Adoptive NK Cell Transfer Therapy: History, Mechanism, and application

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Abstract: Natural Killer Cells (NK cells) are innate immune cells which kill target cells without prior sensitization. This type of immune cell exists in multiple organisms, including human. NK Cells has been closely related to their ability to kill tumor cells ever since the discovery of them. At present, the research on NK cells mainly focuses on its ability to directly kill target cells, a minority of related research studied on its regulatory role in the immune system. Scientists have been able to artificially mass produce NK cells in vitro, and have mastered some factors affecting the immune characteristics of NK cells. However, since there are relatively few studies on NK cell immunotherapy against cancer compared to other therapies. The research on NK Cells have not been deep enough, there are some problems about the theoretical background of NK cells, the mechanism by which NK cells recognize target cells and the specific mechanism by which NK cells cooperate with other immune cells remains unclear or unknown. Secondly, in clinical and laboratory research, the vitro amplification technology and safety had also become one of the bottlenecks of research. This review mainly focuses on summarizing the existing knowledge about NK cells, including the mechanism of killing tumor cells, the ability to regulate or cooperate with other immune cells as well as some examples of clinical application. In the final part, this review summarizes some possible development directions and research suggestions in the future.

1. Introduction

With advances in medicine, immunology and biochemistry, and scientists' progressive understanding of cancer and the human immune system, cancer treatment and anti-cancer technology had a rapid development in the past few decades, in the meantime, the incidence rate of cancer among young people also raised steadily [1]. Research on T cells in cancer treatment has made progress and opened the gate of adoptive cell therapy for scientist and allowed human beings to see lots of possibilities. Immunotherapy like CAR-T Cell therapy grows rapidly in the past few years, recent research results on this topic has been published and brought up front constantly, this technology seems to be more and more steps closer to its maturity. Meanwhile, the development of adoptive cell transfer therapy has also inspired scientists for more kinds of feasible treatments, these include the Natural Killer (NK) Cell therapy.

Like T cells, NK cells are also natural human immune cells. However, unlike T cells, the mechanism of NK cells is more complicated, and the cooperation and regulation between NK cells and other immune cells are more diverse. It is generally believed that NK cells are derived from hematopoietic stem cells (HSC) in the bone marrow. They are different from B cells and T cells. The immune response of NK cells does not require prior sensitization/exposure. Some of the specificity of NK cells also presents its outstanding ability to kill tumors, which brings a good potential clinical practicality.

At present, one of the widely recognized techniques of adoptive cell transfer therapy that has achieved research and clinical results is the CAR-T cell therapy, and NK cells are inextricably related to T cells throughout their life cycle, especially in the early stages. In contrast, according to the current research status, we know relatively little about NK cells. Therefore, the main purpose of this review paper is to summarize current immunotherapy and revisit other related research results about NK cell

immunotherapy. This would be a good way to organize the knowledge in a broad view and can also help us to be better prepared for what's coming next. Since both the experimental and theoretical information with regard to NK cell immunotherapy is relatively limited, this article will also put forward some current limitations and challenges as well as some topics that were needed to be studied but have never been touched, as general suggestions for future research reference.

At this stage, NK cell immunotherapy has also encountered some problems. Studies have established an iPSC-induced NK cell system. In the experimental system, although a seed bank can be established to achieve mass production and a ready-to-use NK cell system, the clinical safety remains to be verified. In addition, NK cell expansion system in vitro is still the main technical bottleneck for the clinical application of NK cells [2]. In other words, the current human medical technology is not enough to ensure the safety of NK cell immunotherapy, that is, large-scale clinical use is still not advisable.

This review composed the following: a brief introduction to NK, including its brief history, an introduction of the mechanism of NK cells, which would be divided into two parts. One is about the mechanism of NK cells recognizing and killing tumor cells, and the other is the mechanism of NK cells cooperating with other cells, which is very important since human immune cells never work independently. In this process, comparison between NK cells and T cells to a certain extent brought a more intuitive display. Later, some laboratory research examples, or clinical applications of NK cell immunotherapy has been provided, which will also cover some data in NK cell culture experiments in vitro. The final part contains discussion about the possible experimental directions in the future, some potential problems found in the in the researching stage, as well as some thoughts on the use direction.

2. The history of NK cell therapy

It is worth noting that the process of human discovery of NK cells has been accompanied by the study of its anti-tumor ability from the beginning. In 1973, Greenberg and his team discovered a new kind of immune cell that can kill target cells without prior immunization, which made immediate distinguishment of it from B cells and T cells. Two year later, Rolf Keissling and his team reported some natural cytotoxicity findings with evidence of gene regulation for this new immune cell and its abilities to kill multiple cancer cells, Keissling gave the name "Natural Killer Cell" to this new cell. One year later, Greenberg and his proposed that NK cells' anti-tumor function is T-cell independent [3].

