Limitations and Overcomes of CAR-NK for Cancer Immunotherapy

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Abstract. Adoptive cell treatment utilising chimeric antigen receptor (CAR) immunotherapy is gaining remarkable breakthroughs. In solid tumours, CAR immunotherapy lags far behind. CAR T cell production, does not have tumor-specific antigens, and is not effective in CAR T cell infiltration and trafficking into tumour sites, therapy-associated toxicity, immunosuppressive TME, and antigen escape are among the primary obstacles for CAR immunotherapy in solid tumours. Compared to CAR T cells, CAR NK cells have a number of benefits, including the ability to be processed from preexisting cell lines with mismatched MHC, the ability to kill bad cells via CAR-independent and CAR-dependent pathway with less toxicity. One clinical study demonstrated the effectiveness and safety of CAR NK cell treatment. However, CAR NK cell therapy also has its drawbacks, including, low persistence, lack of transporting pathway, immunosuppressive tumour microenvironment, and low lentivirus transduction efficiency. Solving these issues are significantly important for this technology to be accepted in future use.

Keywords: CAR-NK, Cancer, Immunotherapy.

1. Introduction

From the sky to the sea, viruses and bacteria are almost everywhere. Although the human body is constantly at risk from pathogenic microbes, we don't always get sick, thanks to the immune system, the body's "guardian army". Immune cells play an important role in the immune system. There are many kinds of immune cells, among which T cells and NK cells have become important research objects in the field of immune cell therapy product development due to their unique advantages [1].

In the human immune system, T cells are like special forces, capable of precise and effective attacks on bacteria, viruses and cancer cells. Based on the properties of T cells, scientists have developed CAR-T products to treat cancer. Unlike T cells, NK cells kill spontaneously. They are highly reactive and do not need to be activated to go straight to the attack. They are considered the first line of defense of the body's immune system. Therefore, scientists are also developing cell therapy products such as CAR-NK to provide more weapons for humans to fight cancer [2].

NK cells have recently gained prominence as a formidable immunotherapy tool. While NK cells and CTLs use comparable killing mechanisms to eliminate malignant or virally infected cells, their target recognition mechanisms differ significantly. Allogeneic NK cells are fairly safe for ACT because they do not normally mediate the development of GVHD. In addition to inhibiting cancer cells by recognizing tumor-surface antigens with SCFV, NK cells can also inhibit cancer cells by recognizing various ligands through a variety of receptors. In addition, NK cells secrete only small amounts of IFN-γ and GM-CSF and do not produce IL-1 and IL-6, which initiate CRS.

As CAR NK emerges as a promising treatment for hematological malignancies, there are still remaining issues with this approach. So this article will focus on introducing limitations and side effects through the collected clinical data and research. Understanding these problems and investigating ways to overcome them will be significantly important for CAR NK cell therapy to be wider accepted.

It has the potential to become a "general purpose" product. Due to the relatively wide range of sources of NK cells and the safety profile of allogeneic NK cells, CAR-NK cells are expected to provide a "universal" product that addresses the long manufacturing cycles and individualized

requirements that beset current CAR-T products. Currently, many CAR-NK products are undergoing clinical trials, most of which are in phase 1/2 clinical trials. In terms of targets, similar to CAR-T, most CAR-NK cell therapy products target common hematologic malignancies.

2. Limitations and overcomes

2.1. Low persistence

The inability of infused cells to survive in vivo without cytokine support is a major limitations therapy. Though it be harmless, could reduce effectiveness of NK-therapy. [3]. Cytokines have been proven to enhance the propagation and longevity of NK cells adoptively; yet they can also induce undesirable side effects, such as the development of inhibiting immunological subgroups such as regulatory T cells [4]. Several investigations have shown encouraging outcomes by modifying NK cells cytokine-encoding transgenes that constitutively released or are expressed. In study, Mice with tumours with NK cells cells transduced expressing IL-15 or IL-2 produced IL-15 or IL-2 had enhanced multiplication and longevity [5]. Increase NK cell proliferation can likewise be achieved by integration, and survival. Anticancer activity in lymphoid patients without boosting generating toxicity. Other CAR-NK cells are currently being developed; nevertheless, there are already existing examples.

