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Advances and Challenges in CAR-NK Cell Therapy for Tumor Immunotherapy

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Abstract. The root cause of tumors lies in genetic mutations. NK cells have shown significant potential in tumor immunotherapy. NK cells possess three anti-tumor mechanisms: direct cytotoxicity, antibody-dependent cell-mediated cytotoxicity, and secretion of cytokines. Compared with traditional αβ T cell therapy, NK cell therapy is safer, and KIR mismatch can enhance its anti-tumor response. CAR-NK cell therapy, through genetic engineering, transfers the synthetic receptor protein CAR into NK cells, enabling precise recognition and killing of tumor cells. The mechanisms by which CAR-NK cells kill tumor cells include the release of cytotoxic granules, induction of apoptosis, and secretion of cytokines. This therapy has remarkable efficacy in treating solid tumors, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, and has advantages such as broad-spectrum anti-tumor activity, wide cell source, relatively simple preparation, and the ability to be used as a "ready-made" product. However, CAR-NK cell therapy also faces challenges such as limitations on NK cell infiltration by the tumor microenvironment, limited cell sources, and deficiencies in immune checkpoint inhibitors. Combining with traditional therapies such as chemotherapy and radiotherapy or using other biological agents in combination is expected to improve its efficacy. In addition, allogeneic NK cell therapy has been applied in hematopoietic stem cell transplantation and adoptive immunotherapy, although it shows significant efficacy in hematological malignancies, it still needs improvement in the treatment of solid tumors. Overall, NK cell therapy has a promising future, but many difficulties need to be overcome to achieve wider clinical application.

Keywords: CAR-NK cells; Tumor immunotherapy; NK cell therapy.

1. Introduction

In today's era, cancer has become one of the fatal diseases around the world. There are countless number of people who died due to cancers such as lung cancer and melanoma. Cancer's high morbidity and mortality severely influenced the average life span and health level of human beings. Especially in developing countries, cancer has caused higher death rate because of the medical standards lag. This phenomenon, however, further negatively affect the improvement of population improvement. In the past, it was hard for scientists to deal with cancer because of the cancer cells' strong proliferation which lead to rapid division and production, as well as the gene mutation that helps them to adapt to the varying environment and further escape medication. However, national governments and charity organizations are investing more money on cancer research. This includes basic studies like looking into the pathogenesis of cancer and exploring the function of oncogenes, as well as clinical studies such as developing immunotherapies and targeted therapies.

Among them, a very promising cancer treatment therapy is undoubtedly immunotherapy based on natural killer cells. Scientists have made a series of advancements in the field of research and development. For instance, the function of NK cells can become more effective through the participation of cytokines and antibodies. Furthermore, scientists have certain scientific and technological reserves to produce CAR-NK cells. In this production process, NK cells need to be first obtained from iPSCs, and then advanced genetic engineering techniques are used to modify the obtained NK cells. CAR-NK cells have great advantages in cellular immunotherapy [1]. There's also the SSB NMs nanoemulsion system to co-deliver a TGF-β inhibitor and SeC for enhanced anticancer effect. It boosted NK92 cells' lytic ability 2.1-fold and made Triple - Negative Breast Cancer

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Cells more sensitive to patient-derived NK cells, increasing cancer lysis up to 13.8 times. By suppressing signaling, it strengthened the immune response. Combined with NK92 cells, it worked in animals, offering a design approach and insights into NK cell therapy for breast cancer [2]. This strategy strengthens the anticancer ability of NK92 cell, making it easier to recognize and kill the cancer cells. However, there are several disadvantages to using a single kind of immunotherapy tumor heterogeneity limits efficacy, tumor microenvironment (TME) suppression and immune escape issue. Therefore, this essay will discuss some novel NK cell based immunotherapies as well as the combination of these therapies to make the treatment more effective.

2. Main Methods of NK Cell to Inhibit Tumors

The root cause of tumors is genetic material mutations. Unidentified mistakes that take place during the process of cell mitosis can result in swift and uncontrolled cell growth and division. Such mutations can either be passed down through inheritance or emerge later in life due to epigenetic factors or exposure to carcinogenic agents like smoking, obesity, radiation, and unhealthy lifestyle habits [3]. NK cells belong to one of the lymphocytes which roam in the humans bodies' innate immune system. Under normal circumstances, these immune cells can restrict the spread of infection effectively.

