells ought to be more capable of activating and eliminating cancer cells than the second [6]. However, According to certain research, there hasn't been a considerable improvement in the killing ability of third-generation.

The fourth generation has been devised primarily to modulate CAR T cells. For example, suicide genes may be added, damage to healthy tissues after tumor removal may be minimized, or cytokines and chemokine receptor structures may be added to promote tumor tissue infiltration and achieve solid tumor killing effects.

The effectiveness of it in hematological therapy cancers has been encouraging [7]. Several studies have found that targeting CD19 are valid in relapsed and refractory acute B lymphocytic leukemia also in large B cell lymphoma. It can play a targeted killing effect and can provide a very high remission rate of 80% to 90%. This discovery not only confirms the clinical value of cellular immunotherapy, but also stimulates clinicians' enthusiasm for the application of other tumor-associated antigens and solid tumor CAR-T treatment.

2. Healing process of CAR-T therapy and hepatocellular carcinoma (HCC)

2.1. The healing process of CAR-T therapy

First, the patient is evaluated and subjected to a number of assessments and exams to ascertain whether T cells can recognize antigens that target tumor cells thanks to CARs, which are proteins [8]. When CAR-T cells are needed for treatment, they can be frozen and transported to a medical facility for use. Patients may receive chemotherapy for cancer before infusion of it. This facilitates the expansion and proliferation of the CAR-T cells that have been introduced into the immune system. Shortly after chemotherapy, patients can be injected with CAR T cells at their next admission using the same injection process. In order to accurately detect their body's effects on treatment, overall recovery, and negative effects, patients need to be hospitalized for several weeks. Finally, patients undergoing such treatment undergo an observation period of approximately two to three months to transition to the risk (recovery)period [9,10]. Throughout this time, the patients were not uncommon to be readmitted to the hospital for complications. In the first four weeks after leaved the hospital, patients require regular follow-up care close to our center. If necessary, a resource specialist can help them find a place to stay during this time.

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2.2. hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is account about 85% of liver cancers [11]. A major risk factor of liver cancer is HCV and chronic HBV infection, it causes chronic inflammation in the liver. Since liver cancer is usually diagnosed at an advanced stage, which cannot be treated by surgery, 70 percent of patients will have a recurrence within five years. Most HCC patients receive local or systemic therapy, for instance chemoradiotherapy, or injection therapy (percutaneous ethanol injection); Other common clinical treatments are radiofrequency ablation and embolization procedure. However, the efficacy of these treatments is not enough. Systemic treatments such surgery, radiation, chemotherapy, and others, however, the efficacy of which is insufficient.

In all advanced cases, the most common is not primary liver cancer, but liver metastases, because cancers of the gastrointestinal tract and others usually metastasize to it [12]. The only treatment is surgical resection, and unfortunately, few patients are willing to undergo this treatment, or few are suitable for it. Therefore, a new and effective treatment scheme is of great significance in clinical research.

2.2.1 Glypican-3 (GPC3). GPC3, a proteoglycan belonging to the heparan sulfate family, is highly expressed in HCC but not or just weakly expressed in healthy liver tissue. Some research confirmed: the expression of GPC3 is related to the progression of HCC. About 72% of HCC patients express GPC3, and 53% of HCC patients have significantly elevated serum GPC3 expression levels [13]. The use of GPC3 CAR-T treatment was investigated in the HCC study. This study comprised thirteen patients with advanced HCC. One patient was still alive after 44.2 months, while the 3-year is 10%, 1-year is 42%, 6-month overall survival (OS) rates 50.3%, respectively [14]. ln 9 cases, cytokine release syndrome (CRS) was seen, 8 of which were grade 1-2, 1 was grade 5, and no grade 3 or 4 neurotoxicity occurred. Several other studies evaluating the efficacy of it alone, or in combination with anti-angiogenic drugs in HCC, also included combination with immune checkpoint inhibitors or are ongoing [13].

2.2.2 Alpha-fetoprotein (AFP). AFP having a number of crucial physiological roles, such as transport, immunosuppression, inducing apoptosis, etc. The concentration of alpha-fetoprotein is highest during fetal development and declines after birth. After birth, albumin essentially replaces alpha-fetoprotein, making blood testing for it problematic, so its content in adult serum is extremely low, however, AFP is re-expressed when HCC develops in adults. In this study, has confirmed that ET1402L1-CAR T cells may specifically bind to AFP protein-positive HCC cells and release them. lysed and confirmed the antitumor activity of this cell in an animal model using an intraperitoneal HCC xenograft [15].

2.2.3 c-Met. c-Met can activate the downstream MAPK, PI3K and STAT3 pathways by linked to HGF by inducing cell growth, proliferation, migration, Invasion and a series of biological effects. Studies have shown that overexpression of it can increase the progression of HCC. [16] In conclusion, it has been thought as a potential target. At present, the c-Met CAR-T cells second generations and third generations have already been successfully prepared, demonstrating the ability of the new treatment to destroy HCC cells specifically [16].

2.2.4 Mucin 1 (MUC1). One transmembrane glycoprotein called MUC1 is over expressed in several hematological, epithelial malignancies. Studies have shown that the MUC1/JNK/TGF- signaling pathway facilitates liver cancer cells' invasion and migration. Therefore, MUC1 is considered as one of the potential therapeutic targets for HCC [13]. MUC1 CAR-T cells have been created, and research has shown that they can specifically destroy HCC cells with high MUC1 expression while barely harming healthy hepatocytes with low MUC1 expression.

