

Role of natural killer cells for immunotherapy in chronic myeloid leukemia (Review)

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Received January 8, 2019; Accepted February 6, 2019

DOI: 10.3892/or.2019.7059

Abstract. The majority of natural killer (NK) cells serve an important role in eliminating malignant cells. The cytotoxic effects of NK cells were first identified against leukemia cells, and it is now hypothesized that they may have a critical role in leukemia therapy. The cellular functions of NK cells are mediated by their cell surface receptors, which recognize ligands on cancer cells. The role of NK cells is specifically regulated by the activating or inhibitory killer cell immunoglobulin-like receptors (KIRs) on their surface, which bind to the human leukocyte antigen (HLA) class I ligands present on the target cells. The association between KIR and HLA is derived from the diversity of *KIR/HLA* gene profiles present in different individuals, and this determines the cytotoxic effect of NK cells on cancer cells. Chronic myeloid leukemia (CML) is a hematological leukemia characterized by the hyper-proliferation of myeloid cells, with the majority of patients with CML presenting with abnormal immune cells. Tyrosine kinase inhibitors are the present standard therapy for CML, but are associated with numerous adverse side effects. Various studies have proposed CML therapy by immunotherapeutic approaches targeting the immune cells. This review summarizes the contents of NK cells and the association between KIR/HLA and leukemia, especially CML. This is followed by a discussion on the development of NK cell immunotherapy in hematological malignancies and research into strategies to enhance NK cell function for CML treatment.

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1. Natural killer (NK) cells and their markers

NK cells are innate lymphoid immune cells comprising 5-20% of the peripheral blood, and serve an important role in the immune control of cancer (1,2). They are identified by the expression of the surface markers cluster of differentiation (CD)56 and CD16, and the lack of CD3; the CD3⁻ subset therefore indicates T-cell depletion. The two major NK cell subset markers, CD56 and CD16, are present in human cells, and induce different functions in NK cells. The CD56^{bright}CD16⁻ subset has specialized proliferative potential, whereas CD56^{dim}CD16⁺ facilitates the cytolytic activity of NK cells. The higher density of lytic granules in CD56^{dim} cells and the capacity to trigger cytokine production of CD16⁺ cells confers cytotoxic activity on NK cells (1-3) (Fig. 1).

2. Types of NK cell receptors

NK cell activity is adapted by a number of gene families encoding inhibitory and activating receptors. The major histocompatibility complex (MHC) class I-binding or non-binding receptors include two principal types of receptors. The NK cell receptor families belonging to MHC class I-binding receptors are the c-type lectin receptors (CLRs), apart from

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Key words: natural killer cell receptors, killer cell immunoglobulin-like receptor-human leukocyte antigen mismatch, natural killer cell signaling, chronic myeloid leukemia therapy

NKG2-D type II integral membrane protein (NKG2D), leukocyte immunoglobulin-like receptors (LIRs) and killer cell immunoglobulin-like receptors (KIRs). On the other hand, natural cytotoxicity receptors (NCRs) and other NK cell receptors recognize tumor cells independently of MHC class I molecules (1) (Fig. 2).

The CLRs of NK cell receptors structurally contain the c-type lectin domain in their extracellular region. Lectins contain a carbohydrate-binding protein domain, and the c-type form requires calcium in order to bind with proteins. These lectins are diversely functional in their immunopathological reactions in the context of NK cell interactions.

The LIRs are a multigene family located on chromosome 19, including the *KIR* gene family, containing Ig-like extracellular domains in their cytoplasmic tails. Even though LIRs bind to MHC class I molecules, they do not have an important role in NK cell interactions since the binding of LIR to UL18 has a higher affinity compared with the binding of LIR-1 to MHC class I. The LIR uses the same binding site to interact with MHC class I molecules or UL18, an example of which being the human cytomegalovirus genes (1,4-6).

There are two types of KIRs: The inhibitory KIR (iKIR) and activating KIR (aKIR). Structurally, KIRs have two or three immunoglobulin-like domains that are transmembrane glycoproteins. The cytoplasmic tail of iKIR contains the immunoreceptor tyrosine-based inhibitory motif (ITIM), whereas the short cytoplasmic tails of aKIR contain the immunoreceptor tyrosine-based activation motif (ITAM) and have a positively charged residue.

CD56^{dim}CD16⁺ NK cells express high levels of KIRs and CLRs compared with CD56^{bright}CD16⁻ NK cells. Furthermore, CD56^{dim}CD16⁺ NK cells have a higher affinity and recognize target cells bearing MHC class I ligands, thereby exerting greater cytotoxicity compared with CD56^{bright}CD16⁻ NK cells. Therefore, this review focuses on the KIRs of NK cell receptors, due to their increased recognition and interaction with MHC class I molecules on target cells (4).

The NCRs and other NK cell receptors recognize tumor cells irrespective of MHC class I. NCRs are of three types: NKp46, NKp44 and NKp30. NKp30 have a crystal and dimeric structure, NKp44 comprises a typical v-type Ig-fold, whereas NKp46 consists of labeled β strands. However, at present, the NCRs and their ligand interactions are poorly understood (5,7).

