

Current progress of immunotherapy based on NK cell in cancer therapy

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Abstract. Cancer generally refers to malignant tumor, including carcinoma, sarcoma and carcinosarcoma. This disease is caused by malignant cell hyperplasia. It is invasive and metastable. It is manifested by the continuous growth of a local mass of the body that destroys the normal tissue structure and can be transferred to other sites. It has been reported that there will be about 20 million new cases of cancer in 2020 and half of all cancer patients will die. Surgery, chemotherapy and radiotherapy, as the three standard treatment methods for cancer, plays an vital role in improving the survival rate and prolonging the survival time of cancer patients. In recent years, researchers have focused on the immune mechanism of tumors to explore the treatment of malignant tumors. Driven by the rapid development of modern molecular biology and gene engineering technology, immunotherapy has been paid more and more attention, showing good effect and application prospects, has become the world's recognized malignancy of the fourth treatment mode. Among these, natural killer cells (NK) fight cancer through their cytolytic function and the production of IFN- γ . One of the primary clinical objectives is to use their abilities for tumour immunotherapy. This article looks at advances in NK cell-based tumor therapy.

Keywords: Cancer, NK cell-based immunotherapy, NK cells

1. Introduction

Cancer affects everyone and imposes a huge burden on patients, families and society. Additionally, one of the main causes of death worldwide, particularly in poorer nations, is cancer. In 2020, there were about 20 million new cases of cancer worldwide, according to GLOBOCAN 2020. Surgery, chemotherapy and radiotherapy, as the three standard treatment methods for cancer, plays a vital role in improving the survival rate and prolonging the survival time of cancer patients. But there is still a long way to go to cure cancer, and it is urgent to develop new therapeutic methods. In 2013, the American journal -- Science published the advent of the era of tumor immunotherapy [1]. In recent years, immunotherapy, as the fourth treatment method for tumor, is developing rapidly and attracting great attention. Immunotherapy is different from traditional standard therapy, which kills cancer cells directly. Instead, immunotherapy drives out and destroys cancer cells by activating the body's immune system. Currently, immunotherapy includes immune checkpoint inhibitors and immune cell therapy represented by CTLA-4 and PD-1. Some people put the success of CTLA-4 and PD-1 in the field of cancer treatment on a par with the discovery of penicillin in the history of antibiotics, heralding a new era in immunotherapy of cancer. Therefore, we can boldly predict that there will be more and more anti-tumor drugs using immune principles in the future. Immune cell therapy involves a variety of different cell classes. Currently, Sipuleucel-T (dendritic cell vaccine) for prostate cancer and CAR T cells for pediatric B-cell leukemia and lymphoma have been approved. As research progresses, more cellular agents for tumor immunotherapy will be developed. Compared to t cells, NK cells can cope with cancer cells, not sensitive or restricted to homologous antigens. Compared to t cells, NK cells may face cancer cells that are not sensitive or limited to homologous antigens. For this new treatment, researchers are still working to develop the case in order to provide the best way to activate, use doses and transfer methods. In the process of participating in the immune response, NK is the first immune cell to attack, and would have to be another sword to defeat cancer. Cancer therapy.

2. Discovery and characterization of NK cells

In the 1970s, Rosenberg et al. first described cytotoxic cells targeting tumor cells [2]. Initially seen as an artifact, these cells were eventually identified as a new group of lymphocytes and named Natural killer (NK) cells. In 1990, Ljunggren and Karre et al. found that NK cells were able to kill cells with a deletion or low expression of major histocompatibility complex (MHC) class I molecules [3], and NK activator receptors combined with their own polysaccharide antigens to produce activation signals. Meanwhile, both NK cells and other normal cells expressed MHC class I molecules in their initial state. Binding with the inhibitory receptors on the surface of NK cells, the inhibitory signals are dominant, so the autologous cells will not be killed by the body NK cells. After that, NK cells kill target cells without cytotoxicity to normal cells. Therefore, it is believed that at least one receptor molecule expressed by NK cells is specifically related to its own MHC, which prevents NK cells from killing autologous cells, while autologous cells with deletion or low expression of MHC class I molecules induced by non-autologous cells and environmental pressure will be recognized and killed by NK cells [4]. Yokoyama proposed in 2006 that NK cells need to be "educated" by MHCI class molecules in the process of development and maturation, otherwise it cannot achieve the function of killing target cells [5]. Subsequently, J Lister and MariaR et al. showed that adoptive transfusion of autologous NK cells could exist long duration in the body, and IL-2 could make it have high activity and tumor killing ability, but it did not have good clinical therapeutic effect on solid tumors.