2.1. Direct Cytotoxicity

NK cells have a special ability to recognize and attach to tumor cells. This special function exists because of the unique receptors on the cell surface. When these receptors bind to the receptors of tumor cells, some cytotoxic substances will enter the cancer cells, thereby eliminating them devastatingly. One of the substances is called perforin. The membranes of tumor cells will develop holes due to the presence of this substance. This kind of hole provides a channel for the entry of granzyme. When granzyme enters the interior of tumor cells, the programmed death of normal cells - apoptosis - is restarted, thereby causing tumor cells to commit suicide [4].

2.2. Antibody-dependent Cell-mediated Cytotoxicity

The second mechanism of action is ADCC. The surface of NK cells contains CD16 receptors, which have the property of binding to the Fc fragments of IgG antibodies. Once IgG antibodies bind to the antigens on the surface of tumor cells, NK cells can precisely recognize and bind to these tumor cells that have been coated with IgG antibodies by means of their own CD16 receptors. Immediately after, NK cells promptly activate their cytotoxic functions, efficiently and powerfully attacking tumor cells, thereby achieving the elimination of tumor cells.

2.3. Secretion of cytokine

The last method is by secreting cytokine: NK cells are capable of secreting a diverse range of cytokines, including tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), among others. TNF-α can directly impede the proliferation of tumor cells and induce their necrosis. On the other hand, IFN-γ can enhance the antigen-presenting functions of macrophages and dendritic cells. It also has the ability to activate T cells, thereby strengthening the overall anti-tumor immune response. Moreover, IFN-γ can inhibit the process of tumor angiogenesis, which in turn restricts the growth and metastatic spread of tumors.

3. Unique Advantages of NK Cell Therapy and KIR Mismatch

Unlike classical T cells, NK cell therapy is less unlikely to cause the secretion of CRS and neurotoxicity, and it will not cause GvHD neither. KIR mismatch can enhance the anti-tumor response. It happens when KIR and HLA mismatch. There are two main kind of KIR mismatching, inhibitory KIR mismatch and activating KIR mismatch.

3.1. Inhibitory KIR Mismatch

When the inhibitory KIR on the surface of donor NK cells fail to recognize and bind to the HLA class I molecules on the surface of recipient cells, the inhibitory signals that could normally suppress the activity of NK cells cannot be generated. In this situation where the inhibitory signals are absent, once the activating KIR on the surface of donor NK cells successfully binds to the corresponding ligands on the surface of recipient cells, an activating signal will be triggered, thereby activating the NK cells and enabling them to exert their immune killing function.

3.2. Activating KIR Mismatch

As for the second kind of KIR mismatch, When the surface activating killer cell immunoglobulin-like receptors (KIR) of the donor's NK cells, which are originally ligands corresponding to those on the recipient's cells, have abnormal binding situations, this will lead to excessive activation and stimulation of the NK cells of the donor. Under the condition of being over-activated, the donor's NK cells will launch more powerful attacks on and eliminate tumor cells within the recipient's body. From this perspective, it can enhance the therapeutic effect of transplantation surgeries such as hematopoietic stem cell transplantation. It will be safe for patients to use NK cell donated from other healthy people [5].

4. CAR-NK Immunotherapy

4.1. Advantages of NK Cells

When mentioning CAR therapy, NK cells should be the most ideal ones. They have the advantages of being able to kill cancer cells without pre-sensitization, having a low risk of CRS, and being applicable in an allogeneic setting. Some clinical trials have yielded promising results. For example, FT596 induced objective responses in both monotherapy and combination therapy. Engineered NK cells also showed good efficacy in treating B-cell lymphomas with no severe treatment-related toxicity.