3. Fundamentals of NK cell alloreactivity (inhibitory receptors)

Since NK cells spontaneously circulate throughout the human body via the peripheral blood, it is essential to have inhibitory receptors that impede their attack on healthy tissues. The majority of inhibitory receptors recognize the MHC class I molecules present on the surface of cells. Since MHC class I molecules are downregulated in tumor cells or virus-infected cells (unhealthy cells), the NK cells are able to selectively eliminate these unhealthy cells via a process termed 'missing-self' (8) (Fig. 3). CD94/NKG2-A/NKG2-B type II integral membrane protein, the principal inhibitory receptor of NK cells, which binds to the MHC class I molecule HLA-E, is present on target cells (9). The other inhibitory receptors are

primarily iKIRs, which bind to the MHC class I molecules HLA-A, B and C (9,10). Since NK cell receptors are involved in the binding of ligands, it is important to establish strategies that kill tumor cells via NK cell cytotoxicity.

Inhibitory NK cell receptors exert their effect by obstructing the activation of NK cells. The inhibitory NK cell receptors generally express the ITIM, which recruits and phosphorylates Src homology (SH)-containing tyrosine phosphatase (SHP)-1 and SHP-2. Inhibition by ITIM-bearing receptors hampers the tyrosine phosphorylation of essential signaling components, including tyrosine phosphoprotein SLP-76 (SLP-76) and proto-oncogene vav (Vav), to activate signaling in NK cells. However, non-MHC-binding inhibitory receptor mechanisms have various signaling pathways (11-14) (Fig. 4). Specifically, 2B4 of NK cell receptors bind to CD48, which exists on all human hematopoietic cells. 2B4 comprises four tyrosine motifs (TxYxxI/V) in the cytoplasmic tail, and the immunoreceptor tyrosine-based switch motif interacts with SLAM-associated protein. The function of 2B4 has been demonstrated to involve the recruitment of signaling components of other activating receptors (12).

4. Fundamentals of NK cell alloreactivity (activating receptors)

The activation of NK cells requires external signals via their activating receptors that bind to the ligands on target cells. Other important activating receptors include the NKG2 members (excluding A and B), NCRs and aKIRs. NKG2C, E and H bind to the MHC class I molecule HLA-E, whereas NKG2D binds to MHC class I chain-related genes A and B (*MICA* and *MICB*) and UL-16 protein-ligand family 1 and 6 (ULBP1 and ULBP6). aKIR binds to the MHC class I molecules HLA-C or G, and another unknown receptor (3,14-16).

The activating NK receptors possess small transmembrane-anchored adaptor proteins that express ITAM in addition to ITAM-bearing polypeptides (CD3 ζ , FcR γ , DAP10 and DAP12) in their intracellular membrane. ITAM-bearing receptor complexes facilitate the recruitment of tyrosine-protein kinase SYK (Syk) and tyrosine-protein kinase ZAP-70 (ZAP70). Moreover, since CD3 ζ and FcR γ facilitate CD16 signaling in NK cells, the ITAM-bearing receptor complexes phosphorylate src family kinases. The recruitment and phosphorylation of Syk and ZAP70 promote the signals that trigger the activation of SLP-76, SHC-transforming protein 1 (Shc), linker for activation of T-cells family member 1 (LAT), growth factor receptor-bound protein 2 and SH3 domain-binding protein 2, which are linkers for the activation of proto-oncogene Vav and Ras-related C3 botulinum toxin substrate 1. These signals produce downstream events, namely the activation of the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathway (11-14) (Fig. 4).

One example is the NCRs, comprising three major proteins (NKp46, NKp30 and NKp44) that have an adaptor protein, ITAM. Activation of the NCRs is associated with ITAM-bearing receptor complexes that induce signaling pathways such as MAPK and ERK. Other examples are the NKG2 members, which are associated with TYRO protein tyrosine kinase-binding protein/DNAX-activation protein (DAP) 10 or

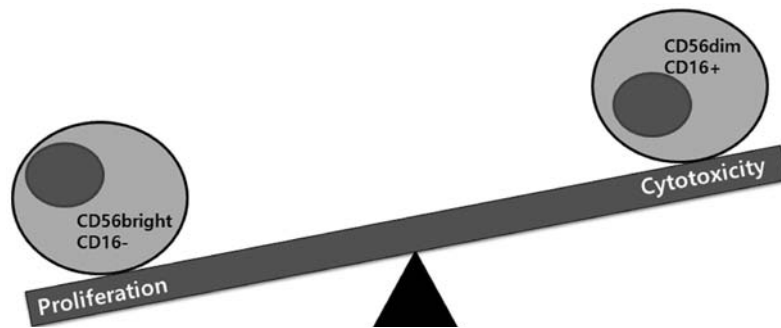


Figure 1. Functions of human CD56^{bright}CD16⁻ and CD56^{dim}CD16⁺ NK cells. The principal NK cell markers that induce different functions in NK cells are CD56 and CD16. The CD56^{bright}CD16⁻ subset has proliferative potential, whereas the CD56^{dim}CD16⁺ subset possesses cytolytic activity. CD, cluster of differentiation; NK, natural killer.