Like T cells, NK cells are also human innate immune cells. But unlike T cells, NK cells release perforin or granzyme to kill cells/cancer cells directly [4]. Moreover, NK cells are also part of the T cell regulation system. Its role runs through the whole process of T cells: NK cells release cytokines to promote the proliferation and initial differentiation of T cells as well as to trigger further T cells immune response [5], like killing cancer cells in our case.

At this point, one may be aware of the advantage of the NK cell immunotherapy, not only can it kill cancer cells directly like other types of adoptive cells, but it can also play a role in the regulation of other immune cells, enhancing the cooperation between different kinds of immune cells will enhance the human immune system from a different perspective, to play a more powerful effect of cancer treatment.

3. The mechanism of NK cell transfer therapy

NK cells are the first immune cells found to have anti-cancer ability. They can kill cancer cells without being activated [5]. Understanding how NK cells immunotherapy works and the principles behind it can make us better contribute to its future developments. Based on the current research, compared with our knowledge of immune transmission mechanism for T cells, we certainly know less about the NK cells. We do know that T cells uses their T cell antigen receptors (TCRs) to distinguish between body cells and cancer cells, NK cells recognize cancer cells differently, unlike T cells, there's no such a major specific receptor for NK cells, their patterns of recognition are rather innate [7]. At

present, we clearly understand that like T cells, NK cells also have surface receptors, which are inhibitory receptors and activating receptors respectively.

According to the known research results, we can conclude that NK cells generally use inhibitory receptors to recognize cells. In normal cells, the inhibitory receptor of NK cells recognizes a substance called major histocompatibility complex I (MHC I), which is used by cells to "indicate identity". If the receptor of NK cells does not recognize MHC I, the killing mechanism of NK cells will be activated. In addition, the activating receptors on NK cells will directly activate the immune response of NK cells after recognizing certain activators, that is, after recognition of foreign cell, NK cell will force those cells to go into apoptosis. Here, people may be able to understand a general approach of NK cell immunotherapy: find the substances that can activate NK cell activation receptors on cancer cells and carry out such transformation on NK cells in vitro (perhaps without modification at all). However, due to such a mechanism of NK cells (active use of inhibitory receptors), tumor cells with MHC I can easily escape immune system monitoring. The current research is inconsistent on whether the stimulation intensity received by the receptor can determine the final behavior of NK cells. In other words, when NK cells receive signals to stimulate and inhibit both receptors at the same time, there is no clear evidence that the stronger one will dominate the immune performance of NK cells [8]. Therefore, one of the foci of immunotherapy is to allow cancer cells that have escaped immune screening to be re-recognized by newly implanted immune cells.

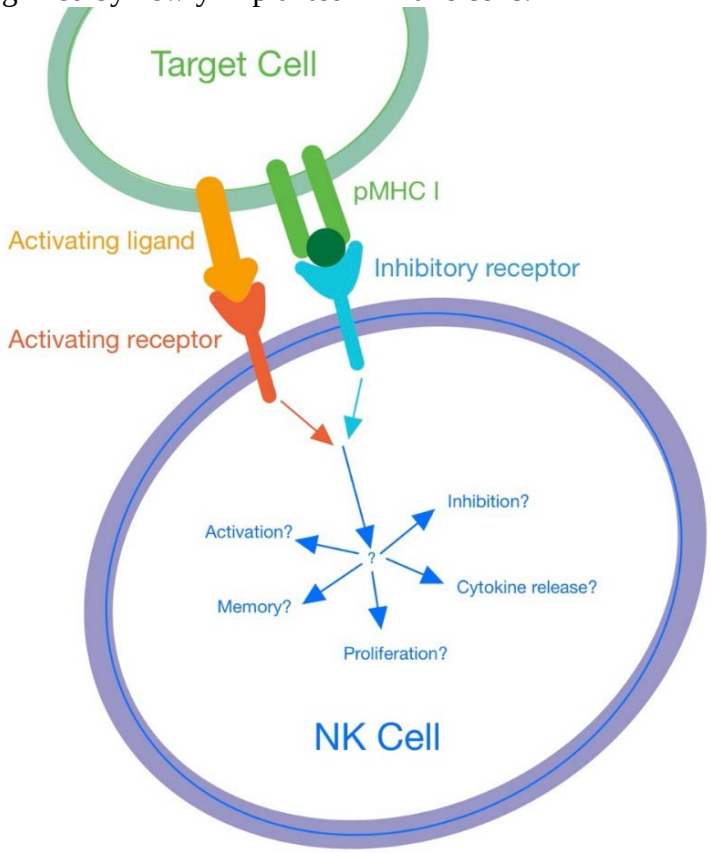

Figure 1. The mechanism of NK cell therapy.

