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Review

Exploring the dynamic of NKG2D/NKG2DL axis: A central regulator of NK cell functions

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Abstract: Natural killer (NK) cells are cytotoxic innate lymphocytes that represent the first line of defense against pathogen infections and tumor growth thanks to their ability to kill cancerous or infected cells and release pro-inflammatory cytokines. NK cell activation is regulated by the expression of a wide array of inhibitory receptors for MHC-I molecules and activating receptors, including NKG2D, which recognize self-ligands upregulated on stressed and damaged cells. Even though the expression of NKG2D ligands (NKG2DL) flags the target cells for NK cell-mediated elimination, a persistent interaction of NKG2D with its ligands promotes receptor downregulation, mainly through the internalization and lysosomal degradation of NKG2D/NKG2DL complexes, thus leading to an exhausted phenotype. On both human and murine NK cells, this phenotype is characterized by the down-modulation of the cytolytic machinery and the upregulation of inhibitory receptors.

In this review, we discuss the current knowledge on the contribution of the NKG2D/NKG2DL axis in both NK cell-mediated clearance of infected and transformed cells and in the dysregulation of NK cell activity due to chronic exposure to NKG2DLs during tumorigenesis.

Keywords: Innate immunity; NK cells; NKG2D; NKG2D ligands; immune surveillance

1. Introduction

Natural killer (NK) cells are cytotoxic innate lymphocytes which share many functional features with $CD8^+$ T cells. However, while T-cell recognition and activation primarily rely on antigen-specific

T-cell receptors (TCRs), NK cells utilize a diverse set of germline-encoded receptors. Their activation is influenced by a balance between inhibitory and activating receptors [1–3].

The crucial inhibitory signal for NK cells comes from MHC class I molecules. Receptors that interact with these molecules include Ly49 receptors in mice and Killer-cell Immunoglobulin-like Receptors (KIRs) in humans, which bind classical MHC-I molecules and CD94/NKG2A heterodimers and recognize HLA-E in human and Qa-1(b) in mouse. Ligand binding to these receptors initiates inhibitory signals that preserve healthy cells from lysis [4,5].

Among the activating receptors, NKG2D (Natural Killer receptor group 2, member D), Natural Cytotoxicity Receptors (NCRs), and DNAX-associated molecule-1 (DNAM-1 or CD226) are key molecules which recognize self-proteins that are either classically absent or expressed at low levels on healthy cells but are upregulated during stress conditions [6–12]. A notable feature of these receptors is their ability to bind multiple ligands. Specifically, human NKG2D ligands (NKG2DL) include MHC-I-related proteins such as MICA/B and ULBP1-6, while murine ligands include retinoic acid early inducible-1 (Rae-1), murine UL16-binding protein-like transcript 1 (MULT-1), and H60 [7,9,11].

DNAM-1 interacts with members of the Nectin/Nectin like family of adhesion molecules, namely Nectin2/CD112 and PVR/CD155, while NCRs, that include NKp46, NKp44, and NKp30, have been shown to recognize a broad spectrum of ligands ranging from viral- and fungal-derived ligands to cellular ligands [10,12,13]. However, the full identification of such ligands remains incomplete.

NKG2D is a C-type lectin receptor expressed not only by NK cells but also by $CD8^+$ αβ T cells, invariant NKT cells (iNKT), and γδT cells [9,11,14–16]. The gene that encodes NKG2D, *KLRK1*, is on human chromosome 12p13.2 flanked by *KLRD1* (CD94) on the centromeric side and by the cluster of *KLRC4* (NKG2F), *KLRC3* (NKG2E), *KLRC2* (NKG2C), and *KLRC1* (NKG2A) genes on the telomeric side. Orthologs of *KLRK1* are present in the genome of all mammals, which indicates that the gene is highly conserved during evolution [17,18].

In early NK precursors, the NKG2D levels are relatively low, but its expression increases over time and is kept high in mature NK cells [19]. Experiments in NKG2D deficient mice revealed an unexpected role of NKG2D in NK cell development [20–22] and showed differences in receptor repertoire, with altered levels of c-kit (CD117), inhibitory Ly49 receptors, and the adhesion molecule DNAM-1 [20,22]. In particular, $Klrk1^{-/-}$ mice were more susceptible to developing tumors that expressed NKG2DLs [23], thus demonstrating a pivotal role for the NKG2D receptor in NK cell-mediated anti-tumoral immune surveillance. Moreover, they showed an increased resistance to cytomegalovirus infections likely due to the hyperreactivity of NCR1 receptor [20,21].

Recently, a new role for this receptor in the recognition and killing of fungal pathogens has been envisaged [24], thus expanding NKG2D roles in immune surveillance.