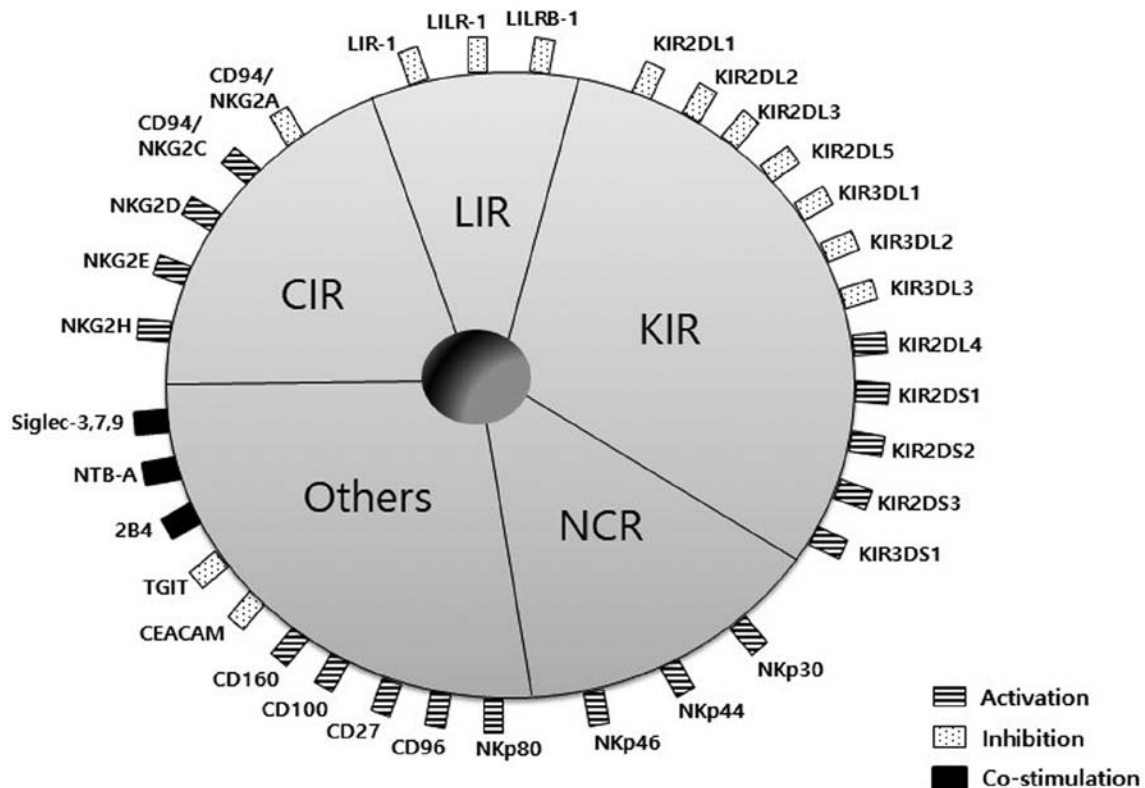


Figure 2. NK cell receptors. The different types of NK cell receptors are presented: CLR, c-type lectin receptor; LIR, leukocyte immunoglobulin-like receptor; KIR, killer cell immunoglobulin-like receptor; NCR, natural cytotoxicity receptor; CD, cluster of differentiation; NK, natural killer.

DAP12 adaptor proteins. In particular, NKG2D is expressed on the NK cell surface and binds to its ligands on target cells (MICA, MICB and ULBP) in humans, resulting in the expression of DAP10 and DAP12. NKG2D is further classified as the short form (NKG2D-S) or the long form (NKG2D-L), in line with the size of its cytoplasmic tail. NKG2D-S binds to either DAP10 or DAP12, but NKG-L is able to bind only to DAP10. In humans, NKG2D pairs only with DAP10 subsequent to the stimulation of the p85 PI3 kinase-associated pathway. In addition, the NKG2D signaling pathway has cross-linking signals that are associated with the activation of the signal transducer and activator of transcription (STAT)5, tyrosine-protein kinase JAK2 (JAK2), ERK, MAPK and phosphatidylinositol 3-kinase/RAC- α serine/threonine-protein kinase signal transduction pathways (11,13).

5. KIRs

Due to their individual diversity, KIRs serve a pivotal role in human NK cells with respect to targeting cells. In terms of gene content, KIR receptors are classified as iKIR (2DL1, 2DL2, 2DL3, 3DL1, 3DL2, 3DL3, and 2DL5A and B) and aKIR (2DL4, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1). The *KIR* gene content defines the KIR haplotype of an individual. KIR haplotypes with distinct gene content are distinguished as 'A haplotype' and 'B haplotype'. The A haplotype exists in all populations conventionally, and has a fixed gene content of 9 genes (3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, 2DS4 and 3DL2). Different B haplotypes exist among different people (2DS1, 2DS2, 2DS3, 2DS5, 2DL2, 2DL1 and 3DS1). It encodes more active KIRs compared with the A

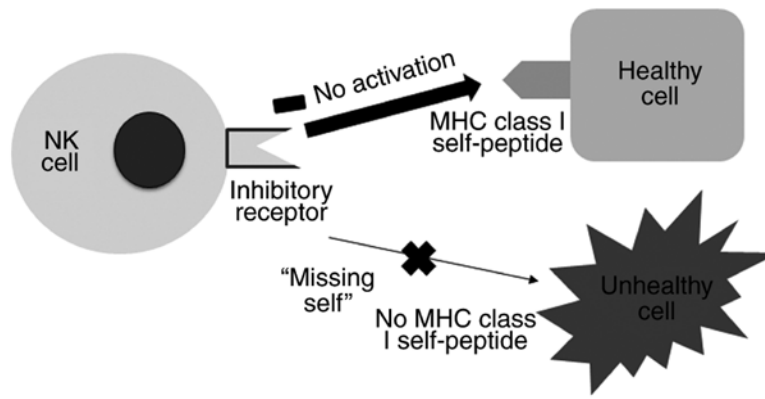


Figure 3. Concept of ‘missing-self’ with respect to NK cells. The ‘missing-self’ concept is a professional killer cell response, allowing NK cells selectively remove only unhealthy cells. NK, natural killer; MHC, major histocompatibility complex.

