

# Natural Killer Cells: From Defense to Immunotherapy in Cancer

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## Abstract

Natural killer (NK) cells are central components of the innate immunity. The numerous mechanisms used by NK cells to regulate and control cancer metastasis include interactions with tumor cells via specific receptors and ligands as well as exerting direct cytotoxicity and cytokine-induced effector mechanisms. NK cells are also clinically important and represent a good target for anticancer immune therapy in which the host immune system is harnessed for anticancer activities. They also display impaired functionality and capability to infiltrate tumors in cancer patients. In this review, we provide an overview of our current knowledge on NK cell in oncology and immunotherapy. Although NK cells might appear to be redundant in several conditions of immune challenge in humans, their manipulation seems to hold promise in efforts to promote antitumor immunotherapy. Therefore, efforts to enhance the therapeutic benefits of NK cell-based immunotherapy by developing strategies are the subject of intense research.

**Keywords:** Cancer; Immunology; Immunotherapy; Natural Killer cells

## Background

Efficient immune responses to pathogen invasion in body are counteracted by coordinated interplay between both adaptive and immune effector systems. Natural killer (NK) cells are effector cells of the innate immune system that exert direct cytotoxic functions. These are determined by a finely tuned balance of signals delivered by inhibitory and activating receptors. There are two types of adaptive immune responses: humoral immunity, mediated by antibodies produced by B lymphocytes, and cell-mediated immunity, mediated by T lymphocytes. The innate immune system includes: Natural killer cells, mast cells, eosinophils, basophils, and the phagocytic cells which include macrophages, neutrophils, and dendritic cells, and function within the immune system by identifying and eliminating pathogens that might cause infection.

NK cells are unique, however, as they have the ability to recognize stressed cells in the absence of antibodies and major histocompatibility complex (MHC), allowing a much faster immune reaction. The discovery that a unique type of lymphocyte was responsible for natural or spontaneous cytotoxicity was made in the early 1970s by Rolf Kiessling and Hugh Pross [1]. NK cells are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. They are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation. With all the studies conducted on NK cell biology it helps to understand the regulation of NK cell function and thereby helps to utilize role in immunotherapy for treatment of cancer [2].

## Biology of Natural Killer Cells

Natural killer cells (also known as NK cells, K cells, or killer cells) are the effector lymphocytes of the innate immune system. The primary role of NK cells is to provide frontline defense against tumors and microbial-viral infection [3]. They are known to exert direct cytotoxic effect on the target cells by the release of interferon  $\gamma$  (IFN $\gamma$ ) which further boosts the cell immunogenicity. They also have role in adaptive immune response through interaction with dendritic cells [4], macrophages, T cells and endothelial cells [5]. They possess a distinct characteristic to differentiate between target cells and other healthy

'self' cells which in order controls the initiation of the cytolytic activity and avoids the damage of the tissues [6]. Initiation of cytolytic activity involves variety of cell surface activating and inhibitory receptors. NK cell typically expresses three to four inhibitory receptors and several activation receptors. The inhibitory receptors consist of killer immunoglobulin-like receptors (KIR) or Ig like receptors (CD158), the C type lectin receptors (CD94-NKG2A) and leukocyte inhibitory receptors (LIR1, LAIR -1) which recognize self-MHC class I molecule and prevent NK cell activation. The activating receptors are the natural cytotoxicity receptors (NKp46, NKp44), C type lectin receptors (NKG2D, CD94-NKG2C) and the Ig-Like receptors (2B4) [7]. The activation of NK cells is triggered when they encounter cell which lacks self MHC class I molecules by a phenomena classically known as 'missing self' hypothesis [6]. When the cells undergo stress the constitutive expression of MHC Class I molecule is lost which allows the selective toxicity towards the stressed cells. The apoptosis of the target cell once recognized by NK cells takes place either through the involvement of perforin or through caspases [8]. One more mechanism of killing of tumor cells by NK cells is antibody dependent cellular cytotoxicity (ADCC) as NK cells express low affinity receptor for IgG Fc $\gamma$ RIII (CD16) [9].

Human NK cells are classified into two subsets on the basis of immunophenotype and function: CD56dimCD16bright and CD56brightCD16dim. More than 90% of NK cells in the peripheral blood and spleen are CD56dimCD16bright, they express perforin, produce IFN $\gamma$  on interaction with variety of tumor cells *in vitro* and have high cytotoxic activity [10,11]. The remaining 10% of NK cells present in lymph nodes and tonsils are CD56brightCD16dim, they lack perforin and are involved in production of cytokines and exerts

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low cytotoxicity. The cytokines produced by these cells in turn regulate the downstream adaptive immune responses by T cells. The cytokines which regulate the NK cell functions are IL-2, IL-15, IL-12 and IL-18 [12]. IL-15 has the pivotal role in NK cell development and maintenance [13]. The cytokines responsible for inhibition of NK cell activation and function are transforming growth factor-beta (TGF- $\beta$ ) [14] and IL-10 [15]. Tumor cells secreting TGF- $\beta$  lead to down-regulation of activating receptors Nkp30 and NKG2D which results into NK dysfunction. IL-10 produced by acute myeloid leukemia (AML) blasts, up regulates NKG2A leading to impairment of NK cell function [16].

