

CAR T-cell Therapy for Neuroblastoma

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Abstract. Children aged under 5 being the main patient group of neuroblastoma has been widely acknowledged according to clinical diagnosis results. Traditional therapies like monoclonal therapy and chemo therapy have achieved relatively high curing rate, the 5-year survival rate of these therapies are still not acceptable, followed by strong side effects to microenvironment. To help improve the prognosis of patients who have high risk and lift the survival rate, CAR T-cell therapy for neuroblastoma can be an achievable option. In this review, I summarize the mechanism of CAR T cell therapy, the general information about neuroblastoma and the current clinical experiences advancement in this area.

Keywords: CAR T, Therapy, neuroblastoma.

1. Introduction

Neuroblastoma is a kind of cancer composed of neuro crest cells, which appear during the development of sympathetic nervous system. This type of cancer is one of the most common cancer among infants and are seldom found in children over 5 years old. Though the cause of neuroblastoma is still under research, evidence prove that this cancer is associated with the mutation in oncogene and tumor suppressor gene. Neuroblastoma have similar symptoms to some other cancer, including fever, sweating, fatigue and etc., which is caused by the secrete of chemokines. The treatments including chemotherapy, medication, immunotherapy and etc. Due to the irreversible damage from chemotherapy and relatively low survival rate, researchers have been focusing on the immunotherapy, especially the CAR T therapy. Surgical pathologists usually make decision on the diagnosis of neuroblastoma, the procedure of which also include the clinical performance, microscopic observation, and other laboratory results. Although the cancer is curable after the diagnosis confirmed the exact location of the lesion, children tend to have less life if the disease has come to further stage due to aggressive medical approach, such as chemotherapy, which can cause strong side effects. The neuroblastoma tend to be easy to cure if one is still in the early or intermediate stages, and the treatments can even depend on traditional chemotherapy. However, therapy for high-risk neuroblastoma the past two decades resulted in cures only about 30% of the time [1]. With the advanced researches precede in the area of immunotherapy, the survival rate of high-risk neuroblastoma increased significantly. In this review, we will be focusing on the CAR T therapy for neuroblastoma.

Chimeric antigen receptors (CARs) are a kind of genetically modified receptor that can provide an immune effector cell particularqualities. These receptors can acquire the specificity of a monoclonal antibody directed against a particular tumor cell. Clinical cancer therapies can benefit from the remarkable accuracy with which CAR T-cell therapy binds specific tumour antigens and associated checkpoints. After years of advances, this therapy has come to reality nowadays. Scientists and researchers are anticipating it to be a universal therapy for many types of cancer, including neuroblastoma. CAR are receptor proteins that have undergone genetic engineering to endow T cells with novel antigen-targeting capabilities. Researchers take T cells from volunteers, genetically modify them, and then inject the modified CAR T cells back into volunteers to fight cancer cells. Both CD4+ and CD8+ CAR T cells are capable of producing synergistic antitumor effects when present in comparable amounts [2]. Both healthy donors and the patient's own blood T cells are acceptable sources of CAR T cells. After being taken from a certain person, scientists will genetically

alter these T cells to be able to express a specific CAR that directs them to seek an antigen located on tumor surfaces.

2. Different staging of neuroblastoma

According to the "International Neuroblastoma Staging System," neuroblastoma is staged. (INSS), which was created in 1986. At Stage 1, a patient's tumor is localized and ought to remain confined to its point of genesis. Stage 2A follows, which is characterized by symptoms such a unilateral tumor with imperfect gross excision and palpable ipsilateral and contralateral lymph nodes that are tumor-free. At Stage 2B, unilateral tumors often undergo total or partial gross excision, with the ipsilateral lymph node being proven to be positive for the tumor at this time. At Stage 3, there is a midline tumor that has infiltrated both sides of the body, either unilaterally or bilaterally, and there may or may not be involvement of the regional lymph nodes. Stage 4 malignancy has spread to far-off lymph nodes, organs such as the liver, bone, and bone marrow (not including those organs that are defined as stage 4S) Age 1 year old with a confined initial tumor as described in Stages 1 or 2, and little liver, skin, or bone marrow spread at Stage 4S, which should be analyzed as under 10 percent of tumor cells contributing to the nucleated bone marrow cells.