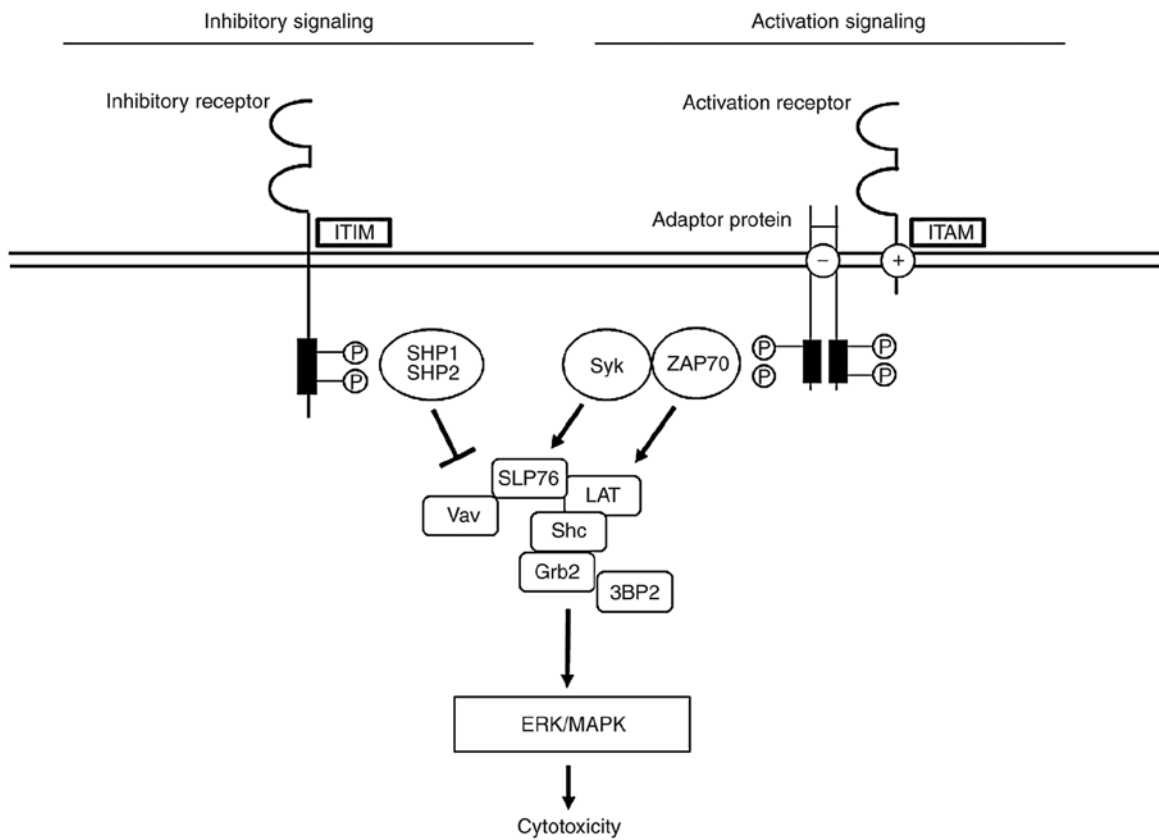


Figure 4. NK cell receptor signaling pathways associated with ITIM- or ITAM-containing adaptor proteins. In these models, the phosphorylation of ITIM leads to the recruitment of SHP-1 and SHP-2, which disturb SLP76 and Vav and are associated with activating signals. On the other hand, the phosphorylation of adaptor proteins with an ITAM triggers the recruitment of Sky and ZAP-70, which activate various signaling molecules, including SLP-76, LAT, Shc, Gb2 and 3BP2. Signals for cytotoxicity result in the activation of downstream activating pathways, via the ERK/MARK pathway. ITIM, immunoreceptor tyrosine-based inhibitory motif; ITAM, immunoreceptor tyrosine-based activation motif; NK, natural killer; SHP, Src homology-containing tyrosine phosphatase; SLP-76, tyrosine phosphoprotein SLP-76; Vav, proto-oncogene vav; Syk, tyrosine-protein kinase SYK; ZAP70, tyrosine-protein kinase ZAP-70; LAT, linker for activation of T-cells family member 1; Shc, SHC-transforming protein 1; Grb2, growth factor receptor-bound protein 2; 3BP2, SH3 domain-binding protein 2; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase.

haplotype (1,17,18). Specifically, iKIRs have a long tail in their intracytoplasmic region, denoted by the letter ‘L’; aKIRs have comparatively shorter tails, indicated by the letter ‘S’. Also, KIR polymorphism is characterized by a different structure, wherein the iKIR contains the ITIM in the cytoplasmic tails, and aKIRs contain positively charged amino acid residues in the transmembrane regions (19).

