Prospect Of CAR-NK Cell In Tumor Immunotherapy

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Abstract. The innate immune system has gained significant interest expected to the impressive natural killer (NK) cells ability to surveil and combat pathogens, regardless of major histocompatibility complex. Following the resounding success of CAR-T cell therapy, there has been a burgeoning interest in harnessing the NK therapeutics potential in recent years. Owing to its clinical triumphs, which those achieved by CAR-T cell therapy, this subject has garnered a discernible level of fascination. A captivating realm of investigation within tumor immunotherapy lies in delving into chimeric antigen receptor (CAR) engineering applied to. In the wake of the astounding achievements witnessed with CAR-T cell therapy for CD19 hematologic disorders, CAR-NK cells are emerging as an auspicious adjunctive strategy to cellular therapy following CAR-T cells. It is intriguing that CAR-NK cells exhibit comparable efficacy against malignancies as demonstrated in CAR-T cell therapy, and preliminary clinical investigations have yielded promising outcomes. review aims to furnish a meticulous synopsis of the advancements in CAR-NK cell treatment, encompassing methodologies for generating and genetically manipulating CAR-NK cells. Additionally, it examines the obstacles and future possibilities associated with utilizing CAR-NK as an advanced form of immunotherapy against cancer. The primary aim of this prospective study is clarify reasons behind implementing CAR-NK as a novel approach to anticancer immunotherapy.

Keywords: Natural killer (NK) cells, CAR-T cell therapy, CAR-NK cell therapy.

1. Introduction

The science of immunotherapy has recently seen an increase in interest in using NK cells, a particular subgroup of ILCs with a variety of mechanisms for removing targets. The application of NK cell therapy attempts diverse advantages over alternative forms of immunotherapy and has demonstrated significant promise in cancer treatment due to its ability to independently target major histocompatibility complex (MHC) antigens, enhanced safety profile, and convenient off-the-shelf production feasibility [1]. Within the tumor microenvironment (TME), NK cells encounter functional inhibition due to various immunosuppressive factors, particularly TGFβ [2]. Additionally, unlike CAR T cells derived primarily from autologous sources, NK cells can be procured from allogeneic donors without eliciting GVHD, thereby facilitating the augmentation of well-characterized cellular products suitable for clinical applications. Both peripheral blood and cord blood serve as sources for harvested NK cells; however, standardizing doses with PB-NK cells poses challenges due to donor variability. Conversely, CB-NK cells exhibit a higher prevalence of immature phenotypes and reduced diversity [3]. Moreover, iPSC-derived NK cells hold promise as a viable option for cell therapy; however, they lack the necessary activation markers. Additionally, there are challenges associated with expanding and activating CAR-NK. One potential solution involves utilizing human iPSCs to systematically generate NK cells and genetically modify them for CAR production [4]. IPSC-derived NK cells, however, face limitations in terms of activation markers. Challenges arise when applying CAR-NK cell therapies due to issues with expanding the cells, reduced antigen-binding capacity caused by CAR structure, and cytokine requirements. Additionally, obtaining NK cell sources and exogenous cytokines can be problematic. The present paper also delves into the impediment posed by immune cells within the tumor microenvironment on the anticancer efficacy of NK cells. This attitudinizes challenge for therapeutic NK cell therapy. The research on CAR-modified NK cell therapies has witnessed an increase in recent years. Similar to CAR-T cell therapies, CAR-modified NK cell therapies involve genetically modifying immune cells to express a synthetic receptor [5]. The redirection of these immune cells towards tumor surface antigens leads to their

elimination through the cytotoxic activity of these immune cells. Utilizing allogeneic NK cells for therapy offers several advantages using T cells, thus driving advancements in ex vivo amplification techniques for NK cells and the development of various CARs and NKG2D chimeric receptors [6,7]. The aspiration of this review is to appraise and synthesize the diverse variations NK cells, alongside the methodologies employed for preparing CAR-NK cells. Additionally, it compares CAR-NK cells with other cellular therapies in terms of CAR modification, while addressing the current limitations and future prospects of CAR-NK cells. The aim is to provide valuable acumen both clinical and fundamental research in cancer treatment.