However, accumulating evidence has demonstrated that a persistent interaction of NKG2D with its ligands, both in the context of infections and tumor transformation, promotes receptor downregulation and contributes to the establishment of an exhausted phenotype in both human and murine NK cells [25,26].

This review recapitulates the contribution of NKG2D in the NK cell-mediated clearance of infected and transformed cells, as well as the functional consequences of chronic exposure to NKG2DLs during infections and tumorigenesis.

2. NKG2D-Triggered signaling pathways

NKG2D is a C-type lectin receptor composed of two disulfide-linked type II transmembrane glycoproteins, each possessing a short intracellular tail that cannot signal independently. Consequently, to initiate intracellular signaling, human NKG2D and a long splice-variant form of mouse NKG2D must associate with the transmembrane adaptor DNAX activating protein 10 (DAP10). Together, they form a hexameric complex composed of one NKG2D homodimer paired with two DAP10 homodimers [27].

DAP10 displays a tyrosine-based motif (YINM) in its cytoplasmic domain, which is critical for recruiting phosphatidylinositol 3-kinase (PI3K) as well as the complex formed by the growth factor receptor–bound protein 2 (Grb2) and the guanine nucleotide exchange factor Vav1 [28–31]. Upon activation, PI3K initiates survival pathways by activating Akt, while the Grb2/Vav1 complex promotes the phosphorylation of Vav1, which, in turn, leads to the activation of Phospholipase C gamma (PLCγ2) and the Src homology 2 (SH2) domain-containing leukocyte protein of 76 kD (SLP-76) [31]. Notably, DAP10 signaling is independent of Syk-family protein tyrosine kinases [30].

Moreover, murine activated NK cells express an alternatively short-spliced isoform of NKG2D that can associate with either DAP10 or DAP12 [32,33]. The latter adaptor contains an immune tyrosine-based activation motif (ITAM) in its cytoplasmic region, which recruits Syk and ZAP70 tyrosine kinases, thus leading to the phosphorylation of PLC and the activation of PI3K; additionally, similarly to DAP10, it is able to engage the Cdc42 family exchange factors, such as Vav2 and Vav3 [34].

All these signals lead to the reorganization of the actin cytoskeleton and the reorientation of the microtubule organizing center (MTOC) to the immunological synapse [35]. This polarization is essential for the delivery toward the abnormal cell of lytic granules which contain perforin—responsible for forming pores in the target cell membrane—and granzymes, which enter the cell to activate apoptotic pathways, ultimately leading to cell destruction [31,36].

In addition to its role in triggering cytotoxicity, the activation of NKG2D on NK cells contributes to the innate immune response by inducing the production of various chemokines and cytokines [32,36].

In mice, DAP10-PI3K signaling is sufficient to trigger NK-cell cytotoxicity against certain tumor cell lines; however, it is not enough to induce cytokine secretion (e.g., interferon-γ) [32,37]. On resting human NK cells, NKG2D often requires additional ITAM-mediated signals derived by other activating receptors, such as 2B4 or NKp46, to trigger degranulation and to initiate cytokine production [38–40].

In parallel with the trigger of these intracellular signals, NKG2D engagement induces clathrin- and dynamin-dependent endocytosis in both murine and human NK cells [41,42], leading to receptor down-regulation [42–44]. In particular, mouse membrane-bound NKG2DLs, including Rae-1 and HL-60, share the ability to induce NKG2D down-modulation with the human counterparts, which leads to the functional impairment of NK cells [45,46].

However, in humans, NKG2D internalization is differentially regulated depending on the ligands involved: engagement with MICA leads to an increased rapid internalization and lysosomal degradation compared to ULBP2 [44]. This difference likely arises because the ubiquitin ligase c-Cbl is required for MICA-induced, but not for ULBP2-induced, NKG2D endocytosis. Specifically, the ubiquitination of DAP10 at Lys84 drives the internalization and sorting of the NKG2D-DAP10 complex to lysosomes for degradation [42]. Interestingly, before being degraded, internalized NKG2D can trigger signals from the endosomal compartment, which is required for the full activation of NK cell effector functions [42].