Although they recognize identical MHC class I alleles, the inherent differences between iKIR and aKIR lead to different outcomes, namely inhibitory or activating responses, respectively. As previously described in this review, iKIR signaling results from the existence of ITIMs that induce inhibitory signaling in connection with SHP-1 and SHP-2. Conversely, aKIR signaling involves ITAMs and may produce

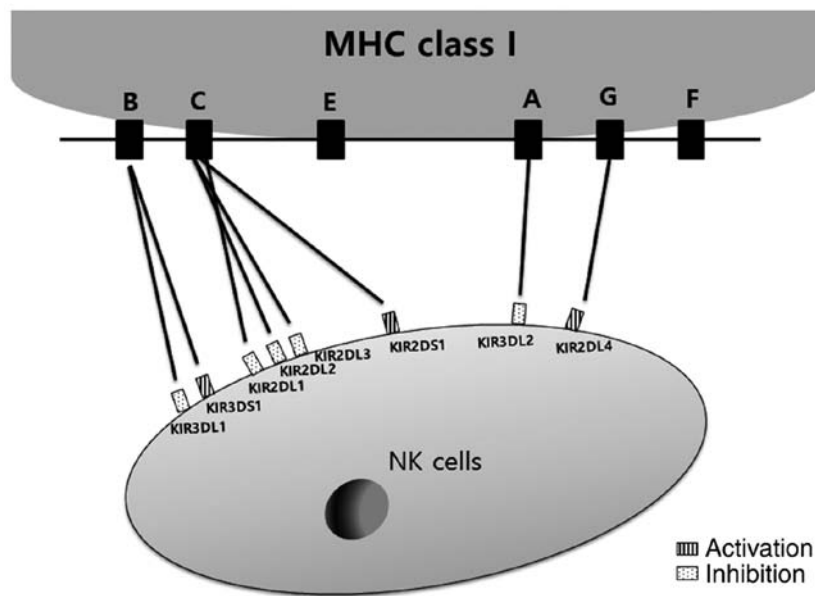


Figure 5. Binding between KIR and specific ligands of MHC class I subunit. Since NK cell receptors have ligand specificity, the NK cell repertoire with diverse combinations of KIRs required further research to investigate matching or mismatching between KIRs and KIR ligands. MHC, major histocompatibility complex; NK, natural killer; KIR, killer cell immunoglobulin-like receptor.

ITAM-bearing receptor complexes that activate signaling molecules, including Syk, ZAP70, SLP-76, Shc and LAT, resulting from the activation of downstream signals, including the MAPK and ERK pathway (4,19) (Fig. 4).

6. Models to determine NK cell KIR alloreactivity for cell therapy

Functional capacity is induced by the interaction between KIR receptors and specific KIR ligands. Inhibitory KIR receptors predominantly bind to distinct MHC class I molecule HLA subtypes, but the ligands for activating KIR receptors are unknown. It has been identified that the activating KIR2DS1 recognizes MHC class I molecule HLA-C subtype ligands (1,18). Thus, the repertoire of NK cells with diverse combinations of KIRs requires more detailed study to investigate KIR-KIR ligand matches or mismatches due to their requirement for type specificity (3,17) (Fig. 5). Notably, an A haplotype KIR-KIRL mismatched donor-recipient has been described in allogeneic hematopoietic transplantation for the treatment of leukemia (20). The process of 'licensing' of NK cells may be beneficial as a graft-versus-host (GVH) effect. This means that the iKIR of licensed NK cells is not recognized by KIR ligands, and the absence of MHC class I molecule HLA subset expression on the leukemia cells of the recipient is able to trigger NK cell alloreactivity. Donor NK cell alloreactivity has a critical role in the GVH effect compared with donor T cell allografts, since the alloreactivity of T cells may negatively affect healthy recipient cells (18,21).

7. Mechanism of NK cell cytotoxicity in cancer

NK cells release various cytokines and acquire certain cytotoxic effects. NK cells recognize tumor cells by their surface

markers or receptors in order to activate NK functions via either of the two tumor-recognizing models: 'Missing-self recognition' and 'stress-induced recognition'. The missing-self recognition model encompasses a loss of the inhibitory effect of NK cells resulting from the absence of MHC class I molecules on tumor cells. In the stress-induced recognition model, damaged proteins on tumors bind to the activating receptors of NK cells and stimulate the cytotoxic functions of NK cells (22-24).

NK cell activation facilitates killing of tumor cells either through direct or indirect means. In the first mechanism, granules are released, including perforin and granzymes, which induce apoptosis in tumor cells (22,25). The second mechanism includes antibody-dependent cell-mediated cytotoxicity, wherein the Fc region of the IgG antibody binds to antigens on the tumor cells in addition to the CD16 receptor on NK cells, forming cross-linking via CD16. The cross-linking of CD16 promotes the granule-dependent mechanism that kills the tumor cells via the activated NK cells by apoptosis (22,26,27). A third mechanism is death receptor-dependent apoptosis. This mechanism involves the Fas ligand and tumor necrosis factor (TNF)-related apoptosis-inducing ligand, which induce caspase-dependent apoptosis in tumor cells (22,25,28). An indirect model of activated NK cells involves the secretion of cytokines and chemokines, including interferon (IFN)- α , TNF- α , C-C motif chemokine (CCL)3, CCL4 and CCL5. A number of cytokines enhance the proliferation and activation of immune cells, such as monocytes, macrophages, dendritic cells (DCs), B-cells and cytotoxic T lymphocytes (CTLs). These cells are cytotoxic towards tumor cells, which die due to the immune response associated with apoptosis and necrosis (22,25,26,29). Further studies are required to focus on NK cell immunotherapy for cancer, since NK cells serve an important role in the immune response and are capable of killing cancer cells.