4.2. Three Steps of CAR NK Cell Therapy

CAR NK cell therapy mainly consists of the following steps. First, autologous or allogeneic NK cells are extracted by collecting peripheral blood. Then, cell separation technology is used to separate NK cells from other blood cells. Next, genetic engineering technology is applied to transfer CAR, a synthetic receptor protein, into NK cells. CAR is mainly composed of three parts: an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain. The antigen-binding structure in CAR can recognize the glycoproteins on the surface of cancer cells. When the two bind, specific signals are transmitted into the cell by the transmembrane domain, activating the intracellular signaling domain and initiating the killing function of NK cells against cancer cells. After the genetically modified CAR NK cells are cultured in vitro for a short period of time and reach a sufficient quantity for cancer cell treatment, they are infused back into the human body. Relying on the synthetic receptor protein CAR, they can accurately locate the target antigen, activate the NK cells, and launch attacks on tumors through multiple mechanisms.

4.3. Mainly Mechanisms by Which CAR NK Cells Kill Tumor Cells

4.3.1. The Release of Cytotoxic Granules

CAR NK cells possess unique and powerful attack capabilities and can release cytotoxic granules containing perforin and granzyme. Perforin is like a "molecular scissor" that can precisely cut tiny holes in the cell membrane of tumor cells. The formation of these holes provides a channel for granzyme to enter. After granzyme enters the interior of tumor cells through these small holes, it is like activating a "death switch", triggering the apoptosis pathway within the cells and prompting tumor cells to die according to a programmed process, thus effectively eliminating tumor cells.

4.3.2. Induction of Apoptosis

Important death receptor ligands are expressed on the surface of CAR-NK cells, such as FasL. These ligands play a very important role in immune surveillance. They can detect abnormal receptors existing on tumor cells and cancer cells with great precision and efficiency. When in contact with these receptors, the ligands on the cell surface will bind relatively, transmitting a message to the tumor cells that they will start to undergo apoptosis like normal cells. Once the tumor cells acquire the ability of programmed death, they will gradually be destroyed, thereby maintaining the internal balance and health of the body.

4.3.3. The Secretion of Cytokines

CAR NK cells are like a powerful "immune factor factory" that can consistently secrete many kinds of cytokines, such as IFN-γ and TNF-α. These cytokines are like "immune regulators". After being released into the body environment, they can finely regulate the immune system. They can awaken and enhance the activity of other immune cells, enabling these immune cells to participate in the fight against tumors with higher efficiency, cooperate with each other, and form a powerful anti-tumor synergy. Moreover, these cytokines can also act directly on tumor cells like "precision strike weapons", inhibiting the growth process of tumor cells and inducing tumor cells to initiate the apoptosis program, prompting them to die, thus effectively curbing tumors.

4.4. Potential in Clinical Applications

CAR NK cell therapy has unlimited potential in clinical applications. First, it can be used for the treatment of solid tumors such as neuroblastoma and colon cancer. Recently, chimeric antigen receptor NK cells (CAR-NK) have achieved satisfactory results in inhibiting glioblastoma multiforme (GBM). The research by Wang et al. shows that given the tumor heterogeneity and various immunosuppressive characteristics of the TME of GBM, the development of multifunctional genetically engineered human NK cells (CD73. mCAR pNK) can produce effective anti-GBM activity [6]. Targeting autophagy as an immunomodulator can promote the chemotactic aggregation of CAR-NK cells with effector functions towards the GBM tumor site, and reprogram the TME of GBM to make it more sensitive to CAR-based targeting. Lin fused the extracellular domain of the NK cell receptor NKG2D with DAP12 to successfully construct a CAR aiming to enhance the response of NK cells to tumors [7]. To effectively avoid risks, they employed RNA electroporation technology, which is a means capable of facilitating the transient expression of CARS. Experimental data show that the expression level of NKG2D RNA CAR has been significantly increased, significantly enhancing the cytolysis activity of NK cells against various solid tumor cell lines in an in vitro environment. Moreover, in mouse models with established solid tumors, these modified NK cells also exhibit significant therapeutic effects, bringing positive impacts on tumor treatment in mice. Based on the promising experimental results, they attempted to transfer this therapy into clinical application. Among three patients with metastatic colon cancer, the first two showed a significant reduction in ascites after treatment, and for the third patient with liver metastatic tumors, they observed a significant regression of the metastatic tumors, further highlighting the optimistic treatment prospects of NK cell therapy modified with RNA CAR. In addition, HLA-G is recognized as an ICP that most of the cancer cells are able to express. JAN found that NK cells transduced with HLA-G CAR exhibit powerful effects of cytotoxicity on cancers such as pancreatic cancer and brain cancer [8]. Moreover, the growth of xenograft tumors in orthotopic mouse models are strongly restricted by these cells and they can prolong the median survival time of the mice. This method will be advantageous in treating solid tumors in a longer term.

