

Car-NK cell therapy for overcoming solid tumors

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Abstract. There are many standard treatments for solid tumors, including surgery, chemotherapy, radiotherapy, or combination therapy, but all of them are difficult to maintain long-term anti-cancer effects. Recent immunotherapies such as Car-T have achieved remarkable results in hematologic cancers. However, clinical success of immunotherapy for solid tumors remains difficult to achieve due to the specific nature of solid tumor microenvironment and impediments to drug delivery. NK cell therapies can be divided into two main types, those that directly use unmodified NK cells to kill cancer cells and CAR-NK cell therapies, which genetically engineer NK cells to bind to specific CAR structures, thereby increasing specificity and thus reducing damage to normal cells when NK cells target cancer cells. Therefore, researchers are currently focusing more on the second type of NK cell therapy. Compared with CAR-T cells, CAR-NK cells have benefit in significant immune rejection, more effectiveness in solid tumor.

Keywords: CAR-T, CAR-NK, Immunotherapy.

1. Introduction

According to NCI, solid tumor refers to a solid mass of cancer cell containing abnormal tissue, as opposed to a liquid tumor. There are many standard treatments for solid tumor, including surgery, chemotherapy, radiation therapy, or combination therapy relies on multiple factors including the type of cancer, the location of the tumor, and the patient's own physiological conditions. However, clinical success of immunotherapy for solid tumors in many cases. For example, surgical resection is hardly applicable to patients with advanced solid tumors, and chemotherapy based on the cytotoxicity, due to its lack of specificity, also attacks normal cells, which potentially causes serious adverse effects. In addition, radiotherapy alone cannot cure most types of cancer, so it usually needs to be combined with chemotherapy, and even with these combined treatments, the problems of side effects and cancer recurrence still exist, meaning that cancer control remains a major challenge to be overcome.

It is a consensus that the immune system vital in tumorigenesis, and, therefore, immunotherapy vital in tumor treatment. In recent years, cancer immunotherapy is becoming a major research hot spot in cancer treatment for its specificity and the longer-lasting anti-cancer effects. To date, there have been various immunotherapies in clinical trials with success, most of which involve the use of monoclonal antibodies, and ACTs, and have achieved success against hematologic cancers [1].

However, new diagnoses of hematologic cancers accounted for only 9.9% of total cancer diagnoses in the US in 2020, while for solid tumors, which account for approximately 90% of cancer diagnoses, relevant clinical trials have obtained fewer encouraging results, related to their mechanism of action. The effectiveness in tumors has been limited for several obstacles, including an immunosuppressive TME, inefficient delivery. In addition, the high intratumoral pressure of solid tumors further exacerbates this situation, posing challenges to the investigation of solid tumor immunotherapy. Moreover, drug delivery method also affects the efficacy of CAR-T therapy in solid tumors. CAR-T therapies for hematologic cancers are generally administered via intravenous infusion. Therefore, CAR-T cells can enter the circulation and are more accessible to target cells in hematologic malignancies than in solid tumors. Furthermore, systemic infusion of immunotherapy might lead to off-target toxicity within the tumor (OTOTT) and the impaired transport of CAR-T cells to the tumor site will decrease the therapeutic effect [2].

Therefore, successful immunotherapy of solid tumors requires a deeper understanding of TME, as well as the selection of innovative cell engineering protocols and delivery technologies, which has led to the need for research on the Car-NK cell therapy. The Car-NK therapy is a promising therapy

that has the potential to overcome the challenges in solid tumor that do not exist in hematologic malignancies.

As multiple areas of Car-NK cell therapy are under active investigation, here we review five aspects that are relevant to Car-NK therapy against solid tumors: (a) the current state of treatment for solid tumors, (b) how current limitations have led to research on Car-NK therapy, (c) the mechanism of action of the Car-NK cell therapy, (d) the clinical efficacy and safety based on undergoing clinical trials, and (e) ultimately its possible limitations and future perspective.

2. Tumor microenvironment (TME) of the solid tumor related to obstacles in immunotherapy

Clinical success of immunotherapy against solid tumors is difficult to achieve due to the unique pathology and physiology of solid tumors, as these challenges posed by solid tumors that do not exist in hematologic cancers need to be overcome [3].

2.1. MDSCs and Tregs

The physiology of solid tumors is significantly at the microenvironment level, posing many challenges for the immune system and providing investigable targets for cancer immunotherapy [4]. Solid tumor microenvironment (TME) mediates the immunosuppressive program through a complex network of cells and soluble mediators.