2.2. Difficulties in transporting to the required tumor site

Tumour beds is critical for the success therapy it also is mediated by complex between NK cell-produced chemokines and tumour cells. The controversy about the efficacy of tumour locations has prompted efforts to enhance it. After that, other researchers have examined various techniques for enhancing NK cell homing. Electroporate the NRP-body was employed to enhance NK cell trafficking and tumour penetration. Furin found pancreatic cancer cells, hence NK cell infiltrates better through the ERK signalling pathway. Additionally, CAR-NK cells have been engineered to enhance their ability to migrate to tumour locations. It has been revealed that CAR-NK cells engineered to generate CXCR4 confer chemotaxis to glioblastoma cells, resulting in enhanced tumour shrinkage and survival [6].

NK cells do not undergo somatic gene rearrangement and do not express antigen-specific receptors. They are non-specific tumor killer cells. NK cells are regulated by a series of activation and inhibitory receptor signals, and their lethality depends on the balance between inhibitory and activating signals. Tumor cells with low HLA expression are more likely to be killed by NK cells due to decreased KIR-mediated inhibition. NK cells also kill target cells through antibody-dependent cell-mediated cytotoxicity (ADCC). CD16 on NK cell membrane can recognize the Fc part of IgG bound to target cell surface, activate NK cells through ITAM signaling pathway, and induce apoptosis of target cells through various ways. D-2HG and L-2HG have the same intracellular concentration, D-2HG can significantly inhibit the proliferation of T cells, and this inhibition is reversible [7]. The lysosomal membrane of CD8+T cells fuses with the cytoplasmic membrane to release cytotoxic proteins by exocytosis and release granzyme, thereby killing target cells.

2.3. Immunosuppressive tumor microenvironment

The tumour microenvironment, which consists of immunosuppressive cells, immunosuppressive soluble compounds, and an unfavourable optimal immune cell functioning environment, is a significant hurdle to the efficacy of CAR-NK cell therapy. Adenosine, IDO, PGE2, and TGF-b are metabolites identified. MDSCs, fibroblasts, platelets and various unfavourable metabolic variables including acidity, hypoxia and food deprivation produce immunosuppression in the malignant environment [8]. Therefore, scientists are attempting to create CAR-NK cells that can mitigate numerous immunosuppressive effects. Engineering TGF-b resistance has proven to be a good method. Using CRISPR/Cas9 technology, the TGFbR2 gene was eliminated in NK cells, leaving growth factor without impairing efficiency against AML. In vitro and in vivo use of an anti-miRNA against miR-

27a-5p, increased NK cell effector activity. Moreover, inhibiting A2A on natural killer (NK) cells increased anticancer activity in animal models of cancer [9]. Checkpoint molecule interaction is an additional crucial mechanism by which TME triggers NK cell death. Some studies focus on NKG2A and discovered that NKG2A-deficient NK cells are more cytotoxic against HLA-E-expressing cancers [9]. Recent research indicates that the combination of CAR engineering and checkpoint deletion is effective. Recently, the use of checkpoint blockage to enhance NK cell effector function has been extensively addressed. Review of the scientific literature suggests that genome editing may be able to improve the constraints and the obstacles presented.

More studies are needed to determine how oxygen availability is linked to metabolite reprogramming in the tumor microenvironment, as well as the complex interactions between multiple other metabolites and cells in the tumor microenvironment. As the technical challenges of metabolite detection in the tumor microenvironment begin to be solved, our understanding of how tumor cells contribute to immune escape in a cellular non-autonomous manner will continue to deepen.

Multiple studies have also identified the critical role of TCF-1+ Tim3-CD8 +T cells in PD-1 ICB response in solid tumors. The presence of tumor antigen-specific memory CD8+T cells in tumor draining lymph nodes confirms the critical role of this population in PD-1 ICB therapy. At the single-cell level, the transcriptional characteristics of TdLN-TTSM cells are more similar to those of classical memory cells, but different from those of depleted T cells. Further analysis of ATAC-seq data also obtained consistent conclusions. Sequencing data showed that there were different degrees of chromatin opening at multiple sites in tumor draining lymph nodes, Tpex cells derived from chronic virus infection and Tex cells isolated from chronic virus infection environment

2.4. Low lentivirus transduction efficiency

A transduction system based on lentivirus is a major prevalent method. NK cells are resistant due to their innate properties, making lentivirus-based transduction difficult. Several substances have been employed. Using protamine sulphate or dextran or polybrene, for instance, the electrical charge on cell membranes can be eliminated. Also, in HSCs and cyclosporine A, progenitor cells, and rapamycin may facilitate the elimination of various lentiviral restriction barriers. Intriguingly, it has been observed that inhibiting intracellular antiviral defense mechanisms increases the effectiveness. It has also been observed that rosuvastatin increases the efficiency via elevating LDLR levels [10]. Colamartino ABL et al. have also demonstrated a robust and efficient method for NK cell transduction.