2. CAR NK Cell Therapy

2.1. NK cell biology

As the initial category of ILCs, NK cells primarily engage in apoptosis induction and secretion of pro-inflammatory cytokines [8]. Unlike B cells together with T cells, NK cells lack antigen receptors that endure somatic disturbance. Instead, they rely on a random assortment of activating and inhibitory receptors to discern between healthy and abnormal cells. The reliance on these receptors is predominantly depends on the delicate balance between the messages they mediate, which are the stimulatory and inhibitory signals [9]. CD16, also known as FcγRIII, serves as a primary activating receptor for NK cells through its interaction with the Fc region of IgG. This interaction triggers signaling via the FcεRIγ chain and the CD3ζ chain, thereby stimulating NK cell activity [10]. The natural killer cell receptors - NKp46 - possess autonomous recognition capabilities for tumor cells externally relying on MHC molecules. These receptors initiate targeting of cancerous cells by specifically identifying heparan sulfate present on the exterior of tumor cells.

The functions of NK cells encompass a multitude of mechanisms that impede the growth and spread of malignancies. Initially, Cancer cells that inhibit MHC-I molecules as far as avoid being recognized by cytotoxic CD8+ T lymphocytes can be found and dealt with by NK cells. Furthermore, therapeutic monoclonal antibodies (mAbs) that precisely target antigens linked to malignancies might cause NK cells to increase the cytotoxicity of cancer cells by virtue of ADCC. This not only enhances the response of cytotoxic CD8+ T cells, but further increases the capability of macrophages to initiate an immune response. NK cells may encounter certain limitations that could potentially affect their Anti-tumor nature and efficiency. These constraints include a relatively brief lifespan of only 1 to 2 weeks without cytokine support. These cells are limited numbers repeatedly necessitating ex vivo multiplication and stimulation, as well as vulnerability to the suppressive tumor microenvironment which can hinder their movement and function. Progress in engineering has allowed scientists to overcome some of these restrictions. As a result, they effectively inhibit cancer cell proliferation, angiogenesis and apoptosis by exerting potent effects. [8,9]. Additionally, NK cells secrete CCL5, XCL1, and XCL2 to recruit cDCs effectively in the tumor microenvironment, further aggrandize the anti-tumor effects.

2.2. NK cell source

An assuring strategy for NK cell treatment is the use of autochthon transplantation of autologous natural killer (NK) cells. This method offers various benefits such as convenient accessibility within one's own body, minimal risk of graft-versus-host reaction occurrence, with elimination of commitment considering immunosuppressive treatment. Nevertheless, employing autologous NK cells does present some limitations when compared to allogeneic counterparts. For example, augmenting the quantity of circulating autologous NK cells might not yield the intended therapeutic response due to potential inhibition caused by their own HLA molecules [11]. Furthermore, challenges may arise concerning restricted expansion efficiency and diminished cytotoxicity levels observed among a considerable portion of previously treated patients.

In the context of allogeneic NK cell infusion treatment, differences between inhibitory KIRs and HLA ligands in recipients lead to alloreactivity of donor NK cells. Non-myeloablative chemotherapy

prior to adoptive transfer is necessary to inhibit repudiation by the recipient's immune system. Studies have demonstrated that AML patients who receive high-dose cyclophosphamide and fludarabine treatment followed by haploidentical NK cell infusion achieve complete remission. Notably, lymphodepletion conditioning significantly enhances endogenous IL-15 levels crucial for conferrer NK cell expansion along with long-term presence.

Enhanced strategies intention at optimizing the competency of NK cell therapies encompass a diverse array of approaches to intensify both the ex vivo development and functionality of NK harnessing various source. Subsequently, cytokines like IL-2, IL-15, and IL-18 are employed to activate these cells and amplify their cytotoxicity towards target cells as well as IFN-production [11]. Once an adequate quantity and quality of haploidentical NK cells have been generated through these methods, they can be infused into patients. Ex vivo modification precisely recognize tumor-associated antigens (TAA) represents an alternative approach to enhance the efficacy of targeted therapy. In vivo strategies encompass the utilization of cytokines serving as IL-2 or IL-15 to stimulate the expansion and activation of NK cells. Additionally, immune-checkpoint inhibitors (ICIs) can be employed to alleviate inhibitory conditions induced by conventional inhibitory receptors on cancerous MHC-I molecules that interact with KIRs, NKG2A, and LIRs present on the surface of NK cells, thereby inducing a state referred to as 'dominant inhibition' [9]. Antibodies targeting these receptors can enhance the anti-cancer abilities regarding natural killer (NK) cells aside competing with DNAM-1 binding ligands CD155/CD112 on cancer cells and inhibiting the activity of TIGIT, a receptor found on NK cells. Therefore, inhibiting TIGIT efficiently can prevent the fatigue of NK cells that infiltrate tumors and increase their powerful anti-cancer activity. The intercommunication and synergy between CD47 expressed on tumor cells and SIRP expressed on NK cells impedes phagocytosis by the NK cell, surpassing conventional PD-1/PD-L1 signaling cascade [2]. The combination of PD-L1 antibody and CD47 antibody possesses the potential intensify functionality of NK cells, thereby eliciting a more robust immune response against cancer.

