

Chimeric Antigen Receptor-T Cell Therapy with Breast Cancer

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Abstract. Breast cancer (BC) poses an increasingly serious threat to the women's health. In 2020, there will be 2.26 million new cases of BC, surpassing lung cancer for the first time and becoming the world's "first cancer". BC is distinguished by its intense invasion, extreme malignancy, and dismal outlook. High attention is needed. Chimera Antigen Receptor-T(CAR-T) has the potential to modify T cells and direct them towards particular tumor cells. It has made unheard-of advancements in hematological cancers since it was originally authorized for use in B-cell derived malignant tumors in 2017. In recent years, it has greatly reshaped the pattern of cancer treatment. At present, accumulated evidence suggests that this therapy may become the feasible treatment strategy for the solid tumors. This review will describe the main goals of this therapy for BC, list the challenges it faces, and offer the solutions to these problems, offering a unique strategy for breast cancer treatment.

Keywords: CAR-T, breast cancer, cancer treatment.

1. Introduction

The high degree of the malignancy and the poor prognosis of breast cancer (BC) pose a grave danger to women's health. This disease can be divided into four subtypes: basal, lumen A, lumen B, triple negative, and HER-2 amplification. Treatment for this type of cancer is still largely focused on surgery, chemotherapy, and radiotherapy. The therapeutic effect of breast cancer is severely limited by the fatal complications of damage, recurrence and metastasis of regular breast tissue after treatment. However, trastuzumab and other targeted therapies have been used in a continuous manner to improve the prognosis of HER2 positive breast cancer patients with fewer adverse reactions. However, their overall therapeutic effectiveness remains restricted and necessitates an urgent requirement for alternative treatments.

CAR-T cell therapy has a good clinical effect in the treatment of blood cancer, and its effectiveness has led people to wonder whether it has the same effect in solid tumors such as BC. This review aims to list prospective breast cancer targets and clinical trials that using this treatment and talk about the challenges and its engineering approach faced.

2. How CAR is Built Up

Divided into four distinct components, CAR is structured and functions in a variety of ways; these comprise the intracellular signaling domain, the transmembrane domain, the hinge domain, and also the antigen-binding domain. Optimizing the four structural domains in the development process of CAR can significantly increase the safety and effectiveness in tumor treatment (as shown in figure1).

