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Adoptive cell therapy using engineered natural killer cells

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Abstract

The generation of autologous T cells expressing a chimeric antigen receptor (CAR) have revolutionized the field of adoptive cellular therapy. CAR-T cells directed against CD19 have resulted in remarkable clinical responses in patients affected by B-lymphoid malignancies. However, the production of allogeneic CAR-T cells products remains expensive and clinically challenging. Moreover, the toxicity profile of CAR T-cells means that currently these life-saving treatments are only delivered in specialized centers. Therefore, efforts are underway to develop reliable off-the-shelf cellular products with acceptable safety profiles for the treatment of patients with cancer. Natural killer (NK) cells are innate effector lymphocytes with potent antitumor activity. The availability of NK cells from multiple sources and their proven safety profile in the allogeneic setting positions them as attractive contenders for cancer immunotherapy. In this review, we discuss advantages and potential drawbacks of using NK cells as a novel cellular therapy against hematologic malignancies, as well as strategies to further enhance their effector function.

Introduction

Adoptive cell therapy has become a powerful treatment modality for advanced cancers refractory to conventional therapy. Remarkable clinical results have been reported in patients receiving autologous T cells for the treatment of B-lymphoid malignancies [1–7]. The first two T-cell therapy using gene-modified chimeric antigen receptor (CAR), tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta), were approved in August 2017 by the Food and Drug Administration for the treatment of relapsed B-cell acute lymphoblastic leukemia (ALL) and refractory or relapsed large B-cell non-Hodgkin lymphoma (NHL) [7, 8]. The third product – Tisagenlecleucel (Kymriah) was approved for refractory lymphoma in May 2018. However, CAR-modified T cells still have a number of limitations: (i) it can be clinically challenging to generate autologous products for each individual patient, (ii) the price of CAR T-cell therapy, which include the manufacturing costs, administration of lymphodepleting chemotherapy and the need for inpatient care may ultimately be economically unviable for many health care systems; (iii) the longer time that is required to generate CAR T-cells may result in unavoidable delays in therapy, especially for patients with rapidly advancing disease. Although allogeneic products have the potential to overcome

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these limitations, allogeneic T-cells (even if HLA-matched) can mediate graft-versus-host disease (GVHD) [9] through their native T-cell receptor. Natural killer (NK) cells, on the other hand, may provide an attractive and safe source of allogeneic cells from immunotherapy. In contrast to other lymphocytes such as T cells, NK cells do not express rearranged, antigen-specific receptors; rather, their effector function is dictated by the integration of signals received through germline-encoded receptors that can recognize ligands on their cellular targets. Functionally, NK cell receptors are classified as activating or inhibitory and, upon recognition of specific cellular ligands, induce a positive or a negative signal, respectively [10–14]. Therefore, NK cells are of great clinical interest for CAR engineering for the following reasons: (i) allogeneic NK cells do not cause GVHD [13, 15–18], (ii) their relatively short life-span can allow an effective antitumor activity while reducing long-term adverse events such as cytopenia; and (iii) since CAR-NK cells recognize and target tumor cells through their native receptors, the possibility of tumor escape by down-regulating the CAR target antigen is less likely to happen than with CAR T-cells [19]. Another attractive possibility could be to select donors for CAR-NK production based on killer cell immunoglobulin receptor (KIR)-ligand mismatch with the recipient or haplotype B *KIR* gene, as both of these have been reported to be beneficial in the setting of allogeneic stem cell transplantation [20–23]. Thus, CAR-NK cells have the potential to be used as an off-the-shelf cellular immunotherapy for immediate administration as clinically needed and could overcome some of the obstacles related to logistics and costs.

Genetic modification of NK cells to enhance their function for cancer immunotherapy

Genetic modification to improve NK cell persistence

A major drawback of using NK cells for adoptive transfer is their inability to persist in the absence of cytokine support [24]. Recent studies have shown that in vivo proliferation and persistence of NK cells following adoptive transfer may predict clinical response [25]. A number of groups including ours have developed novel strategies to genetically manipulate NK cells to express cytokines for autocrine proliferation [26–28]. NK cells engineered using a retroviral construct to express *IL2* and *IL15* were shown to mediate superior in vivo growth and activity in tumor-bearing mice, without the need to add exogenous cytokines [26, 27]. In addition, our group recently demonstrated that ex vivo expanded NK cells transduced to express a CAR against CD19 and to ectopically produce IL-15 can dramatically enhance the in vivo antitumor activity and persistence of CAR-NK cells in a mouse model of lymphoma [28].