### Natural Killer Cells In Oncology

NK cells have predominant role in several disease conditions. There are several *in vitro* studies performed using human cell lines and *in vivo* studies using mice and rats which indicate that the tumor cells are being recognized as NK cell targets [17,18]. One of the primary functions of NK cells involves the immuno-surveillance of the body and inhibition of metastases formation by recognition and killing of tumor cells [19]. For the *in vivo* studies, syngeneic tumors are implanted in mice. These tumors are either genetically deficient for NK cell function or are depleted of NK cells by use of antibodies which target NK1.1 or the glycolipid asialo-GM-1 [20]. The evaluation of studies conducted using antibodies need to be done wisely. Targeting NK1.1 also affects the invariant NK T cells and other NK1.1<sup>+</sup> cells and targeting asialo-GM-1 expression do not selectively deplete NK cells since several cell types like myeloid cells, epithelial cells and T cell subsets express it on its surface [20]. The mouse NK cells *in vivo* are involved in rejection of transplanted tumor depending upon the presence and absence of NK cell receptor ligands expressed by the tumor. Tumors which lack MHC Class I or have up-regulated NKG2D ligands are susceptible to NK cell mediated lysis. Moreover cytokines like IL-2 and IL-12 promote perforin mediated anti-tumor activity by NK cells through NKG2D pathway while IL-18 support the anti-tumor activity depending on FasL expression by tumor cells [21].

It is observed in some of the experimental models that there is development of tumor specific T cell response to parental tumor cells which is induced by NK cell mediated elimination of tumor cells [22,23]. In a recent study, implantation of MHC class I low tumor cells led to the release of IFN $\gamma$  by NK cells which in turn stimulated and matured the dendritic cells (DC) into a IL-12 producing DC1 phenotype that resulted in a strong and protective anti-tumor CD8<sup>+</sup> T cell response [24]. NK cells also have role in controlling the growth of B cell lymphomas that arise spontaneously in mice which lack both perforin and  $\beta$ 2-microglobulin [17].

There are clinical and experimental evidences which suggest that NK cells have important role in tumor surveillance which was derived from various correlative studies. A 11 years study correlates low NK-like cytotoxicity of peripheral blood lymphocytes to increase risk for cancer [25]. In different carcinomas, it has also been reported that when tumors were infiltrated by NK cells they act as a positive prognostic marker [26-28]. In case of established tumors there are only few infiltrating NK cells which do not contribute significantly for elimination of tumors [29]. The inefficient homing of NK cells into malignant tissues leads to low NK cells in tumors which could be overcome by cytokine mediated activation [30]. A remarkable increase in post transplantation survival, absence of GvHD and protection from relapse was observed in selected malignancy of acute myeloid leukemia where patients lacked HLA class I ligands for donor inhibitory KIR [31].

### Natural Killer Cells In Cancer Immunotherapy

All evidences about NK cells in tumor immune control appeal for NK cell based immunotherapeutic approaches. The early studies of autologous NK cell therapy focused mainly on increasing the antitumor activity of the endogenous NK cells [32]. Immune suppression is a primary requirement for NK cell infusions. Mainly IL-2 was administered to activate the endogenous NK cells or adoptive transfer of NK cells activated using IL-2 were used as treatment [33]. This therapy has been experimented for the treatment of renal cell carcinoma, malignant glioma and metastatic breast cancer [34]. Infusion followed by administration of systemic cytokines did provide additional stimulation and support the expansion but this strategy had limited success due to involvement of various factors [35]. The cytokine stimulation resulted into NK cell activation followed by greater cytotoxicity against malignant target *in vitro*, but *in vivo* it demonstrated very limited cytotoxicity [35,36]. The failure of systemic IL-2 administration is mainly attributed to development of severe side effects, expansion of regulatory T cells and inhibition of NK cells by self-HLA molecules. This redirected towards the allogeneic NK cell therapy where adoptive transfer of *ex vivo* activated or expanded cells were used. In AML patient it has been demonstrated that on adoptive transfer of *ex vivo* expanded haploidentical NK cell resulted in expansion of NK cell *in vivo* without inducing graft vs host disease (GvHD). Studies were also conducted by adoptive cellular transfer of allogeneic NK cells from haploidentical donors for treatment of renal cell carcinoma, metastatic melanoma, refractory Hodgkin's disease and refractory AML [37]. A pivotal pilot study in NKAML patients indicated the use of donor-recipient HLA mismatched NK which may reduce the risk of relapse in childhood AML [38]. Since the allogeneic NK cells have the advantage of being derived from healthy donors and have more cytotoxic activity, the trial conducted provided valuable information on the *in vivo* persistence, donor chimerism and antitumor potential in different indications [10]. The absence of life threatening GvHD and major treatment related toxicities make this method advantageous.