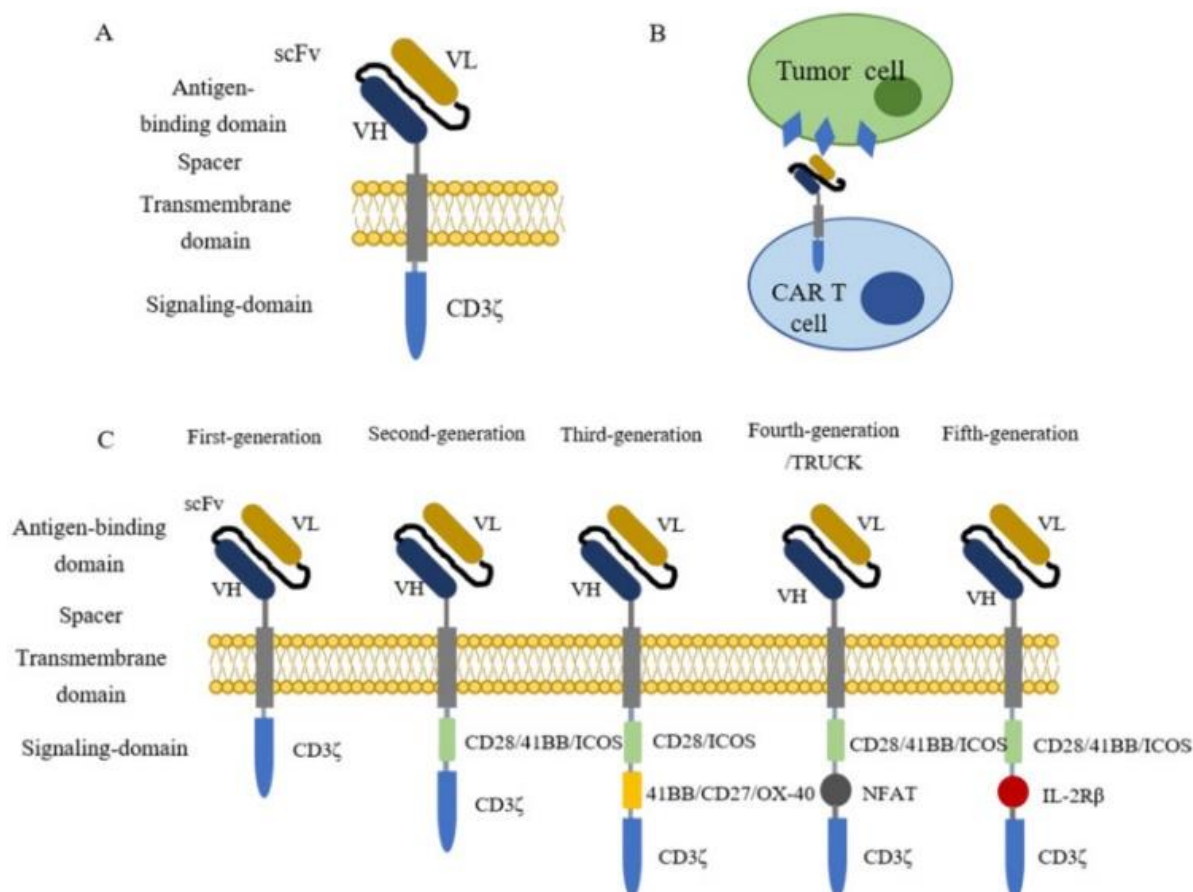


Figure 1. Four parts of CAR. (B) CAR-T cells identify the tumor cells by binding to antigens on their surfaces. (C) CAR-T cell research and development are underway. A single signaling domain (CD3) is present in the first generation, whereas two signaling domains (CD3 plus CD28/41BB/ICOS) are present in the second generation. Three intracellular signaling domains are present in the following generations. The third generation includes 41BB/CD27/OX-40, CD28/ICOS, and CD3.

The conventional two signaling domains in the fourth and fifth generations were added to a separately distinct domain called NFAT and IL-2R [2].

The most important structure of CAR is the part that recognizes tumor cell antigens, which originates from the antibodies scFv structure. Unlike the TCR of T cells, scFv can theoretically recognize all cell surface antigens (after in vitro antigen binding screening). The TCR recognition of normal T cells is mostly based on peptide segments within the target cell, and recognition must rely on the display of MHC molecules in the target cell. While CAR-T recognizes surface antigens of target cells through the scFv, increasing breadth of this target recognition, meanwhile, it does not have limitations of MHC. In addition, a certain distance needs to be maintained between normal T cells and target cells to form immune synapses, the intercellular distance between the CAR-T cells and the target cells changes with size and position and may be regulated through scFv. The design of scFv also has certain flaws, as artificially chimeric scFv is prone to forming oligomers, leading to T cell depletion [1].

Antigen binding domains have flexibility to overcome spatial obstacles when associating with tumor cells, which facilitates these cells' recognition and interaction with such cells.

The transmembrane domain links the intracellular and the extracellular domains, thus solidifying its fundamental structure on the membrane of the cell. Type I proteins are the primary components of this region, like the CD3, CD4, CD28 or CD8 α --found that compared to containing CD3. The transmembrane domain containing CD28 is more stable than the transmembrane domain containing CD3.

CAR's intracellular localization component, typically composed of an activation and co-stimulatory domain, is known as the intracellular signaling domain. CAR-T cells receive recognition signals from tumor antigens, then activating the signal domain and thus promoting the T cell elimination of tumors - this being accomplished through both the activation and co-stimulation domains. Most of the CAR activation domains originate from the CD3 based on the tyrosine activated motifs immune receptors. But relying solely on CD3, the activation signals transmitted are not sufficient to induce T cells to produce a sustained immune response. Consequently, the CAR's structure was augmented with stimulatory structures such as OX40, 4-1BB, or ICOS. Research has found that activating the co-stimulatory domain can significantly improve the anti-tumor influence and the persistence by producing cytokines for example the IL-2.

3. The Therapeutic Target of CAR-T

3.1. HER2

The over-expression of HER2, a molecule that has been extensively researched in this field, is associated with the high rate of BC metastasis and also the recurrence. Upon activation, HER2 activates various downstream signaling pathways that induce expression of the genes that encoding epithelial-mesenchymal transition (EMT)- thus initiating tumor metastasis [3-5]. In 1998, the FDA gave trastuzumab the green light to be used in breast cancer treatment, revolutionizing the concept of HER2 positive breast cancer. Despite its success in treating patients with good outcomes, drug resistance has still hindered its effectiveness. The HER2 targeting CAR-T cells developed have improved efficacy and safety in sarcoma clinical studies and are capable of actively recognizing tumors. Mutations in the somatic HER2 gene, activating HER2 signaling, can also be linked to breast cancer development [6].

3.2. MSLN

MSLN is typically expressed on mesothelial cells of certain tissues [7]. MSLN targeting CAR-T cells raises possible safety issues for breast cancer therapy because multiple studies have shown that it shows a tendency of overexpression in BC and other malignant tumors. So the third-generation MSLN-targeted CAR-T cells with the CD28 and the 4-1BB co-stimulatory domains was created. In both vitro and vivo xenotransplantation model of BC, it specifically destroyed the MSLN positive breast cancer cell line, significantly hindering the growth of BC tumors. Additionally, it was discovered that T cells and cytokine secretion levels augmented significantly when CAR-T cells were present.