2.2.1 The exquisite NK cells in peripheral blood and umbilical cord blood.

The distribution of NK cells in peripheral blood lymphocytes is typically around 10-15%, although it can vary significantly between different healthy individuals ranging from no detectable levels up to 60%. These NK cells may be categorized into binary fundamental subdivision based on their phenotype: CD56brightCD16dim or CD56dimCD16bright, with the latter being more prevalent in peripheral blood. The advantage of peripheral blood-derived NK cells lies in their ready availability and manifestation of a fully mature phenotypic profile. While genetic factors primarily control the regulation of inhibitory receptors to maintain self-tolerance, NK cell-activating receptors display remarkable adaptability in response to various environmental stimuli triggered by pathogens and malignancies. However, due to individual differences among donors, it becomes challenging to standardize dosage since there is no consistent and renewable source for these peripheral blood-derived NK cells. Furthermore, phenotypic diversity base on these NK cell subsets appears to be specific to each donor and highly variable across individuals.

Umbilical cord blood (CB) is a precious inception for acquiring NK cells, which possess a higher absolute number and fraction in CB compared to peripheral blood (PB). In fact, they constitute approximately 20-30% of the lymphocytic pool in CB [12]. Furthermore, CB-derived NK cells exhibit reduced diversity and display characteristics of immaturity when juxtaposed with PB-derived NK cells. Additionally, T-bet, eomesodermin, perforin, and granzymes are maturation indicators with reduced expression levels in CB-NK cells, according to transcriptome research. NK cells derived from CB units represent a valuable asset for immunotherapy, because to their accessibility through international CB banks, ability to manufacture CARs through genetic modification and growth using feeder cells and cytokines. However, akin to PB-NK cells, there exist significant variations in their phenotype and yields across different donors, posing a challenge due to the absence of a singular renewable source.

2.2.2 Induced pluripotent stem cells

Some sequential approaches can effectively harness human iPSCs for the engenderment of NK cells. Following exposure to a particular mix of cytokines that promote iPSC development into NK cells, stromal cells generated from bone marrow are initially co-cultured with iPSCs. Additionally, these NK cells from iPSCs show the presence of both activating and inhibitory receptors, including CD16, KIRs, and natural cytotoxic receptors, which are characteristic features observed in fully matured NK cells. Additionally, these iPSC-derived NK cells demonstrate potent anti-tumor activity both in vitro and in vivo. To expand the population of differentiated iPSC-derived NK cells, Co-culture with feeder cells, including aAPCs that have undergone genetic modification, is an option [9]. The expression of CARs has been facilitated in genetically modified iPSC-derived NK cells [8]. The initial experiments focused on infecting HIV by introducing CARs into these cells. In these studies, to increase the exertion and capability of iPSC-derived NK cells to eradicate malignancies, tumor-specific chimeric antigen receptors have been added [11]. In conclusion, NK cells derived from iPSCs remain a homogeneous and exceedingly promising source of cellular therapy. These cells are capable cultivate in vast quantities, possess the capacity for genetic manipulation, and exhibit a prepared phenotypic contour among potent antitumor cytotoxicity. However, it is crucial to acknowledge that there are certain limitations associated with them in terms of lacking essential activation markers, which may hinder their ability to effectively eliminate tumor cells. Therefore, further investigation is imperative to demonstrate their efficacy in both animal models and clinical settings. The utilization of NK cells derived from iPSCs offers additional advantages such as easy genetic engineering, selection of clones after modification, and eliminates the need for donor cell collection. However, scaling up and manufacturing NK cells from iPSCs can present challenges; nevertheless, it has consistently been successfully accomplished.