Genetic modification of NK cells to improve their cytotoxicity

NK cells express an array of activating and inhibitory receptors on their cell surface and therefore provide a unique opportunity to skew their signaling balance towards activation as a way to enhance killing of target cells. This can be achieved by introducing strategies to block inhibitory receptors, enhance the function of activating receptors or redirect the specificity of NK cells.

Blocking inhibitory receptors

Inhibitory receptors KIRs (iKIRs) are constitutively expressed on NK cells and bind to HLA class I molecules to block NK-cell cytotoxic response against healthy cells. This interaction explains the 'missing-self' hypothesis, which postulates that NK cells survey tissues for normal levels of the ubiquitously expressed MHC class I molecule [14]. If there is downregulation of HLA class I on target cells, (i.e. 'missing-self'), then the NK cell becomes activated if an activation receptor is also engaged [29, 30]. In the setting of haploidentical stem cell transplantation, patients with acute myeloid leukemia that have a KIR ligand-mismatched donor have superior relapse-free survival [13]. Thus, pharmacological inhibition of inhibitory receptors to enhance NK cell function is an area of active research. One such approach is to simulate KIR-ligand mismatch by preventing KIR-HLA interactions using an anti-KIR monoclonal antibody (Lirilumab) either alone or in combination with other immunotherapeutic agents. This approach is under investigation for hematologic and solid tumors [31, 32]. NKG2A, a C-type lectin receptor, is another inhibitory receptor that interacts with HLA-E, a nonclassical HLA class I molecules, and it is upregulated on cells from many cancers [33]. Silencing NKG2A triggers an NK cell "missing-self" response and promotes higher NK cell cytotoxicity against HLA-E-expressing cancer cell lines both in vivo and in vitro [33, 34]. Monalizumab (previously IPH2201) is a novel anti-NKG2A blocking antibody that is currently under investigation as monotherapy or in combination with other immunomodulatory agents in phase I/II clinical trials.

Enhancing NK cell functional activity

NK cells also express an array of activating receptors on their surface which include the natural cytotoxicity receptors NKp46, NKp44, and NKp30, the C-type lectins NKG2D and NKG2C, and a number of activating co-receptors including 2B4 and DNAM-1. Strategies to upregulate expression of these molecules include exposure to cytokines such as IL-2 or IL15 or gene transduction [24, 26]. NK cell antibody-dependent cellular cytotoxicity can also be directed towards specific targets through the NK cell Fc receptor CD16 (FcγRIIIa). Strategies under investigation include the use of bispecific killer cell engagers (BiKEs) or trispecific killer cell engagers (TriKEs) (Reviewed in [35]).

CAR-engineered NK cells

The cytotoxic activity of NK cells can be enhanced by redirecting their specificity against a specific antigen using a CAR, thus allowing the killing of previously resistant targets. The feasibility of genetically engineering NK cell lines and primary NK cells to express CARs has been shown in pre-clinical studies against multiple targets including B and T cell malignancies, multiple myeloma, glioblastoma, neuroectodermal tumors, epithelial cancers, and melanoma (Reviewed in refs. [36, 37]). Although NK cell lines such as NK92 cells are easily expanded in culture and can provide an unlimited source of cells for immunotherapy [38], they are derived from patients with NK lymphoma/leukemia and have a number of shortcomings. Irradiation of these cells is required to reduce potential safety issues such as in vivo tumorigenicity, which will likely affect their in vivo proliferation, efficacy, and persistence. Given the above-mentioned limitations of CAR-engineered NK cell lines, others

have developed approaches to introduce CARs into primary NK cells derived from peripheral blood, umbilical cord blood (CB), or induced pluripotent stem cells (iPSCs) (reviewed in ref. [37]). Our group at MD Anderson Cancer Center has developed a GMP compliant pipeline for the generation of CAR-NK cells obtained from CB. A first-in-human clinical trial to test the safety and efficacy of off-the-shelf CB-derived NK cells engineered to express a CD19 CAR and IL-15 to support their autocrine proliferation and persistence in vivo is under way at our center (NCT03056339).

Concluding remarks

Adoptive cell therapy using genetically engineered immune cells has emerged as a powerful treatment for certain cancers, as recently demonstrated by the use autologous CAR T-cells for the treatment of patients with B-lymphoid malignancies [1–6]. NK cells are strong contenders for allogeneic effector immune cells for cancer immunotherapy. Indeed, recent advances in our understanding of NK cell biology and their feasibility to be genetically modified to express a CAR against various cancer targets provide a unique opportunity to develop novel NK cell-based cancer therapeutics. Future genetic manipulation strategies using TALEN or CRISPR/Cas9 provide unprecedented opportunities to further enhance the activity of NK cell-based therapeutic by enhancing their effector cytotoxic function while preserving their safety profile, and to improve their persistence and antigen specificity.

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