Scientists believe that there is a certain balance between the inhibitory and activating behavior of NK cells, such operator or receptor mechanism has yet to be determined [9].

There are two main points about NK cells in the direct fight against tumor cells. The first is to directly kill tumor cells, and the second is to inhibit the growth of tumor cells. Like T cells, NK cells are also cytotoxic. These cells secrete perforin to kill target cell through apoptosis [10]. NK cells make cancer cells enter apoptosis by letting perforin form channels on the cell membrane of cancer cells and destroy the selectivity of cell plasma membrane [11]. Such a channel that allows substances in the environment to enter the cell by osmosis will directly make the cell no longer independent of the environment and die rapidly. Activated NK cells pass through IFN- γ to help inhibit the growth of

tumor cells. This molecule has been proved to directly inhibit the proliferation of tumor cells in vitro. In vivo, it indirectly inhibits and interferes with tumor growth by inhibiting other factors, such as inhibiting the formation of blood vessels in tumor tissue - by inhibiting human interferon-inducible protein 10 (IP10) [12].

The cooperation between innate immune cells plays an important role in the body's immune function, which ensures the information transmission in the process of immune response. Such cooperation is particularly important when the immune cells are working against cancer. Although the current research on NK cell immunotherapy focuses on how to make the implanted NK cells directly kill cancer cells, it is also important to enhance the cooperation between NK cells and other immune cells.

As described, NK cells have good anti-tumor ability as an immune cell itself, but another important point in this paper is that the cooperation between various cells of the immune system is also very important in fighting cancer, especially now that we have CAR T cell immunotherapy technology. In one study, scientists have shown that activated NK cells can enter lymph nodes and place themselves near the location of were all the unmatured T cells grow. This made possible for the NK cells to impact the size, strength and therefore the quality of future immune response of matured T cells, all in the early stages [13]. This makes NK cell immunotherapy have the potential to work together with CAR-T cell immunotherapy in cancer treatment.

Although our current research on NK cells is very limited compared with the research on T cells, one thing that cannot be ignored from the previous research is that NK cells have the ability to activate T cells. This also makes it possible for multi-party cooperation. First, we need to realize that the success of T-cell immunotherapy is very limited by now. Secondly, although NK cells have been discovered for a long time, and people also understand that NK cells are a kind of immune cells that can directly kill cancer cells, there are few studies on NK cell immunotherapy. Here, my view is that multi-immune cell cooperation against cancer seems to be a more effective means at present and may exceed single immunotherapy. Besides, NK cell's impact on T cells is not limited to the growth and differentiation of cytotoxic T cell, but also the activation and quick response of memory T cells, which is critical when fighting against recurrent cancer or other diseases. The specific mechanism of this impact is a classic example of multi-faceted cooperation of immune cells include dendritic cells (DC), NK cells and T cells. In the recall response of the immune system, researchers used Ovalbumin (OVA) to simulate the antigen, in that infection model, memory CD8 T cells are activated by the secretion of DC cells. Using the secretion of DC cells, the reactivation of memory CD8 T cells are improved. In the process of T cell recall, NK cells are activated rapidly and become the early source of IFNγ, which is a substance that will further improve the function of DC cell secretion, so that CD8 T cell will have an even stronger and faster recall response. With that being tested, research still showed the effect of NK cells reduces the formation of memory T cells during differentiation [14].

In addition, since NK cells can interact with macrophages, T cells and dendritic cells [15-16], it will play an important role in cancer immune regulation, especially when dealing with tumor cells that escape immune screening. If future research can clarify the specific mechanism, it will bring a great breakthrough to NK cell immunotherapy.

4. The application of and clinical effects of NK cell therapy

At present, the only effective method for the treatment of acute myeloid leukemia (AML) is allogeneic hematopoietic cell transplantation, but it cannot prevent the recurrence of AML.

In one experiment, Ruggeri and his team demonstrated that allogeneic NK cell transplantation can eliminate the recurrence of leukemia and even eliminate the rejection reaction [17]. This research result proves the role of NK cells in immunotherapy. In view of its effectiveness in preventing the recurrence of AML and eliminating the immune rejection after transplantation, NK cell therapy has been widely used in the field of hematopoietic stem cell transplantation [18].

Studies have shown that the existence of NK cells in tumor tissues is an important factor in delaying the development of cancer [19]. This may reflect another use of NK cell immunotherapy. That is to

delay the progress of cancer by increasing the content of NK cells in tissues. At present, this seems to be a good option. For many cancers, time can make patients more likely to be exposed to treatment.