What's encouraging, the CD19-targeted CAR-NK cell therapy shows good efficacy, with an ORR as high as 73%, and the number of patients with CR reaching 7 American researchers announced the promising results of a phase I/II study on cancer patients receiving CD19-targeted CAR-NK cell therapy derived from cord blood [9]. The data shows that within 100 days after treatment, the OR rate of patients reaches 48.6%. It is particularly noteworthy that in the group of patients with relapsed or refractory B-cell malignancies, the one-year PFS is 32%, and the overall survival rate OS is 68%. Generally speaking, in terms of the objective response rate, progression-free survival rate, and overall survival rate, CAR NK cell therapy has strong curative potential and brings new hope for cancer treatment [10].

4.5. Advantages of CAR NK Cell Therapy

CAR NK cell therapy has many advantages. First, it has broad-spectrum anti-cancer properties [11]. NK cells have unique advantages. They do not require specific recognition of tumors to function and are not restricted by the inhibitory activity generated by the major histocompatibility complex (MHC) on the cell surface. CAR-NK cells combat cancer cells through diverse pathways. On the one hand, they can effectively inhibit the growth and proliferation of cancer cells through CAR-dependent pathways and NK cell receptor-dependent pathways; on the other hand, NK cells can also precisely eliminate tumor cells through the CD16-mediated ADCC mechanism, demonstrating powerful anti-cancer capabilities. The second advantage is its wide source and relatively simple preparation. NK cells are widely present in various clinical samples and have an extremely rich source. NK cells can be obtained from PB, UCB, human HESC, IPSC, and NK-92 cell lines. Among these sources, iPSC have outstanding advantages. Its preparation process only requires a small amount of seed cells and can achieve large-scale culture and expansion, not only greatly reducing the preparation cost but also enabling autologous supply. At the same time, it has low immunogenicity and greatly reduces the risk of immune rejection. The NK-92 cell line is known for its unlimited proliferation characteristics and has low sensitivity to freezing and thawing, which provides convenience for the large-scale storage and long-term use of NK cells. It also has the unique advantage of being an "Off-the-Shelf" product. Relying on its high safety, NK cell therapy demonstrates great clinical application value, and the most remarkable one is that it can be used as an "Off-the-Shelf" product. This means that NK cell therapy can be widely applied to patients of different individuals without the need for individual customization for each patient. Moreover, NK cells can be cryopreserved for a long time and can be quickly used when patients urgently need treatment, winning precious treatment time for critically ill patients. In addition, this "Off-the-Shelf" characteristic can also effectively reduce production costs, making the terminal selling price more affordable. This not only reduces the out-of-pocket expenses of patients but also alleviates the pressure on medical insurance payments to a certain extent, enabling more patients to benefit from NK cell therapy.

4.6. Challenges CAR NK Cell Therapy Faces

Even so, it still needs to face a series of challenges. The limitation of NK cell infiltration by the microenvironment of solid tumors is one of the difficulties of this type of therapy [12]. NK cells are sparsely distributed in solid tumors. The expression of chemokines such as CCL27, CXCL12, and CCL21 that recruit NK cells is reduced. The expression of CXCR5 and CXCR6 in tumor-infiltrating NK cells is increased, while the expression of CX3CR1 and S1PR1 is decreased, resulting in the preferential recruitment of CD56bright NK cells with lower cytotoxicity. The migration of NK cells to the TME is affected due to the obstruction of their migration and maturation from the bone marrow. Second, the limited source of cells: Although there are various sources of NK cells, each has certain problems. The number of NK cells in peripheral blood is limited. The "off-the-shelf" NK cell line (such as NK92) has the problem of genetic instability. The therapeutic effect of UCB NK cells is affected by the KIR haplotype. iPSC-NKs have advantages, but they still face challenges in large-scale production and quality control. Third, the defects of immune checkpoint inhibitors (ICIs): ICIs have a relatively short half-life. Although they are easy to administer and control, they also limit their therapeutic effect and increase the treatment cost.

