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Perspective

A multifaceted approach targeting NK cells for better treatment of cancer: focus on hematological malignancies

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Introduction

Natural killer (NK) cells are innate lymphoid cells that are endowed with an intrinsic ability to kill tumor cells without the expense of prior activation. With an array of germ line-encoded receptors that recognize specific ligands on target cells, they sense and selectively kill tumor cells and thus play a pivotal role in the first line of defense against the development of cancer [1]. Owing to such an innate selectivity against tumor cells, NK cells have gained considerable attention as promising therapeutic measures for cancer immunotherapy [2, 3]. Moreover, NK cell functions are often impaired in a variety of cancer patients, and the extent of NK cell dysfunction correlates with clinical prognosis. Thus, NK cells can be used as useful prognostic biomarkers. NK cell decision to kill target cells is determined by a subtle balance of signals transmitted from diverse activating and inhibitory receptors [1]. NK cell activation is tightly controlled by the requirement for the engagement of multiple activating receptors upon encounter with target cells. Except for CD16 that mediates antibody-dependent cellular cytotoxicity (ADCC), neither single activating receptor is sufficient but requires co-engagement of specific pairs of activating receptors to trigger cytotoxicity of freshly isolated NK cells. For example, 2B4 requires combination with NKG2D or with DNAM-1 for synergistic activation of NK cells. Cytokines such as IL-2 or IL-15 lower the threshold for NK cell activation such that cytokine-stimulated NK cells can respond to single activating receptors for triggering of killing activity [4]. In addition, inhibitory receptors such as KIRs and CD94-NKG2A, specific for MHC class I, dominantly antagonize such activation and thereby provide protection of healthy cells from killing by NK cells. The down-regulation of MHC class I for inhibitory receptor and/or up-regulation of specific ligands for activating receptor (e.g. NKG2D) are frequently observed on transformed/malignant cells, thus cooperatively allowing their effective destruction by NK cells [5]. In this respect, many efforts have been made to enhance the therapeutic benefit of NK cells for cancer immunotherapy by manipulating NK cell effector function. Here, we describe recent advances in NK cell-based cancer immunotherapy with focus on NK cell activation and discuss its future perspectives.

Strategies circumventing inhibition

Autologous NK cell deficiency is a well-known feature observed in many cancer patients, especially in patients with hematological malignancies. The several mechanisms suggested include up-regulation of inhibitory receptors on NK cells and down-regulation of the ligands on tumor cells for NK cell activating receptors [3]. Thus, infusion of NK cells without this deficiency holds promise in the treatment of cancer. The initial studies of NK cell immunotherapy focused on adoptive transfer of *ex vivo* activated autologous lymphokine-activated killer (LAK) cells or NK cells followed by systemic administration of IL-2 to support their *in vivo* stimulation and expansion. Although some objective tumor

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regression, this approach resulted in limited efficacy and poor clinical outcome in patients with lymphoma [2, 6]. These were explained by several factors including the suppression of autologous NK cells by "self"-MHC molecules present on tumor cells, and IL-2-induced severe toxic effect (e.g., vascular leak syndrome) and expansion of suppressive regulatory T cells. Several strategies have been developed to overcome these barriers for NK cell-based immunotherapy such as the use of allogeneic NK cells, KIR-blocking antibody, and NK cell-boosting cytokines (e.g., IL-15, IL-21) other than IL-2 (Fig. 1) [3, 6, 7]. Numerous studies have shown that allogeneic NK cells in both hematopoietic stem cell transplantation (HSCT) setting and as adoptive cell therapy are safe in terms of graft-versus-host disease and can induce remission or suppress relapse in patients with acute myeloid leukemia (AML) and multiple myeloma (MM). Variegated expression of MHC class I is observed on the surface of AML and MM cells. Thus, donor NK cell alloreactivity has emerged as a promising strategy that removes autologous checkpoint afforded by tumor MHC and creates a situation of "missing self" recognition of tumor cells. In this respect, numerous clinical trials for patients with hematological malignancies are ongoing to validate the feasibility of allogeneic NK cells, especially with haploidentical KIR-MHC mismatch and show some favorable outcome [2, 6]. Another approach that employs similar "missing self" concept is the use of anti-KIR monoclonal antibody (e.g., IPH-2101) that blocks the interaction of inhibitory KIRs on autologous NK cells with their cognate HLA class I. The clinical trials are currently underway for this treatment approach in patients with AML and relapsed/refractory MM. It is useful when there is a problem with appropriate donor selection or side effect related to conditioning regimen. Recent studies also suggested that IL-15 and IL-21 are crucial for NK cell differentiation, maturation, and *ex vivo* expansion, and they do not exert adverse effects associated with *in vivo* IL-2. Thus, their therapeutic benefits are currently being assessed as an alternative to IL-2 for NK cell immunotherapy [7].