3. Classification and function of NK cells

CD56dimNK cells and CD56brightNK cells are the predominant NK cells in vivo. In the blood, CD56dimNK cells are the dominant subset. Compared to CD56brightNK cells, this subset showed more cytotoxic activity. In addition, CD56dimNK cells better express the FC-activated CD16 receptor, giving them the ability to be ADCC cytotoxic [6]. CD56brightNK cells express CCR7 chemokine receptors and L-selective proteins, which encourages migration of CD56brightNK cells to secondary lymphoid organs. On the other hand, CD56dim expresses a high density of CX3CR1 and CXCR1, which favors the migration of CD56brightNK cells to peripheral tissues. Interleukin (IL-2) and IL-15 encourages all NK cells to be activated and proliferated. However, CD56dim expresses a receiver of low dimmer affinity for IL-2, while CD56brightNK cells express elevated levels of trimmer IL-2R (CD25-CD122-CD132) [7-10].

4. The mechanism by which NK cells kill target cells

4.1. Perforin/granulozyme pathway

Granase and perforin are stored in the cytoplasmic granules of NK cells. N cells bind to the Fc segment of IgG bound to target cells with its FcRH1. Activated NK cells release guanase and perforin into the intercellular space together. The permeability of the cell membrane increases and finally causes the target cell to undergo osmotic lysis. Perforin - formed membrane channels facilitate granulozyme to enter target cells. In addition, perforin also caused the redistribution of guanase in the cytoplasm and nucleus of the target cells, so that granase concentrated in the lysate site, which was conducive to the lysate of the target cells. Finally, the target cells were apoptotic [11-14].

4.2. Fas/FasL pathway

The Fas molecule (APO-1 or CD95), is a member of the type I transmembrane glycoprotein family. Binding of FasL to Fas allows Fas to deliver "death signals" to cells within hours of apoptosis. The apoptosis signal of Fas is mainly mediated by the death domain protein (FADD) associated with its cytoplasmic region. After Fas is combined with FdsL, the receptor polymerization occurs, and the cytoplasmic death domain protein (DD) also polymerization occurs, so that FADD located in the cytoplasm can bind to the receptor cytoplasmic DD through its C-terminal DD. On the one hand,

FADD binds to Fas through DD at C-terminal, on the other hand, FAdd binds to DEM at N-terminal of Caspase-8 through death response domain (DEM), which induces activation of effecting caspase protease by caspase-8, degrade DEM and eventually leads to cell apoptosis [15].

4.3. TNF-a pathway

NK cells can secrete cytokine TNF-a, and TF modifies the stability of cell lysosomes by leaking a variety of hydrolytic enzymes. It impacts the phospholipidic metabolism of the cell membrane [16]. Target cell glucose metabolism is altered to lower tissue pH and target cell endonucleolytic activity is activated, resulting in genomic DNA degradation, programmed cell death, or other mechanisms. TF-induced cell death was significantly slower than that of cells in the performance lysate.

4.4. ADCC(antibody-dependent cell-mediated cytotoxicity) pathway

By using therapeutic mAbs that is directed against tumour-associated antigens, one can take advantage of ADCC, another important mechanism of NK cell-mediated killing of cancer cells. (Fig. 1) When NK cells are bound to a target cell via the CD16 receptor, phosphorylation of CD3 and FcR, adaptor proteins, activates p56lck tyrosine kinase, which then recruits Syk and ZAP 70 and phosphorylate phospholipase C2 tyrosine. The PI3K signal transduction process will eventually start as a result [17]. All other NK cell receptors, with the exception of CD10, activate their own signals while also co-activating another receptor [18]. In addition, unlike the receptors discussed above, the binding of ICAM-1 to the integrin LFA1 receptor on the NK cell is mediated by a distinct mechanism. The interplay of 2B4 signaling, which comes into contact with LFA1-ICAM conjugates as a co-pathway, increases this LFA1-dependent cytotoxicity [19].