5. CAR-NK Cell Combination Therapy

The emergence of this combination therapy is applicable to advanced cancer and NK cells where the TME is inhibited. However, for some cancer cells with strong mutation ability, the combined therapy of CAR-NK cells alone is far from sufficient. Combining with traditional therapies. This combined therapy can be combined with existing traditional therapies in clinical practice, such as chemotherapy and radiotherapy. People will take this measure because radiotherapy has many advantages, such as directly destroying tumor cells or re-establishing the TME. Although the side effects of radiotherapy are relatively large, through combined use with CAR-NK cell therapy, the side effects can be minimized as much as possible. Not only that, the synergistic effect can also be manifested when the cell binds to the tumor antigen.

5.1. Allogeneic NK Cell Therapy

Another NK cell based immunotherapy is Allogeneic NK Cell Therapy. The function of autologous NK cells in cancer patients is limited by KIR ligand matching. However, the mismatch between KIR receptors of allogeneic NK cells and their ligands on the surface of target tumor cells can promote the generation of strong anti - tumor activity and limit GvHD. Unlike T cells, NK cells do not cause GvHD, and the toxic effects of infusing allogeneic donor NK cells are relatively small.

5.2. Application of Aallogeneic NK cells

Hematopoietic stem cell transplantation or adoptive immunotherapy provides a platform for the application of allogeneic NK cells. During the process of hematopoietic stem cell transplantation, the side effects caused by NK cells are smaller compared with those of ordinary T cells. For example, the incidence of GvHD is lower, and this feature is applied in hematopoietic stem cell transplantation with T cell depletion. Meanwhile, the first batch of lymphocytes reconstructed after allogeneic hematopoietic stem cell transplantation is also one of the main characteristics of this type of cell. Scientists can obtain NK cells by isolating products from substances such as umbilical cord blood and NK92 during the process of adoptive immunotherapy. So far, a relatively feasible way has been discovered to improve the treatment of blood diseases and the efficacy of solid cancers, that is, to co-culture NK cells with feeder cells and various cytokines. So far, NK cell donors can be selected through various methods, such as based on the genotypes of their KIR and KIR ligands. Of course, scientists are not limited to this one method. They are actively exploring other effective methods.

5.3. Challenges and Copping Methods of Allogenetic NK Cell Therapy

Finally, allogeneic NK cell therapy still faces many challenges, but fortunately, scientists have already developed some solutions. For instance, the therapeutic effect of allogeneic NK cells is not obvious for all conditions. Although it is highly effective in hematological malignancies, in the environment of solid tumors, these cells are often damaged. So far, solutions that can relatively improve the therapeutic effect of solid tumors have been developed. Targeted drugs such as checkpoint inhibitors and cytokines can enable NK cells to play a better role in solid tumors. The combined treatment of specific drugs will make the therapeutic effect more significant.

6. Conclusion

Being an emerging immunotherapy for cancer treatment, NK cell therapy has demonstrated great potential and unque advantages. From the perspective of its anti-tumor mechanism, whether it directly kills tumor cells or regulates immunity through antibody-dependent pathways and cytokine secretion, it provides multi-level strategies for combating tumors. CAR NK cell immunotherapy has shown great success in clinical trials, especially in the treatment of solid tumors and hematological malignancies. It brings more hope to patients who are suffering from these cancers. Meanwhile, advantages such as broad cell sources, large-scale capabilities and the trait of ready-made product have provided convenient conditions for the promotion of clinical applications.

However, there are still challenges that NK cell therapy has to face. Issues such as the obstruction of NK cell infiltration by the microenvironment of solid tumors, the limitation of cell sources, and the deficiencies of immune checkpoint inhibitors all constrain its further development. Although allogeneic NK cell therapy has achieved certain success in the treatment of hematological malignancies, its limitations in the treatment of solid tumors also urgently need to be addressed. Therefore, future studies have to focus on overcoming these barriers like enhancing the adaptability of NK cells towards the solid tumor microenvironment, exploring more stable and efficient cell origin and innovating more effective immune response to improve the treating affect of NK cell therapies. With the continuous improvement of technology and the in-depth research, NK cell therapy is expected to make greater breakthroughs in the field of tumor treatment, providing safer and more effective treatment options for cancer patients and becoming an indispensable part of comprehensive tumor treatment.

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