MDSCs include myeloid cells and are abundant in TME with potent immunosuppressive functions. They expand, differentiate, migrate, and activate in cancer growth. MDSCs mediate immunosuppression via multiple mechanisms. Firstly, MDSCs produce ROS and peroxynitrite (ONOO-) and express nitrogen oxidase 2 (iNOS) in response to oxygen stress [5]. The increased ROS concentrations impede T cell signaling activation, proliferation, and activity, thereby inhibiting adaptive immune responses. In addition, ONOO- is rich in aggregation sites for MDSCs and tumor cells, which can nitrate TCR and CD8 molecules. Moreover, MDSCs express iNOS that produces NO, which can inhibit IFN- γ signaling and the JAK/STAT5 pathway, express MHC class II, and induce apoptosis of T cells and NK cells in tumor cells, directly impairing T cell function.

Secondly, MDSCs promote amino acids consumption required for the body, such as arginine, cystine, and tryptophan, the lack of which would impair T cell proliferation, activation, and normal function. In addition, cell-cell interaction is another way in which MDSCs suppress immune responses. As mentioned above, MDSCs and Tregs are important components of the TME, and the interaction between the two contributes to the establishment. MDSCs enhance Tregs production and induce immune tolerance in tumors through a cell contact-dependent mechanism. Furthermore, MDSCs promote immunosuppression by crosstalk with cells. MDSCs promote tumor development by impairing the antitumor effects of NK cells. Research has revealed a relationship between the reduction of NK cells and a significant increase in MDSCs. Intercellular contacts have been shown in tumor-bearing mice, which would reduce attack tumor cells, demonstrating the significant effect of MDSCs to inhibit NK cell function in TME.

2.2. Tumor vasculature

The physiological differences between the normal and tumor tissues include multiple aspects [6]. The tumor vasculature consists of two types of vessels, the vessels in the normal tissues that are invaded by the tumor, and the tumor micro vessels forming via neovascularization due to the increased expression of proangiogenic factors.

In contrast to normal tissues with well-organized vessels in close enough proximity, the vascular system in tumors tends to be highly abnormal with tortuous vessels, resulting in significantly impaired perfusion, and might cause hypoxic and acidic conditions [7].

Moreover, as the development of a tumor requires continuous growth of new vessels, the TME can promote angiogenesis. Therefore, inhibition of angiogenesis can have a powerful anticancer

effect that is less susceptible to acquired drug resistance (Brown & Giaccia, 1998). Another significant feature of the solid tumor microenvironment is the high intra-tumor pressure, which limits the penetration of cellular therapeutic agents and their ability to reach the tumor mass, thus reducing the therapeutic effect.

3. Car-NK: from natural basis to cancer immunotherapy

3.1. Molecular mechanism and innate cytotoxicity of NK cells

Experiments designed to characterize T cell-mediated cytotoxicity unexpectedly revealed the presence of naturally occurring cytotoxic lymphocytes that have innate anti-tumor properties which were first observed in the 1960s, and later named as NK cells [8]. Natural killer cells account for 0.05-0.2 of the human's circulating lymphocytes and are regarded as frontline responders that can rapidly mediate responses when transformed or infected cells occur.

Instead of expressing polymorphic clonotypic receptors, NK cells use inhibitory receptors, which can be classified into three main classes: KIRs as the primary MHC-I receptors, the cytotoxicity receptors, and the C-type lectin receptors. The coordination of the expression of multiple activating or inhibiting receptors and the NK cell education can facilitate the NK cell recognition of non-self-cells and protect the body's own cell from being targeted.

According to Elliott and Yokoyama (2011), the functional differences between NK cells are also the result of an educating process of NK cells by regulating the interaction between NK cells and MHC-I [9]. To further investigate how NK cells acquire 'education', the NK cells arming, and disarming models were developed (Raulet and Vance 2006). In the arming model, NK cells are considered functionally mature by the interaction among their own MHC-I-specific inhibitory receptors, which might be sufficient to drive the education. Although these processes are only thought to be controlled during NK cell development, these processes may be critical because they may be affected under disease conditions.

As a result, mature NK cells can quickly distinguish between self and non-self [10]. Under healthy conditions, suppressive NK receptors recognize MHC on target cells to block cytotoxicity, whereas if MHC expression is downregulated, the inhibitory effect of cytotoxic response is not activated.