2.3. Creation of CAR-NK cells

The process of CAR-NK cell preparation involves two primary steps: cultivation and genetic modification of NK cells. Currently, there are binary distinct technique for cultivating NK cells: one utilizes feeder cells for stimulation, while the other employs a non-feeder cell culture approach. Genetic modification was first used to give T cells the ability to admit specific targets moreover to mount effective attacks. Traditionally, CARs are categorized into three generations based on their intracellular components and quantities. First-generation CARs solely consist of a signaling domain (typically CD3ζ), which may not sufficiently induce strong killing responses without additional costimulatory domains. In order to improve effector cell activation, one or two additional costimulatory domains, what was mentioned before. CD28, CD137 have been added to second- and third-generation CARs [5].

2.4. CAR-NK cells Current challenges and alternative strategies

2.4.1 Expanding NK cells in vitro for CAR-NK cell immunotherapy

In spite of myriad benefits associated with NK cells, utilization of immunotherapeutic accession poses numerous complexities and presents several formidable challenges in harnessing the potential of CAR-NK cells. The constitutional impediment to CAR-NK cell therapy lies in ex vivo multiplication of NK cells. Given the ins an adequate number of NK cells from a particular donor, it becomes imperative to amplify and activate these cells for their utilization in treatment [10]. Furthermore, the current configuration of CAR utilized in NK cells results in diminished antigen binding and activation due to its positioning and distance from the exterior of CAR-NK cells, dominant to initial magnetic resistance. Additionally, throughout the production process, NK cell development and the synthesis of cytokines normally require two to three weeks. The presence of exogenous cytokines is pivotal for the viability of NK cells, necessitating the provision of external cytokines to bolster their growth and persistence within the organism. Caution must be exercised when employing autologous NK cells that have undergone freezing and thawing procedures, as this can significantly impair their efficacy against tumors and overall survival rate. Furthermore, due

consideration should be given to potential detrimental effects such as fundamental toxicity resulting from the administration of exogenous cytokines.

2.4.2 The safety of CAR-NK cells

The safety profile of CAR-NK cell therapy surpasses that of CAR-T cell therapy, and preclinical research has yielded promising outcomes. The results from some investigation demonstrated a significant extension in the duration of mice's life after CAR-NK cell therapy, without any indications of abnormal proliferation observed upon pathological examination. Subsequently, Enli Liu et al., presented clinical findings on the effectiveness of umbilical cord CAR-NK cells as a theraputric methhod on lymphoblastic leukemia [12]. In this particular clinical investigation, a total of 11 individuals were enrolled. Following the administration of CAR-NK cells, positive therapeutic outcomes were observed in 8 patients, with complete relief experienced by 7 patients. Additionally, after CAR-NK cell therapy,there were no incidences of cytokine storm, neurotoxicity, or anti-host disease associated with therapy. Inflammatory indicators like IL-6 did not rise over the criterion standard, as well. These results imply that CAR-NK treatment has important assurance benefits.

CAR-NK cell safety worries can be seen from two different angles. The first is that in several preclinical and even some clinical investigations, effector cells derived from natural killer (NK) tumor cell lines are utilized, posing a potential risk of tumorigenicity. To mitigate this risk, current approaches involve treating NK-derived tumor cell lines with gamma-ray irradiation and other methods to maintain their cellular activity while inhibiting proliferation. Although techniques like gamma-ray irradiation have demonstrated efficacy in suppressing the growth of NK-derived tumor cell lines, it is crucial to acknowledge that different cell lines exhibit unique anti-tumor functionalities. Therefore, when developing CAR-NK cells using these specific cell lines, it becomes imperative not only to address their tumorigenic potential through methods such as cellular inactivation via irradiation but also consider the variations in their anti-tumor effects and potential safety concerns arising from such discrepancies.