3. Immunologic properties of neuroblastoma

Clinically, although some NB will rapidly advance in spite of extensive multimodal therapy, another fraction will have spontaneous regression or maturation. While segmental abnormalities and MYCN amplification are frequently found in high-risk metastatic NBs, whole chromosomal gains are also observed in low-risk NBs without segmental aberrations or gene amplifications. These clinical and genetic heterogeneities result in the occurrence of two distinct immunologic profiles. In contrast to a hot tumor, NB is a low-risk subtype where spontaneous regression or maturation is less common. The majority of stage 4S tumors have normal levels of HLA class I antigen expression and considerable CD3⁺ T cell infiltration [3]. which shows that T cells are capable of recognizing NB cells. Antineuronal antibodies are associated with the opsoclonus-myoclonus-ataxia syndrome, which can also exist in people with low-risk NB. Visual and motor cortex thickness, as well as the amount of the cerebellar gray matter, can all be drastically reduced. Additionally, there is a significant rise in the concentration of neurofilament light chain in CSF, which is consistent with damaged neurons. These ganglio neuroblastomas or developing NBs have traits including tumor-associated lymphoid follicles and widespread immune cell infiltrates that show a continuous immune response against NB. On the other hand, high-risk metastatic NBs resemble cold tumors and have mechanisms for evading the immune system.

4. CAR T Mechanism

To understand the mechanism and function of CAR T Cell, the structure of CAR shall be the first to catch on. Proteins called chimeric antigen receptors include several features of conventional T cell activation. They connect an internal signaling domain, which activates the T cell when an antigen is attached, to an external antigen recognition domain. The four parts that make up CARs are an antigen recognition domain, an extracellular hinge region and a transmembrane domain. An intracellular T cell signaling domain is also included. We'll focus on the evolution of the intracellular T cell signaling domain and the antigen recognition domain in this review. When the antigen recognition domain is exposed to the outside of the cell, it interacts with potential target molecules. It is also in charge of directing the CAR-T cell to any cell that expresses a certain complementary molecule. Typically, a monoclonal antibody's variable regions form a single-chain variable fragment that is joined together to form the antigen recognition domain (scFv) [4]. Remains that are hydrophilic make constitute the linker connecting the two chains. Additionally, it contains lengths of glycine and serine for flexibility as well as glutamate and lysine for enhanced solubility [5]. Due to their high transduction

effectiveness in T cells, single domain antibodies have been generated and used as antigen recognition domains in the CAR format [6].

Next, let's talk about intracellular T cell signaling. The endo domain of the receiving point, which is located on the inside of the membrane, houses the intracellular T cell signaling region. CAR receptors group together and send out an activation signal when an antigen has been attached to the exterior antigen recognition domain. The receptor's internal cytoplasmic end thus keeps the T cell's internal signaling going [7]. In addition to CD3 signaling, co-stimulatory molecules are also necessary for T cells to survive following activation. The production of a CAR T cell is determined by the intracellular signaling domain that is employed.

Evidence of tons of researches suggests that Therapies using CAR T cells activate the T cell response against antigen-expressing cells, including both benign and malignant tumors. The antigen is bound by the exterior targeting domain, activating the CAR T cell. When CAR T cells are activated, they produce cytokines and other soluble mediators that may aid in the destruction of both target cells that display the antigen and normal cells.

When the patients get the doses with fully engineered CAR T cells, they seem to be able to automatically fight off the cancer cells. CAR T cells connect to and activate when they come into touch with their target antigen on a cell, then proceed to grow and become cytotoxic [8]. CAR T cells kill cells by increasing their cytotoxicity—the degree to which they are hazardous to other live cells—and by boosting the release of cytokines, interleukins, and growth factors, which can impact other cells. These methods include substantial stimulation of cell proliferation. FDA has approved 6 CAR T cell treatments, with the first one being authorized in 2017 [9-10].