3. NKG2D as receptor for stress-induced ligands: Clearance of viral infected and transformed cells

The ligands recognized by NKG2D are a diverse group of stress-induced molecules that play a pivotal role in immune surveillance. Under normal conditions, these ligands are expressed at low levels on healthy tissues; however, they are upregulated in response to stressors such as mitosis, viral infection, or transformation into cancerous cells [7–9,11]. Structurally, NKG2DLs can be classified into three general categories. First, MICA and MICB are transmembrane proteins with three domains analogous to the α1–α3 domains of MHC I proteins. In contrast, the other ligands possess only two domains—analogous to the α1 and α2 domains of MHC I proteins—lacking an α3-like domain. Among these, ULBP1–3 and ULBP6 are glycosylphosphatidylinositol (GPI)-linked, while ULBP4–5 are transmembrane proteins. In mice, there are no orthologs of the MICA and MICB genes; instead, a family of genes homologous to the human ULBP/RAET1 family is located on chromosome 10. These murine genes encode proteins that fall into three subgroups of NKG2DLs: five isoforms of RAE-1 proteins, one isoform of MULT1, and three isoforms of H60 proteins (although not all mouse strains express every isoform). Specifically, mouse RAE1α–ε and H60c are GPI-linked, whereas mouse MULT1, H60a, and H60b are transmembrane proteins [7].

NKG2DL expression is primarily regulated at the transcriptional and post-transcriptional levels in response to different kinds of stressing signals [7–9,11,47]. Additionally, variations in the structural characteristics of NKG2DLs—such as whether they are GPI-linked or transmembrane—affect their membrane localization, binding affinity, and their release on soluble forms, thus influencing the overall immune response.

One of the main pathways that leads to the upregulation of NKG2DL expression is the activation of the DNA damage response (DDR) and the first evidence that transformed cells can activate DDR was provided by Gasser and coworkers, who demonstrated that genotoxic stress conditions are responsible for persistent NKG2DL expression [48]. This mainly occurs through members of the phosphatidylinositol 3-kinase-like serine/threonine protein family, ataxia telangiectasia mutated (ATM), ATM- and Rad3-related (ATR), and the DNA-dependent protein kinase (DNA-PK), which enhance the sensitivity of tumor cells to NK cell-mediated lysis [8,48,49]. Furthermore, MICA and MICB expression could be regulated in some conditions by the heat shock stress [50]; however, p53 could also amplify the transcription of certain human NKG2DLs, thus highlighting the regulatory effects of p53 [51,52].

Ligand recognition by NKG2D plays a pivotal role in combating viral infections. This activating receptor could bind to ligands expressed by the infected cells, thus triggering NK cell activation even in the absence of MHC class I molecule downregulation [1,53]. During viral infections, DDR also serves as the primary signaling pathway that drives the transcriptional upregulation of NKG2DL expression [6,8,54,55].

It has been shown that when NK cells fight against HIV-1 infection, NKG2D engagement co-stimulates CD16 signaling, hence enhancing the ADCC-mediated killing of HIV-1-infected cells coated with the antibody recognizing the viral envelope [56]. Moreover, upon HIV-1 infection, the viral protein Vpr activates ATR kinase, which senses DNA damage and stress and causes a G2 cell-cycle arrest [57]. This state leads to the enhanced expression of specific ligands—particularly ULBP-1 and ULBP-2—on primary $CD4^+$ T-cells, while sparing ULBP-3, MIC-A, and MIC-B. Accordingly,

T-cell blasts infected with *Vpr*-deficient HIV-1 exhibit a reduced susceptibility to NK cell-mediated cytotoxicity [58].

Additionally, during the infection of the Human Cytomegalovirus (HCMV), an endemic β-herpesvirus, the immediate early (IE) gene products play crucial roles in regulating viral gene expression activating cellular pathways such as DDR [59,60]. In particular, IE2 directly binds to sequences within the MICA gene promoter, which activates its transcription, although this effect appears limited to specific cell types such as fibroblasts [61]. This may be crucial for the onset of an early host antiviral response.

Additionally, NKG2D plays a pivotal role in tumor immune surveillance, as evidenced by mice deficient for this receptor that demonstrate a reduced ability to fight cancer [23]. Upon ligand engagement, NKG2D initiates intracellular signaling cascades that lead to the release of cytolytic granules containing perforin and granzymes, thus ultimately inducing apoptosis in the tumor cell [28,29,53]. Moreover, NKG2D activation enhances the secretion of pro-inflammatory cytokines and chemokines, which further recruit and activate other immune cells within the tumor microenvironment [7,9,11].

Despite these immune surveillance mechanisms, viruses and tumors have evolved strategies to evade NKG2D-mediated immunity.

3.1. Viral evasion strategies targeting NKG2D-Mediated immune response

Several viral strategies can regulate NKG2DL expression, which dampens NK cell activation and thereby allows the virus to evade immune detection.

Both murine cytomegalovirus (MCMV) and HCMV are the viruses with the most powerful mechanisms to block the functions of NKG2D. Indeed, there is an array of viral molecules that target NKG2DL and impair the recognition and elimination of CMV-infected cells by NK cells [62–68]. In MCMV, different immunoevasins, such as *m145, m152*, and *m155* gene products, specifically downregulate RAE-1 proteins, MULT1, or H-60, respectively [62–65], while HCMV expresses the viral protein UL16 that downmodulates the surface expression of ULBP1, ULBP2, ULBP6, and MICB without down-regulating the remaining NKG2DLs ULBP3, ULBP4, and MICA [69–71]. Instead, the viral protein UL142 downmodulates the expression of ULBP3 and MICA [72,73].

