

Leveraging properties of Natural Killer cells for cell-based immunotherapy



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Introduction

Pharmaceutical Sciences provide an expanding array of novel drugs against various diseases but there are many medical conditions like cancer, heart diseases, genetic disorders etc. that remain still incurable. To alleviate all these fatal conditions researchers continued their efforts in search of cutting-edge technologies in the field of Biotechnology as well as Regenerative medicine. In nineteenth century, the knowledge of immunology led the scientists to a promising approach for immunotherapy named as Cell Based therapy or Cytotherapy wherein the viable, healthy and normal or engineered cells from self or donor person are injected or transplanted to the patient in order to make the illness less severe by facilitating the cells to exert therapeutic action (1). Cell based therapy uses vast types of cells for the treatment which are mainly stem cells, dendritic cells, lymphocytes, pancreatic cells etc. Out of these haematopoietic stem cell (HSC) based therapy is already a well-established treatment for blood related disorders whereas rest of the cells used are still in experimental phases (2). On the other hand, in recent years Autologous chimeric antigen receptor (CAR) T cell therapy is the first commercialized therapy which led to substantial breakthrough in patients with critical B cell malignancies. However, despite the success, an obstacle remains which is nothing but graft Vs host disease. Natural killer (NK) cells, furthermore, identify their targets in a human leukocyte antigen (HLA)-unrestricted manner and hence do not represent these same concern which makes them suitable components for cell-based immunotherapy (3). This article mainly focuses on the properties and mechanism of NK cells for immunotherapy against various diseases along with associated challenges and recent advances to improve the effectiveness of NK cell-based therapy.

Immunological defense mechanism of Natural Killer cells

NK cells being the crucial component of innate immunity serve as the very first line of defense against tumors as well as a diverse range of pathogens mainly bacteria and viruses (4). Initial findings on NK cells highlight that the cells can discriminate between healthy cells and stressed or infected cells with the aid of inhibitory and activating receptors present on NK cell surfaces. Primarily all healthy cells possess self-major histocompatibility complex (MHC) class I

molecules on their surfaces which are detected by inhibitory receptors of NK cells and considered as self or healthy cells. According to 'self-missing hypothesis', cells lacking MHC molecules are detected by activating receptors and recognized as foreign or infected ones. Activating receptors of NK cells engage themselves by binding to the ligands displayed on pathogen infected cells consequently stimulating the activation of NK cells. Upon activation, NK cells elicit a potent response through the release of cytolytic granules and cytotoxic cytokines such as tumor necrosis factor (TNF)- α and interferon (IFN)- γ (5) . Moreover, NK cells are also capable of identifying antibody-coated cells through their Fc γ RIIIA (CD16) receptor and trigger antibody-dependent cellular cytotoxicity (ADCC) and cytokine production (6). In this way, NK cells exhibit immune regulatory functions which further help shape T cell and B cell responses, and impact the function of macrophages, dendritic cells, and neutrophils.

Leveraging properties of NK cells for cell-based immunotherapy

Based on the literature studies, it can be concluded that NK cells show remarkable potential to treat various diseases like cancer, cytomegalovirus infection, asthma, autoimmune diseases, and HIV-AIDS etc. They also exhibit few unique features which make them ideal and powerful immunotherapeutic agents to be used in the flourishing field of cell-based therapies. Some of the immunological salient features of NK cells are as follows:

- First line of defense: As discussed above, NK cells have the ability to recognize and kill transformed cells which are deprived of antibodies and MHC, resulting a much faster immune response without the need for prior antigen sensitization (7).
- Exhibit greater phenotypic heterogeneity: Evolution in single cell genomics and high-parameter cytometry, claimed that NK cells may demonstrate higher phenotypic heterogeneity which gives rise to diverse cell populations equipped with altered functional properties (8).
- Lacks graft Vs host disease: During the maturation of NK progenitor cells, they do not undergo clonal selection process and lack expression of the clonotypic TCR and the associated CD3 complex which are requisite components for signalling cascade. Therefore, the allogeneic NK cells do not trigger graft Vs host disease in the receiver's body.
- Memory like function: Studies reported that murine NK cells are endowed with memory like functions in case of cytomegalovirus infection. Later Todd Fehniger's group hypothesized that human NK cells should possess memory-like properties in similar manner. After consistent efforts, their study concluded that human NK cells which were preactivated with IL-12, IL-15 and IL-18, followed by 1–3weeks of resting phase, could initiate a robust immune response directed by increased IFN- γ production upon further exposure to cytokines (9) .

