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The Advantages and Challenges of CAR-NK Cell Therapy in Overcoming Solid Tumors

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Abstract. In the past few years, cell-based immunotherapy has made significant progress in treating tumors, especially CAR-T cell therapy, which has shown significant efficacy in hematological tumors. However, in the field of solid tumors, it faces limitations such as high toxicity and side effects, an immunosuppressive microenvironment, and limited tumor infiltration. NK cells possess natural killing and recognition mechanisms, resulting in fewer toxic side effects. Therefore, CAR-NK therapy has become a new research direction for solid tumors and is expected to become a substitute for CAR-T therapy. However, this treatment is in its early stage of development and faces problems such as insufficient persistence, immunosuppressive tumor microenvironment, and a lack of standardized production processes. This review summarizes the latest topics and exploratory projects on CAR-NK cell therapy, elucidates its therapeutic mechanism, emphasizes its advantages and challenges, suggests prospective solutions for various challenges and finally discusses the future prospects of it. In order to advance this treatment method towards clinical application, challenges such as NK cell persistence must be addressed. Technologies like gene editing and combination therapies are being employed to gradually overcome current limitations.

Keywords: CAR-T, NK cells, CAR-NK, Solid tumors, Immunotherapy.

1. Introduction

As everyone knows, intervention and treatment for cancer are particularly important. Surgery, radiation and chemotherapy constitute the Traditional cancer therapies. Although they are widely used in clinical practice, they also face numerous limitations. The rise of immunotherapy is gradually overcoming these limitations. It has the characteristics of strong specificity, minimal side effects, and strong memory, bringing infinite hope to people in treating tumors.

CAR-T cell therapy starts by extracting T cells from an individual, incorporating the CAR structure onto the T cells using genetic engineering techniques, enabling them to recognize specific tumor cells, and then culturing and amplifying them in the lab before reinfusion into the individual. As a result, it has achieved remarkable efficacy in hematological neoplasms. For example, innovative anti-CD19 CAR-T cells with PD1 integration have achieved a complete response rate of nearly 90% in individuals with recurrent B-cell lymphoma [1]. However, there are limitations and challenges in treating patients with solid tumors, such as the presence of an immunosuppressive tumor microenvironment. Solid tumors are tumors that occur within human tissues and have a solid

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structure. The dense structure of solid tumors contains a rich fibrous matrix, making a physical barrier that hinders CAR-T cells from penetrating deep into the tumor tissue. But NK cells have a milder cytokine profile and lack neurotoxicity, rarely causing adverse reactions such as CRS and GvHD, and thus offer higher safety. It possesses the ability to naturally identify and attack tumor cells without MHC-1 (Major histocompatibility complex-1) molecules to recognize tumor antigen molecules, enabling them to directly target tumor subpopulations that T cells cannot recognize. Recent clinical evidence suggests that that when NKG2D CAR-NK cells are intraperitoneally infused into some pre-treated patients with metastatic colorectal cancer, the peripheral immune system is activated, and the condition of some patients is controlled. This further indicates that it has great value in conquering solid tumors [2]. However, it faces limitations such as insufficient persistence, necessitating gradual improvements.

This review elucidates therapeutic mechanism of CAR-NK therapy and its advantages over traditional CAR-T therapy, summarize the evidence-based studies in recent years, present the challenges of CAR-NK therapy, and explore various coping strategies. The aim is to provide references for the optimization and development of CAR-NK methods in the future, assist medical professionals in identifying better research directions, and facilitate the advancement of CAR-NK therapy.

2. Overview of treatment mechanism

NK cells are important commanders of the entire immune response, possessing a natural mechanism for killing tumor cells. The perforin-granzyme pathway is the main pathway for NK cells directly lyse target cells. Initially, the target cell membrane forms pores in the presence of perforin, and granzyme can then be delivered to the cytoplasm. Subsequently, granzyme induces apoptosis by cleaving its substrates. In addition to the release of lysis particles, NK cells can carry death receptors TRAIL and FasL to trigger cells expressing death receptor ligands. Furthermore, it can Release key factors such as TNF-α(Tumor Necrosis Factor-alpha)and IFN-γ(Interferon-gamma)to promote the activation of immune cells and inhibit tumor proliferation. These mechanisms enable NK cells to mediate the destruction of tumor cells.