3.3. Folate Receptor α (FR α)

Breast cancer cell surfaces overexpress FR α , which is a membrane-binding protein that has strong affinity for the folic acid and has the ability to transport it into cells; normal tissues do not express FR α . It is expected to become a target antigen for breast cancer. When these specific cells are associated with the FR α , When co cultured with TNBC cells, they exhibit significant anti-tumor activity. Employing the trans-signaling CAR strategy was used to improve the safety [8]. In addition, its cytotoxic effect is observed in FR α . In cells, the expression was more pronounced than in normal breast cells with negative FR α , yet it was not seen. Contemporary, CAR-T cells are sensitive to FR α . Negative MCF10A normal breast cells do not produce this specific cytotoxicity.

4. Strategies to Surmount the Obstacle of CAR-T Cell Therapy

Restrictions on the use and therapeutic outcomes of this therapies for BC persist, despite the treatments' encouraging findings. These challenges include tumor heterogeneity, an immunosuppressive milieu in breast malignant tissue, tumor non-recruitment and the adverse effects

[9, 10]. In response to these significant challenges, methods for enhancing the effectiveness have been proposed.

CAR-T cell technology needs to address some safety issues in medical applications. By modifying the T cells to have stronger activity of anti-tumor, it may also attack normal cells. Besides, it may trigger severe cytokine release syndrome (CRS) during the proliferation process in vivo, leading to severe side effects such as high fever, low blood pressure, and thrombocytopenia. Therefore, how to control the activity and reactivity and how to avoid unnecessary toxic side effects have become key issues that require further research. At present, CAR-T cell technology is only mainly used to treat hematomas such as lymphoma and acute lymphoblastic leukemia. However, the technical effectiveness of this technology is still limited for other types of hematomas, such as myeloma and chronic lymphocytic leukemia. In addition, it is also difficult to apply to the metastasis and recurrence of systemic diseases and malignant tumors. Therefore, further research is needed to expand the scope of indications.

CAR-T cell technology faces challenges in preparation and production. Currently, the preparation and production process of CAR-T cell technology is complex and costly. Due to the need to extract patients' own T cells and undergo genetic engineering treatment for the preparation, personalized treatment is required for each patient. Therefore, there may be issues with low production efficiency and consistency of the preparation process. To address this issue, researchers are searching for more efficient and networked preparation methods and are committed to developing portable devices and technologies related to the production of the CAR-T cells to better meet the needs of patients.

The prevention strategy for CAR-T treatment related toxicity can be detected through predictive biomarkers of CRS and neurotoxicity, and timely responses can be made through intensive monitoring. On the other hand, it is necessary to design safer CAR-T cell therapies, and the current consensus strategy is to install a "safety switch" on CAR-T, treat it under human control, and promptly apply the brake before adverse reactions occur. At present, clinical treatment for CRS mainly involves reducing CAR-T dosage, using steroid therapy, or blocking IL-6R antibodies, due to the association between IL-6 and severe CRS. Another CRS management strategy being explored is to block IL-1, which is another essential cytokine responsible for CRS. IL-1 participates in the activation pathway of macrophages. The IKZF family of proteins, with its zinc finger domain capable of recognizing DNA sequences and binding to other proteins, has been demonstrated by their research to be significantly enhanced in cytotoxicity when IL-21 is added to trastuzumab resistant breast cancer cells such as HCC1954 and BT474 after HER2 targeting CAR-T cell combination. This also promotes IFN- γ Synthesis and secretion.

5. Conclusion

Ranking first in both incidence and mortality among women, BC is currently the most widely diagnosed form of the cancer worldwide. The successful utilization of CAR-T cell therapy in hematologic malignancies has spurred its growth to treat the breast cancer and the potential targets are believed to be modified expressions of various molecules, as a type of the immunotherapies that utilizes immune cells of patients to combat the cancer.

In addition, it is worth noticing that molecular targeted therapy, such as monoclonal antibodies, has also achieved great success in breast cancer. The preparation and clinical application of monoclonal antibodies, including Trastuzumab on breast cancer cells expressing HER2 has greater convenience than that of CAR-T cell therapy. However, its killing effect still relies upon immune cell activity. Furthermore, epitope masking and steric hindrance must be addressed to properly address drug resistance in order for ADC recognizing the tumor cells and carrying the cytotoxic drugs to eliminate them. The specific recognition of breast cancer cells, the decoupling of antibodies from drugs and drug resistance still impedes the wide application of the ADC drugs towards B.Th. efficacy in the future is undeniable, due to their capacity to recognize and eradicate tumor cells, thus

circumventing the challenges encountered with antibody therapy. In the future, evidence-based research still needs to be increased to provide convincing evidence for clinical application.

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