Table I. Expression of ligands and receptors in patients with hematological cancer.

Author, year	Type of cancer	Expression levels in patients		(Refs.)
		Ligands	Receptors	
Nüchel <i>et al</i> , 2010	CLL	MICA/B, absent; ULBP, absent	-	(30)
Osman <i>et al</i> , 2017	ALL	MICA/B, downregulated; ULBP, downregulated	-	(32)
Osman <i>et al</i> , 2017	AML	DNAM-1, downregulated	NCR, downregulated; NKG2D, downregulated; CD94/NKG2C, downregulated	(32)
Boissel <i>et al</i> , 2006	CML	MICA, upregulated	NKG2D, downregulated	(33)

CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MIC, MHC class I chain-related gene; ULBP, UL-16 protein-ligand family; DNAM-1, CD 226 antigen; NCR, natural cytotoxicity receptor; NKG2D, NKG2-D type II integral membrane protein; CD, cluster of differentiation.

8. Types of leukemia and the therapeutic effect of NK cells

Among hematological cancers, leukemia exhibits rapid proliferation of abnormal white blood cells in the bone marrow. Leukemias are classified into four major types: Acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML). It is important to understand the various leukemia types, since each type requires a different treatment. In leukemia, NK cells exert their potential therapeutic effect by eliminating abnormal leukemic cells due to their cytotoxicity. Various therapeutic options for leukemia have been associated with NK cell receptors (16).

ALL, a cancer of the lymphoid cells characterized by an increased number of lymphocytes, is the most common childhood leukemia. Although ALL is resistant to NK cell cytotoxicity, recent clinical studies have revealed the therapeutic effect of NK cells in pediatric patients (1,17). CLL encompasses the B-cell subtype of lymphocytes (B-CLL) in the bone marrow. Since CLL blasts do not express the MICA, MICB and ULBP ligands, NK cells are capable of eradicating these blasts (30-32). CML involves the constant proliferation of myeloid cells, particularly mature granulocytes, which are clonal bone marrow stem cells. Thus, CML is referred to as a clonal disorder, since the majority of patients with CML bear the distinctive feature of a chromosomal translocation termed Philadelphia chromosome (Ph). Ph is a mutual translocation between the 9th and 22nd chromosomes in all hematopoietic cells; the Abelson (*abl*) gene on chromosome 9 is transferred to the breakpoint cluster region (*bcr*) gene from chromosome 22. This translocation results in an abnormal 'fusion' termed the *bcr/abl* gene. The *bcr/abl* gene develops a tyrosine kinase protein, since the *abl* gene product adds phosphate groups to tyrosine residues (33,34). As a result, CML is a clonal myelo-proliferative disease of abnormal translocation of the *bcr-abl* oncogene, which produces the oncokinase protein BCR-ABL, responsible for the pathogenesis of CML (34,35).

In the search for CML therapies, previous *in vitro* experiments investigated NK-92MI cell line cytotoxicity against the K562 leukemia cell line (36,37). In addition, CML may be treated with tyrosine kinase inhibitors (TKIs) due to the presence of high levels of MICA and low expression of NKG2D (33,36). Therefore, for the effective treatment of cancer using immunotherapy, such as NK cells, it is important to understand the expression of ligands and receptors in patients with hematological cancer, which are summarized in Table I. This review particularly focuses on anti-leukemic effects in CML. Notably, NK cells exert their cytotoxic effect by eliminating CML leukemic cells through lysis.

9. Therapeutic targets in CML

As previously discussed in this review, the *bcr-abl* oncogene is a critical gene in CML (34,35). Thus, novel strategies targeting CML use TKIs, including imatinib mesylate, which provide improved long-term survival of patients (38). In the majority of early studies, researchers proposed that TKIs could be an important therapeutic avenue for CML treatment, although there was limited information about the underlying molecular mechanism. To be specific, statistics indicate that imatinib mesylate is one of the TKIs that induces a complete hematological and cytogenetic response in ~83% of CML patients for 10 years (37,38). However, certain patients with CML are resistant to the therapy due to mutations in the ABL kinase domain; furthermore, the treatment is associated with long-lasting side effects including nausea, cramps and peripheral edema. In addition, TKI therapy involves a high drug cost (39,40). Second-generation TKIs, including dasatinib and nilotinib are under investigation for the treatment of CML. To date, the number of patients with CML who have benefitted from TKI treatment remains unknown (41-44). Therefore, the development of new TKI agents and combination therapies with other treatment strategies is urgently required for TKI therapy. If certain patients with CML exhibit resistance to

Table II. Immunotherapeutic approaches for CML.

Author, year	Pathway or target	Treatment strategy	(Refs.)
Cayssials and Guilhot, 2017; Mahoney <i>et al</i> , 2015	PD-1+ cytotoxic T cells	Monoclonal antibodies	(51,52)
Cayssials and Guilhot, 2017; Mahoney <i>et al</i> , 2015; Mumprecht <i>et al</i> , 2009	PD-L1+ myeloid leukemic cells	PD-1/PD-L1 antagonists	(51-53)
Zhang <i>et al</i> , 2016; Stramucci and Perrotti, 2016; Ågerstam <i>et al</i> , 2016; Arranz <i>et al</i> , 2017; Zhao <i>et al</i> , 2017	IL-1 signaling in CML stem cells	Monoclonal antibodies CAR-T cells	(54-57)
Tarafdar <i>et al</i> , 2017; Levescot <i>et al</i> , 2014; Rocca <i>et al</i> , 2018	JAK/STAT signaling	JAK/STAT inhibitors (e.g. ruxolitinib)	(59-61)

CML, chronic myelogenous leukemia; PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand 1; JAK, tyrosine-protein kinase JAK; STAT, signal transducer and activator of transcription; CAR-T, specific chimeric antigen receptor-modified T; IL-1, interleukin-1.