2.4.3 The anti-tumor activity of CAR-NK cells

T cells and NK cells have very different activation processes. While NK cells are activated by a variety of activating receptors produced on their cellular membranes, T cells are activated predominantly by their own TCR receptors[4]. When constructing chimeric antigen receptors, it is imperative to incorporate four essential components: an external domain responsible for antigen recognition, a flexible linker connecting this recognition region with the membrane-spanning moiety, the membrane-spanning moiety itself, and ultimately an internal segment. Typically, the recognition domain encompasses a scFv molecule. It should be emphasized that optimizing the length of the linker is crucial in facilitating effective communication between effector cells during immune synapse formation. The intracellular costimulatory domain of CAR plays a pivotal role in activating and orchestrating the functioning of effector cells, surpassing these elements in significance. Presently, CD28-CD3ζ stands as the predominant costimulatory domain extensively employed in CAR-NK cell research [11]. In the dimention of classical second-generation CAR-T cell development, CD28-CD3ζ is commonly employed as an intracellular costimulatory domain. However, with the advancement of research on CAR-NK cells, mounting evidence suggests that constructing direct CAR-NK cells using chimeric antigen receptors derived from CAR-T may not be optimal. Consequently, there arises a pressing need for designing chimeric antigen receptors tailored specifically to the unique attributes of NK cells. Encouragingly, significant strides have been made in exploring positively impactful advancements in rational design-based approaches for CAR-NK cells.

2.5. Compare with CAR-T cell therapy

Hematological malignancies capable of treated with CAR T cell therapy, which shown to have substantial potential, yet a major concern associated with this approach lies in the possibility of severe adverse events that could potentially be life-threatening. Two commonly observed adverse events include ICANS and CRS. Alternative treatments for these adverse events encompass anakinra, which

acts as an IL-1 receptor antagonist, and siltuximab, a chimeric antibody targeting IL-6. The underlying mechanisms responsible for CRS and ICANS are intricate. Studies conducted on murine models of CRS have unveiled that monocytes or macrophages serve as the primary sources of IL-1 and IL-6[12].

One drawback of the current CAR T cell therapy lies in its exorbitant cost associated with producing autologous CAR T cells, resulting in a comprehensive treatment expense, up to $500,000 for patients experiencing severe CRS. Moreover, the conventional manufacturing time required for autologous CAR T cells ranges from 21 to 35 days. During this waiting period, patients may necessitate interim therapy and, in certain cases, succumb to rapidly progressing illness without reaping the benefits of CAR T cell therapy. CAR-NK cells harbor potential applications across diverse medical domains. Because CAR-T cell-produced proinflammatory cytokines together with TNF-, IL-1, and IL-6 are commonly blocked by naturally safer NK cells that have been activated, such as because of their natural safety, these cells may provide increased safety compared to CAR-T cells. Additionally, because CAR-NK cells are not finite by MHC restrictions, using them reduces the risk of GVHD. CAR-NK cells possess a diverse array of cytotoxic effects, utilizing both engineered killing capabilities and natural cytotoxic receptors to identify and eliminate targets. In clinical trials, these cells have demonstrated the ability to detect and eradicate residual tumor cells even after prolonged treatment. Due to their dual CAR-dependent and CAR-independent target identification skills, CAR-NK cells may confront a comparable difficulty to that shown in CAR-T cells in vivo when treating solid tumors because of their insufficient effectiveness. This is suggested by studies on allogeneic, activated NK cells which indicate their susceptibility to cellular exhaustion and inadequate infiltration into tumor sites. The likelihood of tumor evasion in CAR-NK therapy is significantly reduced. Additionally, the cost-effectiveness of CAR-NK cells surpasses that of their T cell counterparts, indicating a higher market potential for this innovative therapy.

3. Conclusion

In spite of the distinguished clinical advancements achieved by CAR-T cell therapy in hematological malignancies, there hitherto exist several obstacles and challenges. These limitations associated with CAR-T cells have prompted investigations into alternative immune cells for instance NK cells and T cells. In comparison to CAR-T cells, CAR-NK cells offer potential advantages in terms of safety, reduced risk of immune-related adverse effects, and cost-effectiveness. Furthermore, CAR-NK cells possess target recognition and cytotoxic abilities while also demonstrating the capability to prevent tumor escape incidents. Moving forward, next-generation CAR-NK therapies are incorporating cutting-edge technologies inspired by findings from CAR-T research while utilize the unique capabilities of NK cells. Overall, it is anticipated that CAR-NK therapies will increasingly play a significant role in clinical settings in the forthcoming years.

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