Strategies enhancing activation

NK cells do not require recognition of specific antigen on tumor MHC to mediate killing activity. Instead, NK cells have a multiplicity of activating receptors with different ligand specificity and signaling properties: receptors associated with ITAM-bearing molecules (e.g., CD16, NKp30), DAP10-associated receptor NKG2D, receptors of the SLAM family (e.g., 2B4), and other receptors (e.g., DNAM-1) [1]. Given the complexity and heterogeneity of activating ligand expression on tumor cells, it would be promising to target and modulate signaling molecules that limit NK cell activation and are common to multiple NK activating receptors. We recently reported the identification of signaling molecules in this category such as c-Cbl and GSK-3β (Fig. 1) [1, 8]. Certain tumors including acute lymphoblastic leukemia (ALL) have been characterized by the paucity of activating ligand expression for NK cell activation and thus are resistant to NK cell-mediated killing. In such a situation, *in vivo* targeting and activation of NK cells toward tumor cells can be facilitated by the use of therapeutic antibodies directed against tumor antigen [6]. The Fc portion of these antibodies coated on tumor cells engages FcγRIIIA (CD16) on NK cells and thereby elicits strong cytotoxic response through a mechanism termed ADCC. Such ADCC has been appreciated as a critical part in the elimination of tumor cells by several therapeutic antibodies including rituximab and elotuzumab. Another immunoglobulin-based approach to redirect NK cells towards tumor cells is the use of bi-specific and tri-specific killer engagers (BiKEs and TriKEs) [2]. They are designed in a way to bridge between activating receptors (e.g., CD16) on NK cells and tumor-specific antigens (e.g., CD19, CD30, ErbB2). For example, BiKEs are constructed by fusing single

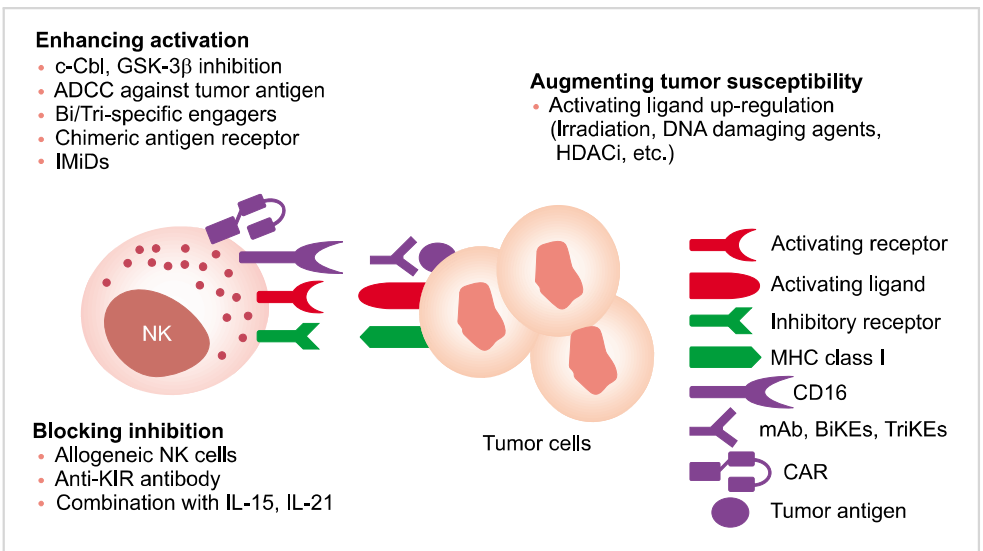

Fig. 1. Strategies aiming at enhancing natural killer (NK) cell reactivity against cancer. Several strategies have been developed to restore or enhance tumor eradication by NK cells: blockade of inhibition, enhancement of activation, or augmentation of tumor susceptibility. These strategies may be combined in a cooperative way to optimize NK cell-based cancer immunotherapy depending on the context.