NK cells have two major cytotoxic mechanisms, perforin and granzyme-mediated granulocyte apoptosis and ADCC. After NK cell activation, granulocyte perforin forms pores in the cell membrane of target cells. After cell membrane rupture, granzyme serine protease is transported to the cytoplasm to induce cell apoptosis.

3.2. Mechanism of action of Car-NK cell therapy in tumor

Role of NK cells in tumor immune surveillance. It lacks clonotypic receptors, which recognize surrounding cells through germ-encoded activation and inhibition receptors. During transformation, genomic mutations are characterized by multiple phenotypic changes leading to a 'missing self' condition, which is caused by down-regulation of the suppressive 'self' MHC-I.

An important mechanism of NK cell anti-tumor immunity works in response to this condition. The increased expression of stress-inducible molecules on the surface of transformed cells can be detected by specific NK cell receptors in a process called "inducible self-recognition [11]. This explains the mechanism by which NK cells can maintain cytotoxic activity. Furthermore, the level of this NK cell function depends on the relative strength of activating and inhibitory receptor signals, which is the coordination of NK cell activity, a concept known as 'altered homeostasis'.

Beyond direct killing tumors, NK cells can also stimulate immune response through interaction with dendritic cells. In addition, NK cell-derived cytokines and chemokines can establish an anti-tumor microenvironment and can, in some case can, clear cancer cells by recruiting cytotoxic T cells.

There are currently two types of NK cell therapies: one directly uses unmodified NK cells to kill cancer cells, and the other is CAR-NK cell therapy, which is genetically engineered to be modified

and combined with a specific CAR structure, which improves specificity and thus reduces the damage to normal cells when NK cells are targeted to cancer cells.

Compared with CAR-T cells, CAR-NK cells have certain goodpoints, one of which is no restriction on HLA (human leukocyte antigen) matching, which enables the use of Car-NK from allogeneic sources, makes it possible to become a universal 'off-the-shelf' cell therapy product. Other benefits of this feature include no graft-versus-host disease or significant immune rejection [12]. Moreover, Car-NK therapy has a lower possibility of tumor cell escape and more advantageous in the treatment of solid tumors. CAR-NK cells generate more effective cytotoxicity in solid tumors, but are also activated via natural cytotoxicity receptors (NCRs) independent of CAR mechanisms.

3.3. Engineering the Car-NK cells for tumor

Like CAR-T cells, genetic engineering is applied for CAR-NK cells to modify NK cells. Compared to CAR-T cells, CAR-NK therapy does not cause serious adverse events. A possible explanation for these advantages might be the short lifespan of NK cells in vivo and the factors, released in the different spectrum during NK cell killing.

In most preclinical studies, lentiviral-or retroviral-based transduction helped NK cells stabilize and sustain CAR expression. With the above approaches, CAR-NK cells can target multiple tumor antigens in pre-clinical studies, not only against hematological malignancies but also against solid tumors.

The CAR consists of three structural domains. The ectodomain domain plays a significant role in specifically recognizing the tumor antigens on cancer cells, with a scFv that is derived from antibodies. The transmembrane structural domains anchor the CAR structure to the effector cell membrane. The cytoplasmic activation domain induces signaling transduction to kill target cells, once the ectodomain domain recognizes a specific antigen and becomes activated.

In addition, NK cells can be obtained from cord blood, iPSC, and PBMC using autologous or allogeneic cells. To overcome the adverse efficacy of CAR therapy, the CAR-NK strategy that has made the most progress in solid tumors is targeting the checkpoint molecules PD-L1, HER2, and MUC1. The poor survival of transferred CAR-NK cells can be addressed in a number of ways.

4. Conclusion

Cellular therapies have been shown to have the potential to be more effective against immunosuppression and in some cases may also be directly tumor killing. New delivery methods also need to be considered to overcome the delivery barriers caused by high pressure within TME and to increase the concentration of therapy in solid tumors. Cell penetration and persistence in tumors can be improved by high-pressure delivery or by limiting systemic toxicity in regional delivery. Meanwhile, the major roadblock for both Car-T and Car-NK therapy is to translate this treatment modality to solid tumors and achieve effective outcomes with higher survival and persistent effect. While autologous CAR-T cell therapy has achieved excellent results, HLA restrictions have prevented the general use of the Car-T cells, making this therapy extremely expensive and time-consuming. However, CAR-NK cell therapy will not face the same problems, as it is not HLA-restricted and has the potential to become a universal "off-the-shelf" cell therapy that will revolutionize immunotherapy in the future. For now, this therapy is still novel and needs to be further validated in the clinic.

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