CAR-NK therapy aims to treat tumors by integrating the self-killing characteristics of NK cells with the precise identification of the CAR structure, which consists of three parts: extracellular domain, transmembrane region, and intracellular signaling domain, but the design of the CAR structure for NK cells and T cells differs. The primary structural distinction lies within the intracellular signaling domain. This region of CAR-T cells usually includes traditional T cell activation receptors, such as CD28 or 4-1BB. This region of CAR-NK cells tends to favor natural signaling molecules that enhance NK cell activation, such as DAP10 or 2B4 [3].

3. CAR-NK vs CAR-T: advantage

3.1. Stronger recognition ability

CAR-T cells can only rely entirely on CAR signaling to target tumor cells, whereas CAR-NK cells can also recognize tumor surface antigens through the natural receptor system without dependence on CAR. NK cells possess multiple natural inhibitory and activating receptors. Normal cells, which express MHC-1 molecules, will bind to the major ligands of NK cell inhibitory receptors (CD94 and KIRs) and be considered healthy cells. However, some tumor cells down-regulate the expression of MHC I molecules, allowing them to escape recognition by CD8+ T cells. However, tumor cells lack

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the main ligands of NK cell inhibitory receptors and do not inhibit NK cells but instead activate them, prompting NK cells to attack tumor cells [4]. Additionally, there are also high expression NKG2D ligands and DNAM-1 ligands in tumor cells that combined with the corresponding activated receptors in NK cells, thereby triggering the specific killing of tumor cells by NK cells. Crucially, such ligands are absent in healthy tissues, making NK cell specificity for tumor recognition. This dual recognition mechanism makes them have a stronger recognition ability.

3.2. Lower toxicity and side effects

CAR-T cell therapy is an approved immunotherapy, but it can lead to some complications in patients, particularly cytokine release syndrome (CRS), immune effector cell-associated neurotoxic syndrome (ICANS), and graft-versus-host disease (GvHD). The mechanism behind CRS caused by this therapy is that on their activation within the host, CAR-T cells release TNF-α (Tumor Necrosis Factor-alpha) and IFN-γ, activating a large number of macrophages to generate elevated levels of pro-inflammatory cytokines. However, NK cells release milder cytokines, such as GM-CSF and IFN-γ, that are not the category of cytokine associated with CRS. Additionally, it has a shorter lifespan, which can reduce the duration of toxicity. Therefore, CAR-NK cell therapy rarely causes toxic and side effects such as CRS and ICANS. Furthermore, NK cells do not express TCR genes and cannot recognize host tissue MHC molecules. Researchers infused electroporation-modified NKG2DCAR-NK cells into two patients with metastatic colorectal cancer who received semi-matched NK cell therapy, and no instances of GvHD were found [5]. Overall, CAR-NK exhibits lower toxicity and side effects in combating solid neoplasms, especially in terms of CRS and neurotoxicity, providing a more favorable safety profile for its clinical application.