Although direct interactions between T cells and tumor cells are the foundation of CAR T cell design, the release of cytokines by activated CAR T cells may improve these cells' anti-tumoral properties. For a live therapy that depends on a very precise and focused approach, it is obviously hard since solid tumor lesions have a large deal of phenotypic variability. Through secondary processes, CAR T cell cytokine production is crucial in promoting tumor lysis. Permanent transgene expression is frequently needed for cell-based gene treatments to produce long-lasting therapeutic results. Lentiviruses and murine gammaretroviruses are two therapeutic gene therapy vector systems that enable CAR transgene expression over an extended period of time. The expression of retroviral vectors in human T cells is safe, according to a substantial body of clinical evidence.

5. Immunotargets for NB

5.1. B7-H3(CD276)

The extracellular domain of the 316 amino acid long type I transmembrane protein known as B7-H3 distinguishes it into two isoforms. Immunoglobulin constant (IgC)-like and immunoglobulin variable (IgV)-like domains make up the extracellular domain in mice, however exon duplication in humans has resulted in either one pair (2Ig-B7-H3) or two identical pairs (4Ig-B7-H3). Most normal tissues express B7-H3 mRNA. The B7-H3 protein, in contrast, has relatively little expression in normal tissues due to the post-transcriptional control of this protein by microRNAs. However, several cancer forms, including neuroblastoma, express the B7-H3 protein often. Because microRNA-29, IFN- γ stimulation, and immunoglobulin-like transcript 4 (ILT-4) signaling, B7-H3 is typically overexpressed in a variety of cancers, immunotherapies that target B7-H3 can avoid off-target side effects [11-12]. Enoblituzumab, a B7-H3-targeting antibody known for being nonreactive to the liver, is presently undergoing phase I studies for a variety of solid tumors, including refractory tumors and pediatric malignancies. Additionally, a clinical research using a dualaffinity retargeting platform-based T-cell-engaging BsAb is now being carried out in patients with advanced solid tumors that are positive for B7-H3. B7-H3 is a particularly enticing tumor target or checkpoint ligand due to the prevalence of B7-H3 overexpression in NB, lung, breast, brain, kidney, and prostate cancers as well as dendritic cells.

5.2. GD2 Immunotherapy and its disadvantages

The highest level of GD2 expression has been seen in NB in the tumor cell lines and tissues studied by the researchers, which has been neuroectodermally derived. It has been reported that there is around 98% expression in all neuroblastoma cell lines. GD2 is an obvious biomarker for NB, with 5–10 million molecules per cell, on average. GD2 is the 12th most efficient antigen for the treatment of cancer. The most efficient immunotherapy to target GD2 is dinutuximab, a chimeric monoclonal antibody created from a mix of mouse and human DNA. In patients with HR-NB, it has a better proportion of event-free survival when taken with immune stimulants. For example, IL-2, GM-CSF, and CRA will turn out to be that result. While patients with advanced solid tumors have showed encouraging outcomes when CAR T-cell therapy is paired with an oncolytic virus that encodes the BiTE gene, GD2 immunotherapy was improved by activating T cells with T-BsAbs. The transport of therapeutic medicines to the tumor was made even more effective by attaching payloads to IgGs. By all means, although GD2 related immunotherapy has been developed for years, yet still stuck in the current situations without the presence of much more improved survival rate.

6. Conclusion

CAR T cell immunotherapy is and will be the main focus on the improvement of therapies for neuroblastoma. Nowadays, the world has witnessed the emergence of several GD2 and other biomarker-based CAR T therapies, however, our understanding of CAR T therapy for NB is still limited. The current therapies still have problems in side effects and precision. With the advancement in gene engineering and protein technology, we will have further insight in using CAR T therapies and protect more children from the risk of NB.

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