chain Fv domain specific to CD16 with single chain Fv targeting a tumor-specific antigen and are highly effective at triggering ADCC by NK cells against AML. Recently, the therapeutic potential of chimeric antigen receptor (CAR)-expressing NK cells has been explored as an innovative approach to enable tumor-specific NK cell targeting and killing. NK cells were successfully engineered to express CARs specific for antigens associated with hematological malignancies such as CD19, and CD20 for B-cell lymphoma and CS1 for MM, and exerted efficient killing activity against tumor target in preclinical setting. These results prompted the recent investigation on the feasibility of CAR NK cells in the clinical setting that target CD19 on B-lineage ALL [2]. In addition, tumor killing by CAR NK cells is not CAR-restricted, and also relies on various activating receptors, which is very useful in a situation where targeted antigen on tumor cells is lost or down-regulated. Of interest, some anti-cancer drugs such as lenalidomide and pomalidomide for MM treatment have been shown to stimulate NK cells including ADCC directly or indirectly via T cells. In this regard, they are referred to as immunomodulatory drugs (IMiDs), which have potential benefit to treat cancer in terms of activation of NK and T cells and inhibition of angiogenesis in addition to their direct anti-cancer effect. However, their therapeutic use warrants further investigation for possible adverse effects depending on dose and combination with other drugs such as dexamethasone [9].

Perspectives

Despite significant progress made in the role of NK cells as a key sentinel in tumor surveillance, the mechanisms that control NK cell responses remain elusive. Accordingly, current efforts for NK cell based therapy have largely relied on the strategies that manipulate inhibitory receptor function using allogeneic NK cells and KIR-blocking antibody. Recent advances in understanding the mechanisms of NK cell activation, including those mediated by diverse activating receptors, provide new therapeutic options to enhance NK cell reactivity against cancer. In this regard, strategies that augment sensitization of tumor cells to NK cell-mediated killing such as up-regulation of the activating ligands on tumor cells and modulation of immunosuppressive tumor microenvironment have also been pursued (Fig. 1) [7, 9]. Furthermore, several reliable protocols for GMP-grade NK cell expansion in large scale *ex vivo* have been established, which renders NK cell immunotherapy a feasible treatment modality [10]. As we gain better understanding of the mechanisms governing NK cell reactivity towards cancer, development of an optimally designed cancer immunotherapy by targeting NK cells either alone or in combination with other therapeutic agents will be possible in the near future.

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Author's Diclosure of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. Annu Rev Immunol 2013;31:227-58.
2. Rezvani K, Rouce RH. The application of natural killer cell immunotherapy for the treatment of cancer. Front Immunol 2015;6:578.
3. Chouaib S, Pittari G, Nanbakhsh A, et al. Improving the outcome of leukemia by natural killer cell-based immunotherapeutic strategies. Front Immunol 2014;5:95.
4. Watzl C. How to trigger a killer: modulation of natural killer cell reactivity on many levels. Adv Immunol 2014;124:137-70.
5. Cerboni C, Fionda C, Soriani A, et al. The DNA damage response: A common pathway in the regulation of nkg2d and dnam-1 ligand expression in normal, infected, and cancer cells. Front Immunol 2014;4:508.
6. Pittari G, Filippini P, Gentilcore G, Grivel JC, Rutella S. Revving up natural killer cells and cytokine-induced killer cells against hematological malignancies. Front Immunol 2015;6:230.
7. Chretien AS, Le Roy A, Vey N, et al. Cancer-induced alterations of NK-mediated target recognition: Current and investigational pharmacological strategies aiming at restoring NK-mediated anti-tumor activity. Front Immunol 2014;5:122.
8. Kwon HJ, Kwon SJ, Lee H, et al. NK cell function triggered by multiple activating receptors is negatively regulated by glycogen synthase kinase-3β. Cell Signal 2015;27:1731-41.
9. Mentlik James A, Cohen AD, Campbell KS. Combination immune therapies to enhance anti-tumor responses by NK cells. Front Immunol 2013;4:481.
10. Childs RW, Berg M. Bringing natural killer cells to the clinic: ex vivo manipulation. Hematology Am Soc Hematol Educ Program 2013;2013:234-46.