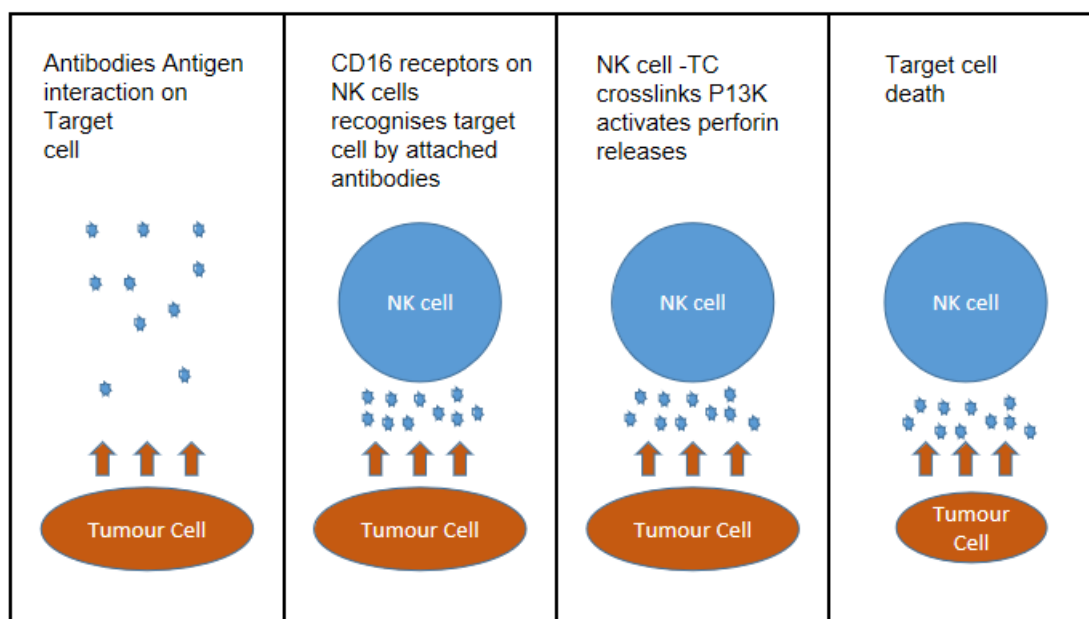


Figure 1. Mechanism of perforin attack by the NK cell - the ADCC pathway

5. NK cell therapy versus conventional cancer therapy

As the main treatment method of solid tumor, surgery is crucial to the healing process of tumor. However, the fact that the recurrence and metastasis of tumor patients after surgery seriously affect the long-term survival of patients cannot be denied, and has already aroused wide attention [20-22]. Animal studies have shown that surgery promotes cancer cell metastasis and reduces survival rate [23,24], which is partly related to the surgery itself. Recent studies have shown that immune cell dysfunction is closely related to postoperative recurrence and metastasis [25,26]. Demichi et al. retrospectively analyzed the survival time of 1173 breast cancer patients who received surgical treatment and 250 non-surgical breast cancer patients, and found that surgical patients had two death

peaks, respectively appearing in the third and eighth years, while non-surgical patients only had a death peak in the fourth year [27,28]. Therefore, some patients may even accelerate tumor recurrence and metastasis due to surgery, leading to shortened survival time of patients. NK cells, as congenital immune cells, may have serious dysfunction during trauma, surgery and critical condition [27]. The degree and duration of NK cell dysfunction are related to injury intensity. NK cell dysfunction may be transient, but its biological effects may persist for a long time, including effects on infection and damage repair. The relationship between surgical resection of tumors and metastasis has been observed as early as 1913, but the role of cellular immune dysfunction has not been discovered until recent studies. In particular, perioperative NK cell dysfunction is closely associated with postoperative tumor recurrence and metastasis. Studies have shown that NK cell function has been reduced on day 1 after surgery and is correlated with the degree of tumor metastasis. The recurrence rate of tumor patients is also closely related to the degree of postoperative NK cell dysfunction. Therefore, perioperative use of cytokines that enhance immune function, such as IL-2, GM-CSF, TNF- α and IFN- α , can reduce postoperative tumor recurrence and metastasis. Since these cytokines can enhance the activity of NK cells, the role of these in preventing tumor recurrence and metastasis after surgery is further verified. The mechanism of NK cell dysfunction after surgery is complex, involving the hypercoagulable state of blood, the release of anti-inflammatory response factors and the increase of immune inhibitory cells after surgery. The focus and difficulty of tumor treatment lie in recurrence and metastasis. Although epithelial-mesenchymal transition (EMT) can escape T cell immunity, its expression of NK cell active molecules can sensitize the surveillance mechanism of NK cell immunity. Therefore, enhanced NK cell activity, including NK cell therapy, will help to stop and prevent tumor metastasis. Due to the complexity of tumor etiology/pathological mechanism, any single tumor therapy has its limitations. Therefore, the need to develop strengths and circumvent weaknesses, comprehensive treatment.