2.2.5 Epithelial cell adhesion molecule (EpCAM). In many cancers, including HCC, the transmembrane glycoprotein EpCAM is extensively expressed. Previous studies have shown that

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epcam-positive HCC cells affect HCC growth, while exhibiting features such as hepatic stem cell-like cell differentiation.

2.2.6 CD133. Another transmembrane glycoprotein with strong expression in pancreatic, lung, brain, and HCC are CD133. Cancer stem cells (CSCs) are thought to be identified by the presence of the CD133 antigen since CSCs are key players in tumor survival, growth, metastasis, and recurrence. Some research found that CD133 cells do not exist in non-cancerous liver tissues, but only in HCC tissues, and its expression in HCC cells, which can make the growth and metastasis of HCC cells. One research demonstrating with high CD133 expression a shorter survival-rate and a greater recurrence-rate in HCC patients, CD133 has been proposed as a molecular target for immunotherapy.

2.2.7 CD147. 80% of HCC cases have been discovered to have high levels of CD147, one kind of I transmembrane glycoprotein that can accelerate growth of tumor, invasion, and metastasize by inducing the release of matrix metalloproteinases. Another report mentions: the expression of CD147 in cells is related to the malignancy of HCC. Currently, the monoclonal antibody specific for CD147 can be used in conjunction with TACE, and RFA has been repeatedly proposed as a novel treatment. These data found that it may become a potential therapeutic strategy for HCC.

*2.2.8 NKG2D.*NKG2D is a type II transmembrane glycoprotein, and its ligand (NKG2DL) is expressed in various tumors HCC excluded, but not in healthy tissues. Two families of human NKG2DL include six cytomegalovirus UL16-binding proteins and MICA and MICB. Previous studies have shown that MICA, MICB, and ULBP1 are highly expressed in HCC. In addition, early recurrence following hepatectomy is closely associated with ULBP1 expression decrease, and lower MICA and MICB manifestation often denotes a markedly elevated risk of tumor development. Therefore, NKG2DL can be a molecular target for HCC immunotherapy.

Some studies have shown that it can typically lyse HCC cells highly expressing NKG2DLs, but do not affect NKG2DLs-negative cell lines. The results show that the cells can eliminate HCC cells with high level of NKG2DL both in vitro and in vivo to provide a possible new treatment option for NKG2DL-positive HCC patients [15].

2.2.9 CEA. CEA is a glycosylated phosphatidylinositol (GPI)-anchored protein. CEA is over expressed in most human tumors. There is no relevant research on CEA with therapeutic target for primary liver cancer, but CEA. The correlation with liver metastasis can be used as a biomarker for anticancer therapy. The first-generation anti-CEA CAR-T cells in liver metastasis using is nonetheless constrained by inconsistent results and serious negative side effects. Eight patients participated in an open-label, single-arm phase I research (NCT01373047) that assessed the effectiveness to increase the tolerance, and durability of anti-CEA. One patient with gastrointestinal malignancies that had spread to the liver lived for more than 23 months, and no other patients occur severe adverse events (AEs) from CAR-T infusion therapy.

2.2.10 Multiple targets. Based on the purpose of improving the recognition specificity of CAR-T cells, the preparation of it targeting two or more antigens is also one of the treatment ideas. In addition, another study prepared dual-target liver tissue-specific protein 1 (ASGR1) and CAR-T cells co-expressing GPC3. Furthermore, GPC3+ASGR1+ HCC tumor xenografts can be effectively inhibited by dual-targeted CAR-T cells [15].

Compared with the achievements in hematological tumors, how it is used in solid tumors still faces many problems. Among them, the selection of therapeutic targets is one of the most serious challenges. First, unlike CD19, which is only expressed in B cells, solid tumor-related tumor antigens are often not only expressed in malignant tissues, it has minimally expression in healthy tissues also, and some antigens even maintain the normal physiological function of tissues. Therefore, in solid tumors, Car-t cells can decline the number of tumor cells that express the same antigen and attack them [16]. it can

be activated and proliferated in non-tumor tissues due to contact with antigens, and then release a large number of inflammatory factors, leading to immune damage to local organs and even induce cytokine storms, resulting in serious or even fatal adverse events (AE). Second, heterogeneity is one of the characteristics of solid tumors. Even tumors in one site of the same patient do not all express the same tumor-associated antigens. Therefore, if only one single-target CAR-T cell is used to treat solid tumors, then theoretically, the highly heterogeneous characteristics of solid tumors determine that they will inevitably recur or cannot be eradicated.

3. Conclusion

Even though CAR-T therapy has treated hematological malignancies with remarkable success, due to the severe off-target effects, as a means of treating solid tumors, it has not been widespread used in clinical treatment. Research and applications still have a long way to go. It should be emphasized that, utilization of it in HCC is still mostly being in the preclinical research and early clinical research. But despite the difficulties, is also worth looking forward to, and a number of clinical studies based on the above targets are also underway. CAR-T cells for precise, multi-target replacement targeting of tumors in the future may change the treatment strategy of HCC and provide patients with more active treatment options. In general, the progress and exploitation of CAR-T cell has allowed us to see some new hopes for tumor treatment, but many problems it faces still need to be solved urgently. Perhaps, when medical technology matures in the future, all problems will be solved naturally. After all, ten years ago, immunological checkpoint inhibitors (monocIonaI antibodies against PD-1, PD-L1), were still immature buds; today, they have blossomed into bright flowers in many tumor treatment fields, and have become a hot anti-cancer drug Cancer weapon. In the future, we look forward to the brilliance of tumor cell immunotherapy, and draw a blueprint of hope for a cure for liver cancer patients.

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