In order to ensure that the masked IL-15 could effectively activate the effector cells in the tumor and play an anti-tumor effect, PD-1 antibody (aPD-1) was added to construct APD-1-IL-15 fusion protein. Due to the limited information on the structure of the fusion protein, the mechanism by which masked IL-15 regains activity remains unclear. Researchers also hypothesized that when aPD-1 antibody binds to PD-1 molecules on cells, the structure between Fc and IL-15 changes conformational, so that the previously covered IL-15 is no longer covered, and the activity of IL-15 is completely released. Based on the structural analysis of the interaction between IL-15 and its receptor, the researchers placed IL-15 at the carboxyl terminus of antibody Fc to form steric hindrance, so that Fc could physically block the binding of IL-15 to the receptor and effectively reduce the activity of IL-15. When injected intraperitoneally into mice, the toxicity of IL-15 was significantly reduced, which ensured the safety of IL-15 use.

The toxicity and antitumor activity of IL-15 are both dependent on the activity of IL-15 itself and are therefore often concomitant, mutually beneficial and mutually destructive. The novel modification strategy proposed by the researchers effectively separates the toxicity and antitumor activity of IL-15 spatially, and reduces the toxicity of peripheral tissues by physically masking and forming steric hindrance to reduce IL-15 activity

3. Possible future approaches to overcome these limits

Identifying consistently expressed target tumour antigens is the most crucial stage in building CARs. consequently, it is inevitable to achieve "on-target, off-tumor" effects. Single-cell clones from tumour may express these TAAs differently due to two key methods for evading immune surveillance. To circumvent this issue, bispecific CARs were created to concurrently target two distinct antigens in cancer, with extremely promising outcomes. Similarly, targeted ultrasound-guided distribution are used to address issues with the trafficking of CARs in solid tumours. In an orthotopic model, pleural injection was shown to be very efficacious. Regional delivery of immune cells based on CAR may potentially reduce the therapeutic dosage. Furthermore, the NK cells has been effectively increased in solid tumours by inhibiting SMAD3.It shown to be very successful in preserving the capacity of UCB-NK cells to generate IFN-g and kill glioblastoma cells. It has been shown that Entinostat (a histone deacetylase inhibitor) increases MICA expression, resulting in enhanced tumour cell identification, and induces circumstances of tumours. TME is also marked by nutritional deficiency and severe hypoxia, which result in acidosis and ultimately decrease immunological responses. Hypoxia promotes tumour growth by interfering with metabolism and boosting the production of many tumour factors. In addition, hypoxia promotes tumour development and metastasis by reducing the expression of many NK cell-activating receptors. Moreover, it has been shown that CD73 induces arginase in the hypoxic condition in order to inhibit NK cell activation.

Not enough attention has been paid to modulating tumour metabolism, an additional significant technique for boosting the function of CAR- NK cells. Under hypoxic circumstances, adenosine is generated by the metabolism of ATP, which are implicated in immune evasion, blocking NK cell transit to tumour locations. In addition, the use has been proven to be extremely effective in boosting the efficacy of targeted treatment for ovarian cancer. Due to its high expression in solid tumours, CD73 may be a significant therapeutic target. After anti-CD73 antibody-based suppression.

Multiple antigens may be addressed concurrently to compensate for antigen loss during CAR treatment. But the high costs associated with producing many vectors and the heterogeneity of CARs, which results in poor clinical analysis, continue to be the most significant limitations of this technique. Designing a CAR capable of recognising many antigens is an additional crucial strategy. This objective may be achieved using 'tandem CAR,' in which two binders are coupled to enhance the effectiveness of the immunological synapse. Additionally, the ribosomal skip sequences may aid in the generation of numerous CARs on a single immune cell utilising a single vector. Recent increases in the number of clinical studies using CARs that concurrently target several antigens are quite encouraging. In the future, we expect further clinical investigations using CARs.

4. Conclusion

On the basis of accumulated evidence, CAR-NK therapy has become a promising and advanced area of research. However, overcoming these limitations and more clinical trials on a larger variety of tumours is required to better understand and apply this technique.

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