TKIs, other approaches are required to improve the treatment of CML. A number of patients with CML have exhibited a complete cytogenetic response with no recurrence within 10 years following IFN- α treatment (45). IFN- α inhibits cell proliferation, regardless of the *bcr-abl* oncogene. There remain certain problems associated with this treatment, since certain CML patients receiving IFN- α therapy experience side effects and develop tolerance to IFN- α (39,45).

Treatment with hydroxyurea, leukapheresis and stem cell transplantation are other options in the treatment of CML. Hydroxyurea is generally administered prior to treatment with imatinib mesylate. Nevertheless, hydroxyurea has demonstrated efficacy in patients with CML of up to 90%, but is not commonly utilized in CML therapy for long-term administration due to its inherent cytotoxicity (46-48). Leukapheresis is another treatment applied to reduce the high levels of white blood cells in patients with CML, and stem cell transplantation has also been considered as another treatment option (49,50).

Considering all of the above, developing novel effective treatment strategies for patients with CML is a clear medical necessity. The understanding of tumor immunology has allowed for considerable progress, which has been applied to the immunotherapy of various tumors. Since immunosurveillance is impaired in patients with CML, the lack of innate immune cells including NK cells, B-cells, T-cells and dendritic cells results in decreased quantitative and qualitative functions in the blood (51).

10. Therapeutic targets in CML by immunotherapy

Previous studies have included various immunotherapeutic approaches for CML by targeting the underlying pathways, such as the programmed cell death protein 1 (PD-1)/PD-1 ligand 1 (PDL-1), interleukin (IL)-1 and JAK/STAT pathways (47-52) (Table II).

The PD-1/PDL-1 interaction may act as an immune checkpoint inhibitor, having a marked effect on the anti-tumor response. To facilitate signaling by PD-1, binding of PDL-1 is essential. PD-1 signaling may be involved in the functional effect of infiltrating T cells in tumors. In patients with CML, PD-1 is highly expressed on CML-specific CTLs, whereas the CML cells express PDL-1. Thus, PD-1/PDL-1 interaction leads to the exhaustion and inhibition of CTLs in patients with CML (53). Therefore, blocking PD-1/PDL-1 binding as a treatment strategy represents a promising therapeutic approach for CML (51-53). IL-1 is a regulator of inflammation and the innate immune response, having numerous roles in immunopathological functions. In hematological malignancies, drugs that target IL-1 have an important therapeutic value. Recently, there have been numerous clinical trials in patients focusing on treatments targeting the IL-1 signaling pathway (54,55). In CML, since IL-1 provides resistance to imatinib in CML, the prevention of IL-1 signaling has the potential to increase the efficacy of TKI treatment. Additionally, when using IFN- α treatment in CML, IFN- α inhibits IL-1 due to its anti-inflammatory effect. Hence, a combination therapy with TKIs and IFN- α may be a more efficacious approach for CML patients. Meanwhile, IFN- α -resistant CML patients have high levels of IL-1, which stimulates IFN- α -sensitive CML cells. One way of the blocking IL-1 pathway is using IL-1 receptor accessory (IL1RAP)-specific chimeric antigen receptor-modified T (CAR-T) cells. IL1RAP-CAR-T cells represent an important alternative therapy for patients with CML presenting with TKI and IFN- α resistance (56-58).

The tyrosine kinase JNK has a critical role in myelopoiesis by transducing cytokine signals, and binds to the cytoplasmic domains of various cytokines and growth factor receptors. Once the binding of ligands to their receptors in the extracellular region occurs, JAK is phosphorylated in the intracellular region. Phosphorylated JAK activates downstream substrates

such as the intracellular transcription factor STAT. The JAK-STAT pathway is involved in facilitating myeloproliferation and is abnormally activated in hematopoietic diseases (59,60). Therefore, JAK-STAT inhibitors merit consideration for the treatment of CML. Furthermore, patients with JAK-mutated CML may benefit from TKI treatment, since the JAK-STAT pathway is not normally activated. Thus, randomized trials are required to confirm the potential advantages of the therapy in this cohort (61).

As a novel therapy for CML, NK cells have potential for use in future therapeutic strategies. NK cells are cytotoxic against malignant cells in leukemia, thereby mounting a defense against tumor cells. In leukemic patients, NK cells decline as the disease progresses from the leukemia blast crisis. Also, NK cells isolated from patients with CML exhibit reduced cytotoxicity. Since NK cells exert cellular cytotoxicity and produce an adaptive immune response following the release of various cytokines, they serve an important role in the antitumor immune response (36). Thus, the anti-leukemic function of NK cells is a novel immunotherapeutic approach for CML.

11. Therapeutic targets in CML by NK cells

NK cells exert cytotoxic activity against tumor cells and malignant cells in leukemia. Previous studies have reported that the number and function of NK cells decreases in leukemic patients. The anti-leukemic action of NK cells is relatively low-level in patients with CML (62-64). However, a previous study demonstrated that T cells and NK cells are observed in patients as a consequence of receiving drug treatment (65).