NK cell-based therapy and application against various diseases

NK cell-based therapy has been recognized as an incredibly promising therapeutic strategy to mitigate numerous severe diseased conditions. Effectiveness of this therapy highly relies on the source from which the NK cells are being transplanted. Predominantly, autologous strategies have been applied but in patients with cancer, NK cells denote a malfunctional phenotype marked by altered gene expression profiles which may affect the feasibility of

autologous strategy and its application by diminishing cytotoxic functions of NK cells (10),(11). Additionally, if patients are unable to provide sufficient cells, it would become more cumbersome for downstream processing and further engineering of NK cells. Therefore, NK cell-based therapy largely depends on allogeneic sources in order to overcome risks associated with autologous strategies. Apart from this, NK cells can also be derived from cord blood (9), (12), peripheral blood mononuclear cells, haematopoietic stem and progenitor cells (HSPCs) (13),(14),(15), immortalized cell lines (16), and induced pluripotent stem cells (iPSCs) (17). NK-92 is the first pioneered NK cell-based immunotherapy to receive Investigational New Drug approval by the US Food and Drug Administration (FDA) for clinical testing. The NK cell-based therapy has wide application to many diseases.

- A. Cancer
- B. Tumor
- C. Acute myeloid leukemia
- D. Hematologic malignancies

Recent Advancement to overcome few incumbrances of NK cell-based therapy

Despite numerous achievements, there are few challenges associated with the NK cell-based therapy which impact on the possible clinical benefits of the treatment. Few of the incumbrances and further recent advancements are as follows:

i)Chimeric antigen receptors (CAR) NK cells: Chimeric antigen receptors (CARs) are synthetic fusion proteins consisting of an intracellular signalling moieties and extracellular antigen-recognition domain that trigger cell activation. To reprogram the specificity of NK cells towards a particular target, CARs can be expressed on NK cells. Recent research data confirmed that these cells can target tumours with efficacy and specificity, by providing a desirable safety profile (18).

ii)Cytokine armouring: It has been observed that freshly extracted NK cells exhibit lower cytolytic potency as compared with NK cells that have been primed (19). Initiatives have been taken ahead aiming to effectively prime NK cells in vivo and/or ex vivo to preserve optimal antitumor function. During ex vivo expansion of NK cells, the cells can be combined with group of interleukin supplementation such as IL-2, IL-15 and IL-21 which demonstrated that these cytokines augmented the cytotoxic function and supported high proliferation rates while keeping the cells in a healthy and non-exhausted state (20). A new strategy has been hypothesized that cytokine armouring might be programmed in such a way that the soluble cytokines can be released into the environment or may be constructed in membrane-bound form to stimulate immune response upon cell to cell interaction (21).

iii)Overcoming immunosuppression: Tumor microenvironment (TME) comprises of a harsh metabolic landscape driven by a heterogeneous mixture of glucose and amino acid deprivation, immunosuppressive metabolites, acidity and hypoxia, which, in consequence, hinder effectiveness of antitumor immunity (22), (23). Since researchers are trying to alter immunometabolism in the TME, it is important to consider the significance of physiological balance, as few of the metabolites are requisite components of regular metabolism. Although most of the strategies are still in the embryonic phase, it has been assumed that combining TME modulation and NK cell

engineering can be plausible to reduce immunosuppression and enhance immune response of cells.

iv) Checkpoint disruption: Most often tumors have transmogrified themselves in a well sophisticated manner to enter immune surveillance, for instance the engagement of immune checkpoints, which may affect the functions of NK cells. Inhibitory KIRs and CD94/NK group 2 member A receptor (NKG2A) have been regarded as potent negative regulators of NK cell function and can revoke any concomitant activating signal when bound with HLA class I ligands. Few of the monoclonal antibodies such as Lirilumab and Monalizumab function to misrepresent the diverse signaling indications perceived by NK cells towards the activation by deactivating these inhibitory checkpoints (24). Hence, researchers claimed that monoclonal antibodies can be a reliable component to modulate the patients' immune cells, in order to maintain or extend in vivo half-life of NK cells. With further advancements in genetic engineering, the genes of NK cells can be stably altered to regulate immunological mechanisms that reinforce NK cell effector function. An example is the genetic disruption of the inhibitory receptor named as NKG2A, resulted in tumour control in xenograft mouse models which were inoculated with HLA-E+ tumours (25).

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