6. NK cell therapy commonly found in cancer immunotherapy

6.1. AutoNK cell adoptive therapy

Adoptive back transfusion of autologous NK cells can improve the antitumor effect since it can be impaired in tumor patients. Clinical studies and experiments have shown that autologous NK cells, after being cultured and amplified in vitro, can be transfused into tumor patients without obvious side effects and have certain therapeutic effects. NK cells are easy to activities to kill tumor cells. Therefore, autologous NK cell transfusion can kill MHC Class I negative malignant cells. It has been shown that primary/primary tumors also lack MHC I molecule, so NK cells also have a scavenging effect on newly formed tumor cells. T According to clinical studies, autologous NK cells dramatically enhance immune function and quality of life in patients with advanced liver cancer while causing essentially no side effects [29]. The function of NK cells in tumor patients will be compromised as a result of the modifications to the tumor cells themselves, and the effectiveness of autologous NK cell transfusion will be somewhat constrained.

6.2. Allogeneic NK cell adoptive therapy

Allogeneic NK cells exhibit better antitumor effects because of the mismatch of suppressor receptor - ligand KUR-MHC Class I molecules, and NK cells are not inhibited by normal cells in the recipient. Allogeneic NK cell therapy is mainly used in hematologic malignancies, and has achieved remarkable efficacy, even tumor regression. When HLA semi-compatible NK cells treat blood tumors, allogeneic NK cells can attack leukemia cells without graft versus host disease, thus alleviating leukemia without serious side effects [30].

7. Chimeric antigen receptorNK (CAR-NK) cells

The immunosurveillance function of NK cells to tumor mainly depends on the active and inhibitory receptors on the surface of the cell membrane. Under normal circumstances, NK cells are in a suppressed state. Once the activator receptor is activated, NK cells change from an inhibited state to an activated state and kill tumor cells through direct lysis and release of perforin. Defective NK cells or receptors will increase the probability of tumor development. According to the function of receptors, the NK cell receptors can be divided into inhibitory receptors and activated receptors. The inhibitory receptors mainly include CD161, CLG1, PD-1, TIM3, LAG3, CD96, TIGIT, NKG2A, etc. The active receptor mainly includes NKG2D, NKp30, NKp44, NKp46, CD16, etc. The NK cell receptor (activator receptor) can directly recognize the corresponding ligand on the surface of virus-infected cells or mutated malignant cells to eliminate and kill these abnormal cells. Based on this principle, during the preparation of CAR T cells, the gene encoding the scFv fragment in the extracellular segment of CAR can be transformed into NKG2D or NKp30 and other NK-activated receptor genes, and then transfected into T cells to prepare CAR-T cells based on NK cell receptors. Cell immunotherapy for tumors. Different from antigen-specific classical CAR-T cells, these NK cell receptor-based CAR-T cells can recognize viral infection or mutated malignant cells expressing their ligands, and have the characteristics of multi-target and broad spectrum. They can not only be used in the treatment of a variety of hematologic and solid malignancies, but also have the characteristics of drug resistance resistance. It has a very good application prospect [31-33].

8. Conclusion

In conclusion, with the development of tumor immunity research, more and more therapeutic drugs and methods utilizing immune mechanisms will enter the clinic in the future. Benefit cancer patients. As immune cells that can directly kill cancer cells, how NK cells play a role in the comprehensive treatment of cancer, especially in the small lesions of tumor, as well as tumor recurrence/metastasis, needs further clinical research.

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