3.3. Greater scalability

CAR-NK has greater advantages, mainly reflected in three aspects: cell source, production cost, and storage and transportation. The source of CAR-T cells is mainly from patient's body, while CAR-NK cells are collected from multiple sources, making sources more extensive and flexible, such as NK92 cell line, peripheral blood mononuclear cells (PBMC), induced pluripotent stem cells (iPSC), and umbilical cord blood (UCB). These sources are all allogeneic, so CAR - NK cells can produce in advance, allowing patients to use ready-made CAR-NK cells without waiting for cell collection, laying a huge foundation for large-scale, standardized production. Additionally, they do not need HLA(Human Leukocyte Antigen) matching, making them suitable for allogeneic applications. The allogeneic nature of CAR-NK allows for large-scale, centralized production, and batch production can save a significant amount of cost. The resuscitation efficiency of CAR-T cells after cryopreservation is low, and their shelf life is typically only 1-2 days. Studies have shown that large-scale mesenchymal stem-like CAR-NK cells generated from human embryonic stem cells can achieve a viability of 70%-80% after being cryopreserved for 6 months and resuscitated. With an extended incubation time of 24-48 hours, the cell viability returns to over 90%. Subsequently, tumor killing assays demonstrated that the resuscitated CAR-NK cells maintain strong ability to remove ovarian tumor cells [6]. This indicates that CAR-NK cells are more suitable for long-term preservation and are easy to transport over long distances. CAR-NK cells have greatly enhanced their scalability due to their advantages such as abundant cell sources, low cost, and ease of storage and transportation.

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4. Application of CAR-NK in solid tumors

Having undergone preliminary testing, CAR-NK cell therapy is now applied to solid tumors including breast, hepatic, and colorectal carcinomas. Although the therapy is still in the exploratory stage, it has demonstrated its potential in treating solid tumors.

About 15-20% of breast cancers exhibit high expression of HER2 (human epidermal growth factor receptor 2), which is called " positive HER2 breast cancer ". A previous team formulated mouse organoid model of this subtype, verifying that engineered HER2-CAR-NK therapy can effectively clear breast cancer cell within organoid microenvironments, giving a new idea for managing HER2-positive breast carcinoma [7]. CAR-NK cells can also target diverse receptors to attack various types of breast carcinoma. For example, CD44v6-targeted CAR-NK cells have shown the competence to resist an immunosuppressive microenvironment in a 3D TNBC (triple-negative breast carcinoma) multicellular tumor spheroid (MCTS) solid tumor model [8].

Liver cancer is a highly dangerous tumors, 5 years survival rate lower than 20%. Transforming growth factor beta (TGF-β) is frequently upregulated in hepatocellular carcinoma. It can directly inhibit NK cell function and accelerate the proliferation of stromal fibroblasts, thereby facilitating tumor metastasis. Therefore, studies have shown that blocking the TGF-β pathway can potentiate the longevity and cytotoxic function of NK cells in a tumor microenvironment rich in TGF-β [9]. How to precisely regulate the TGF-β pathway is also an important research direction for the future of liver cancer.

CD70 is specifically expressed in cancer-associated fibroblasts (CAFs) and tumor tissues, and CAFs are key factors promoting tumor metastasis. Therefore, researchers have explored the role of CD70 for CAR-NK cells, demonstrating its high expression in colorectal cancer (CRC). They also used a lymphoma model mouse to prove that CAR-NK cells stimulated by IL-15 targeting CD70 can effectively eliminate tumor cells [10].

5. Challenges, bottlenecks and countermeasures

5.1. Persistence of NK cells in vivo

Because of their short half-life the lifespan of NK cells is very short. Although this limits their toxicity in the body, it makes it difficult to maintain long-lasting efficacy in patients. Infused CAR-NK cells only survive in patients for about two weeks. To address this issue, some researchers have optimized the CAR design by incorporating the CD28 molecule, which is not expressed by NK cells, into the costimulatory domain of the CAR. In a breast cancer model, mice injected with CAR27-28ζ NK cells exhibited significantly smaller lung tumor areas compared to the control group, emphasizing that CD28 significantly increases NK cell activity [11]. Others have proposed other methods, such as combining interleukin-7 (IL-7) signaling with CAR-NK cells, which was found to increase survival time in the microenvironment of an in vivo neuroblastoma xenograft model, although its tumor control ability was reduced [12]. Future studies need to improve the persistence of NK cells while paying attention to their effector function.