The NKG2D NK cell receptor is activated by recognizing its ligands (NKG2DLs) on malignant cells, provoking the cytotoxic action of NK cells. The majority of tumor cells express NKG2DLs, including MICA, MICB, ULBP1 and ULBP2. MICA ligand is expressed on all leukemia cells and is associated with the immune response and anti-tumor activity. Thus, it is indicated that the NKG2D/MICA interaction has an important role in NK cell cytotoxicity against leukemia (62).

NK cells further influence the treatment of CML when patients with CML are difficult to treat with TKIs. In hematopoietic stem cell transplantation (HSCT), NK cell alloreactivity diminishes the rate of leukemia relapse and protects against an immunological GVH effect. This process is mediated by the 'missing-self' recognition on NK cells, whereas this phenomenon remains incomplete (66,67). Therefore, it is necessary to investigate this process in order to develop an NK cell therapy for CML in the future.

12. NK cell alloreactivity controls the GVH effect

HSCT is also efficacious for myeloid malignancies, which require high doses of chemotherapy (68,69). HSCT exerts its influence by inducing an immunological GVH effect. NK cells are thought to contribute to the GVH effect without directly causing graft-versus-host disease (GVHD) (70). NK cell alloreactivity regulated by their receptors is increased in the absence of HLA ligands. The missing-self represents an inhibitory effect of NK cells, which responds to activating signals to trigger the lysis of tumor targets. In addition, NK

cells work in combination with the adaptive immune response to aggravate GVHD (70,71).

13. Adoptive NK cell therapy in hematopoiesis

In hematopoietic transplants, donors having a KIR-ligand mismatch may be selected, thereby creating an environment in which recipient tumor cells act against alloreactive donor NK cells due to the missing KIR ligand (67,70). The alloreactivity of NK cells relies on the expression of a KIR and its MHC-I ligand in the donor, and the absence of the MHC-I ligand in the recipient. KIR and MHC-I ligand matching displays are summarized in Fig. 5. There are two principal donor-recipient mismatch models. The first mismatch model comprises donor and recipient KIR ligands (ligand-ligand model), and the second model includes recipients having no expression of KIR ligands for donor-inhibitory KIR receptors (missing self-model) (70). In alloreactive NK-cell responses, KIR2DS1 expression offers a striking advantage since it enables the efficient killing of C2/C2 or C1/C2 myelomonocytic dendritic cells and T-cell blasts. The interaction between KIR3DS1 and human leukocyte antigen (HLA)-Bw4 leads to a prognosis of slow disease progression following autologous stem cell transplantation (SCT) in patients with multiple myeloma (71-74). Thus, KIR2DS1 and KIR3DS1 expression in donor NK cells considerably increases the efficacy of the alloreactive NK-cell subset. A previous study reported a higher risk of developing CML due to the prevalence of tolerogenic NK cells, resulting from an increase in inhibitory signals generated by binding of iKIRs to ligands in the homozygous state. Other studies have reported a notable response to first or second generation TKI therapy to increase NK cell activity, mediated at least in part by aKIRs or a mismatch between iKIRs and their respective ligands (74,75). The reason why the presence of active NK cells is paradoxically associated with a disadvantageous therapeutic response in CML remain poorly understood. One study undertook to further investigate the role of KIR receptors and their ligands at the gene level of a homogeneous group of CML patients and controls (76,77). It was reported that KIR-HLA mismatches had a negative impact on the disease course of CML, and that the KIR-ligand combination KIR2DS2/KIR2DL2-absent/HLA-C1-present was markedly reduced in patients with CML (76,77).

14. KIR genes in patients with leukemia

Another study analyzed the KIR genotype in patients with hematological diseases (35). Sequence specific primer-polymerase chain reaction (PCR-SSP) analysis was performed for the amplification of six inhibitory *KIR* genes (KIR2DL1-2DL4 and 3DL1-3DL2) and six activating *KIR* genes (KIR2DS1-S5 and 3DS1). The PCR-SSP analysis determined the KIR genotypes of 54 patients with leukemia, including patients with AML, ALL, CML myelodysplastic syndrome and acute myeloid-lymphoblast leukemia. The results of the present study demonstrated that the frequency of activation of the *KIR* genes 2DS1, 3DS1 and 2DS3 was increased in standard-risk patients with acute AML compared with high-risk patients with acute AML, but there was no association with CML (35).

15. Conclusions

This review has determined that understanding of NK cell functions and signals may improve the treatment of tumors, particularly in CML. Previous and current CML therapeutic strategies were summarized, from TKI treatment to immunotherapy.

Future clinical trials ought to consider that matching or mismatching of KIRs and KIR-ligands (HLAs) may be important for the treatment of CML using immunotherapy. Further research is required to investigate the cellular functions of specific *KIR* genes in patients with CML, and these studies may be applied to the improvement of therapeutic responses in CML.

Acknowledgements

The authors would like to thank the members of Professor Kwang-Hyun Baek's laboratory for their critical comments on the manuscript.

Funding

This study was supported by a grant from the Brain Korea 21 (BK21) PLUS project in Korea (grant no. 22A20130012771).

Availability of data and materials

Not applicable.

Authors' contributions

HRL and KHB performed the literature search and wrote the paper. KHB approved the final version of the manuscript to be published. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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