The adoptive transfer of NK cells to solid tumor poses a special challenge since the cells need to traffic to the sites of disease and also penetrate the tumor to exert the effector function. The additional requirement is of tumor to be inherently susceptible to NK mediated cytotoxicity [39]. Studies in solid tumors have shown the presence of intratumoral NK cells which is correlated to the delayed tumor progression and improved outcome [40]. Trials conducted in solid tumors like neuroblastoma, renal, colon, gastric and ovarian cancers were benefited along with being safe and efficacious [41,42]. Similar trials were also conducted in patients with recurrent metastatic breast and ovarian cancer [43]. The other strategy is of combining of NK cell based immunotherapy with immune checkpoint inhibitors such as anti-KIR mAb which acts against the inhibitory KIRs, KIR2DL1, L2 and L3 [39]. Similar to KIRs, NKG2A binds to ligands HLA-E on tumor cells resulting in an inhibition of NK cell function. Phase I clinical trial to study the effectiveness of anti NKG2A mAb nomalizumab which blocks the interaction between NKG2A and HLA-E is currently under progress [10].

The involvement of NK cells in viral hepatitis, influenza and HIV infection is well reported [44-46]. The therapeutic role of NK cells has also been studied in several diseases such as asthma, multiple sclerosis, diabetics and arthritides [7]. NK cells can be used as a therapy for other diseases as well after understanding the role of NK cell and their receptors.

## Isolating NK Cells For Immunotherapy

For therapeutic application the allogenic NK cells can be sourced from umbilical cord blood (UCB), adult lymphapheresis products or from NK cell lines. Progenitors from cord blood provide a platform for cell to be expanded and differentiated into cytotoxic NK cells which do not lead to relapse and GvHD after transplantation due to low immunogenicity [47]. NK cells for therapeutic application can also be generated from induced pluripotent stem cells (iPSC-NK) and human embryonic stem cells (hESC-NK) [48,49]. The iPSC-NK and hESC-NK cells expressed common NK cell markers viz. KIRs, CD16, NKp44, NKp45, NKG2D and TRAIL, demonstrated anti tumorigenic activity [48] and was found cytotoxic against hematological and solid tumor cells *in vitro* [49]. Both these cells are superior to UCB derived NK cells in terms of cytotoxic activity and also can serve as unlimited source for adoptive transfer of NK cells. Potential tumorigenicity of these cells needs to be determined before using it in clinical set up [7].

There are also reports describing the derivation of NK cells of both functional and mature form (to be used in the immunotherapy) from the stem cells. Both embryonic and pluripotent stem cells are used as starting cells [2]. It is done by allowing a passive entry of HOXB4 which is a homeoprotein. It promotes the enrichment of human embryoid bodies precursors to possibly differentiate into fully functional and mature NK cells [50].

To further apply NK cell as an immunotherapeutic agent several developments in terms of methods to isolate, expand and enhance its activity for better efficacy are required. For expansion and activation of NK cells cytokines like IL-2, IL-15, IL-18, IL-21 and type I interferons have been used [12]. The most widely used NK cell expansion by IL-2 resulted into mobilization of inhibitory T regs which reduced the NK cell cytotoxicity [51], so the focus was shifted on IL-15 which minimized the capillary leak syndromes and had fewer side effects. Although there are trials conducted which proved the efficacy of these NK cells, more efficient methods by which the *ex vivo* expansion of NK cells could be done. The target is to produce large quantities of purified, functionally active NK cell for clinical use from simple blood draw [52]. Another source of generating clinical grade NK cell is from cord blood derived CD34<sup>+</sup> cells [53]. The other method for the large scale expansion of NK cells is by use of irradiated Epstein-Barr virus-transformed lymphoblastoid cells. These cells possess increased expression of activating receptors and death receptor ligands which results into superior cytotoxicity against tumor cells [54]. The specificity of the NK cells can further be enhanced by approaches like genetic modifications by the use of chimeric antigen receptors (CARs). But the safety profile using CAR engineered NK cell is yet to be established [6]. One of the new dimensions for NK cell immunotherapy is the use of NK- 92 a permanent NK cell line well characterized and possesses superior cytotoxicity. Clinical phase I/II trials conducted using irradiated NK-92 cells in patients with leukemia and other diseases demonstrated its safety and feasibility [55].

## Conclusion

Research for all these years have led to significant advances in understanding the molecular mechanism involved in NK cell mediated antitumor activity. Both inhibitory and activating NK cell receptors play important role in molecular recognition of innate immunity. The *in vitro* studies with human and mouse cells and *in vivo* human data suggest that NK cell have important role in early control of viral infection, in hematopoietic stem cell transplantation, in tumor immunosurveillance and also several other disease. Although little is

known about the fate of NK cells after transfusion, they are the potential tools for cancer therapy both due to cytotoxic ability and the possibility of *ex vivo* expansion. Progress is required to achieve the maximum therapeutic benefit with minimal associated adverse effects. The major focus now is onto the development of therapeutic regimens for various types of disease.

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## Competing Interests

All authors declare that they have no conflict of interests.

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