5.2. Immunosuppressive microenvironment

CAR-NK cells also face certain limitations in an immunosuppressive microenvironment. The immunosuppressive microenvironment is mainly composed of a large number of immunosuppressive cells, such as cancer-associated fibroblasts (CAF), myeloid-derived suppressor

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cells (MDSC), and regulatory T cells (Tregs), many suppressive cytokines, as well as numerous inhibitory cytokines. Such factors compromise NK cell functionality, posing significant challenges to CAR-NK cell therapy. TGFβ signaling is another mechanism of immunosuppression within the TME. The secretion of TGF-β promotes immunosuppression by increasing the concentration of immunosuppressive cells, suppressing activating receptors, and regulating cytokines. Recent research has seen a team design a new chimeric cytokine receptor, TRII/21R, which is integrated into CAR-NK cells. Experimental data demonstrates that it's in vitro killing rate against gastric cancer cells is 30%-50% higher than that of traditional CAR-NK cells. Moreover, source in patients of gastric cancer xenograft model, the tumor volume inhibition rate reaches 72%. It exhibits strong antitumor capabilities both in vitro and in vivo [13].

5.3. Lack of standardized production process

One of the bottlenecks currently facing the clinical adoption of CAR-NK is the lack of standardized production processes. Firstly, the sources of cells are not uniform, including peripheral blood, umbilical cord blood, iPSC, etc. Different sources exhibit varying performance in terms of expansion efficiency, killing activity, and persistence. Secondly, there are significant differences in genetic engineering construction, with no unified design of CAR structures. Different research institutions use vector systems and expression strategies that vary greatly. Lastly, the challenges of quality control and GMP (Good Manufacturing Practice) preparation include the optimization of serum-free media (such as X-VIVO medium, AIMV medium, SCGM medium), the compliant use of GMP-grade cytokines (such as rhIL-2, rhIL-15), and the function of seed cell thawing. There is also the issue of the capacity for large-scale preparation is insufficient. Establishing a unified and standardized CAR-NK preparation system is a crucial step in advancing its clinical translation.

6. Future development direction

The future development will be optimized through combination therapies, genetic engineering optimization, and the development of new cells. In terms of combination therapies, combining with immune checkpoint inhibitors can reverse the tumor immune microenvironment and enhance the anti-tumor efficacy. For example, in preliminary clinical studies of breast cancer and ovarian cancer, NK cells combined with PD-1 inhibitors were used to treat advanced patients, and tumor shrinkage was observed without severe adverse reactions [14]. In terms of genetic engineering, pretreating NK cells, such as chemically stimulating them with cytokines like IL-15 or IL-2, or physically stimulating them with electroporation, can increase their activity. The transduction rate of NK cells can be improved by gene editing tools such as CRISPR/Cas9. Additionally, developing new cell sources is crucial. IPSCs are a revolutionary innovation in regenerative medicine in recent years, particularly in the application of CAR-NK cell therapy. Research shows that targeted CD70 CAR-iNK cells exhibit higher cytotoxicity in xenograft mice carrying human lymphoma and renal cancer compared to non-transduced NK cells. Furthermore, they can kill allogeneic reactive T cells, reducing the risk of GVHD [15]. In the future, it is expected that technologies such as gene editing will drive the progress of iPSC-derived NK cells.

7. Conclusion

CAR-NK cell therapy demonstrates potential surpassing that of CAR-T cell therapy in the field of conquering solid tumors. In addition, the core advantage of it is to identify a wider range of cells

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through multiple recognition mechanisms, while also offering higher safety and lower toxicity during treatment. Furthermore, CAT-NK cell therapy exhibits better scalability, making it easier to achieve large-scale, standardized production. However, it still faces challenges such as insufficient persistence, which may lead to diminished efficacy or recurrence. Additionally, the impact of an immunosuppressive microenvironment can reduce NK cell activity. The lack of standardized production processes is also an issue that needs to be addressed. In summary, it represents a promising future direction in solid tumors. In the future, advanced technologies such as genetic engineering, combination therapies, and improved production processes are needed to improve the durability of NK cells, find effective strategies to overcome immune suppression, and improve product stability and consistency. It is believed that with continued research, CAR-NK cell therapy will become a safer and more efficient treatment method.

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