Although the specific mechanism is still inconclusive, studies have revealed that CD8 + T cells, an important anti-cancer T cell in the immune system and NK cells can cooperate to help each other kill cancer cells when they met in cancer tissue [20].

Some review articles mentioned that NK cell therapy can be used as an alternative to CAR-T cell therapy [7]. At present, when the specific immune response between NK cells and T cells is not clear, it is indeed unwise to rashly combine the two therapies. However, combined with the previous research on NK cells, I think NK cell immunotherapy can buy time for the early preparation of car T cell therapy in the future. In addition, combined with the previously discussed applications, allogeneic NK cell immunotherapy can exceed the limit of Graft versus host disease (GVHD).

In the in vitro culture stage, using different factors to affect NK cells can make the cultured NK cells more specific to a certain cancer or refine the function of NK cells. NK cells in cancer patients are often in a state of imbalance [21], which is one of the conditions that can be changed by NK cell culture in vitro. In one of the previous clinical trials, NK cells with different characteristics were cultured in different NK cell culture environments. In this experiment, researchers found that cultured NK cells can have different functions by changing the purity of NK cells and the initial culture materials. For example, in clinical data, through Protocol feature IL-2, using PBMC with CD3 depleted and CD56 enriched, researchers Improved cytotoxic activity against leukemia and tumors [22]. When change to IL-15 and IL-21, with CD3 depleted only, the improvement had change to the cytotoxic against K562 and patient bone marrow blasts [23].

In Angiollo's experiment, they used a mouse model to prove that IP10 is an important factor in promoting angiogenesis [11]. NK cells were proved to affect the expression of IP10 and further inhibit the formation of blood vessels in tumor cells. The principle is unknown, but it is certain that NK cell immunotherapy is widely used in the field of allogeneic hematopoietic stem cell transplantation and some organ transplantation for the effect of Allogeneic NK cells to avoid GVHR and ability to prevent recurrent leukemia [17].

5. The Limitation and future development of NK cell therapy

In One of the suggestions for further research could be the mechanism of NK cells regulation and activation. In my opinion, among the many immune cells in the immune system, NK cells seem to play a role as an intermediate axis, which can directly or indirectly control the activation or performance of other immune cells. This makes me think that understanding the mechanism will enable the future mature NK cell immunotherapy to have more clinical applications, such as directly or indirectly controlling other immune cells through NK cells cultured in vitro to jointly fight cancer. At this stage, NK cells have unlimited potential. On the one hand, as one of the immune cells, NK cells can directly kill tumor cells. On the other hand, as one of the regulatory factors in the immune system, it can mobilize various immune cells of the body to cooperate better.

In view of the unclear influence of other factors on the immune performance of NK cells except some interleukin and IFN, future research can also pay more attention to this aspect to expand more possibilities for the stage of cell culture in NK cell immunotherapy.

Be aware of that the influence of NK cells on DC cells are bidirectional, and at the same time, these two kinds of cells can both regulate T cells and affect various manifestations of T cells. If we want to pursue the superposition effect of NK cell immunotherapy and T cell immunotherapy, this must be taken into considering. At present, no research can clearly give specific relevant data. Therefore, such superposition therapy is not encouraged, but if we can specifically clarify the relationship between the two systems in the future, superposition of NK cell and T cell therapy will be feasible.

Although NK cells and DC cells cooperate to boost the recall response of memory T cells, there are still data indicating that the formation of new memory T cells will be reduced in the presence of NK cells. This is a very disadvantageous factor for cultivating the immune system against recurrent cancer. If NK cell immunotherapy were desired to put on potentially recurrent cancer, the actual efficacy may

become slightly unsatisfactory for this reason. However, at present, there is no clinical experimental comparison about NK cell immunotherapy against recurrent cancer, so it is difficult to draw a conclusion here, if possible, future research on this field will provide a good reference for this field.

6. Conclusion

In general, NK cells can recognize infected cells through receptors and release cytotoxins to kill them without prior sensitization. They can also participate in a relatively macro immune regulation as a member of the immune system. At present, researchers have a long way to go for the specific immune mechanism behind NK cell. According to the information we have so far, NK cells can cooperate with or even regulate many immune cells, including DC cells, T cells and macrophages. Such characteristics will make NK cell immunotherapy play a greater role. For example, when used together with CAR-T Cell therapy, it can not only leave preparation time for the early vitro culture and modification of T cells, but also have the potential to further stimulate the activity of transferred T cells. However, the risks still cannot be ignored. At present, no experiment has given the exact results of combined therapy. This review summarized the theoretical background, mechanism, development direction, safety, and existing clinical data of NK Cell immunotherapy in a relatively broad perspective. In the future , different alternative paths of NK cell immunotherapy will appear. Specific determination and detail will lean on future researchers and further clinical results.

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