Other viruses may exploit similar or alternative strategies to evade NK cell immune surveillance.

For instance, the HIV-1 Nef protein downmodulates the surface expression of MICA, ULBP1, and ULBP2 [74].

During lytic infection, Kaposi's sarcoma-associated herpesvirus (KSHV) evades NK cell recognition by expressing the viral E3 ligase K5. Particularly, Thomas et al. showed that K5 modifies lysine residues within the cytoplasmic tails of both MICA and MICB with ubiquitin [75]. Consequently, these molecules are internalized from the cell membrane and intracellularly sequestered, affecting the capability of NK cells to recognize and kill infected cells.

Some viruses are also able to alter the expression and/or functions of NKG2D. Among them, the Respiratory Syncytial Virus (RSV) induces the production of high levels of soluble MICA (sMICA). The presence of sMICA can act as a decoy molecule by binding to NKG2D without triggering activation, thus leading to a reduction in the NKG2D activity [76]. In severe pediatric RSV infection, NK cells exhibit diminished NKG2D expression and impaired cytotoxic function [76,77]. Similarly, in SARS-CoV-2 infected individuals with severe COVID-19 disease, a peripheral blood population of

NK cells that show a low expression of NKG2D has been identified, and its presence correlates with a concomitant elevated level of soluble NKG2DLs, especially ULBP2 and ULBP3 [78].

Contrasting results were provided concerning the Epstein–Barr Virus (EBV) modulation of NKG2DLs. In some reports, their expression was described on latently EBV-infected cells; however, in other studies, an upregulation [79,80], a *de novo* expression [81], or even a complete absence [82] in the late lytic cycle have been reported. Moreover, several studies reported the capability of individual EBV proteins or microRNAs to either repress or induce specific NKG2DL expression [83]. Thus, further investigations are needed to better understand whether EBV action on NKG2DLs could compromise the capability of NK cells to control viral infections.

Regarding the regulation of receptor expression, a reduced expression of NK activating receptors, including NKG2D, has been shown by analyzing patients with severe and/or recurrent infection with the Herpes Simplex virus (HSV) [84].

Moreover, during some viral infections, including SARS-Cov-2 infection, a cytokine-mediated downregulation of NKG2D can occur. Indeed, in severe COVID-19 patients, high levels of interleukin-6 (IL-6) in the serum correlate with a reduced NKG2D expression on NK cells [85]. This downregulation contributes to a phenotype in which NK cells are hyperactivated yet functionally impaired. Additionally, a transcriptionally down-modulation of NKG2D expression can occur due to an EBV infection through the production of Kynurenine [86].

The intricate interplay between viral strategies and the NKG2D receptor-ligand axis underscores its critical role in the immune system's ability to counter infections and prevent disease progression, which is summarized in Table 1. By manipulating NKG2DL expression and function, viruses can effectively evade immune surveillance, thus paving the way for persistent infections and associated complications. Targeted therapies designed to either restore the NKG2D activity or enhance the ligand recognition represent a promising avenue to restore immune function and mitigate the impact of on human health.

Table 1. Strategies used by viruses to affect NKG2D/NKG2DL axis on human NK cells.

Virus	Viral Factor	Effects on NKG2D/NKG2DL axis	Consequence on NK Cell Function	References
HIV-1	Vpr	Upregulation of ULBP-1 and ULBP-2 on primary $CD4^+$ T-cells	Enhanced activation and cytotoxicity	[57] Ward (2009); [58] Richard (2010)
	Nef	Down-modulation of MICA, ULBP1 and ULBP2 surface expression	Decreased susceptibility to NK cell-mediated lysis	[74] Cerboni (2007)
HCMV	IE2	Binding to sequences within the MICA gene promoter	Enhanced activation and cytotoxicity	[59] Castillo (2005); [60] Xiaofei (2011); [61] Pignoloni (2016)
	UL16	Down-modulation of MICB expression	Decreased susceptibility to NK cell-mediated lysis	[69] Dunn (2003); [70] Welte (2003); [71] Müller (2010)
	U142	Down-modulation of ULBP3 and MICA expression	Decreased susceptibility to NK cell-mediated lysis	[72] Ashiru (2009); [73] Bennett (2010)

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Virus	Viral Factor	Effects on NKG2D/NKG2DL axis	Consequence on NK Cell Function	References
EBV	Kynurenine-mediated effect	Down-modulation of NKG2D expression	Decreased cytotoxicity	[86] Song (2011)
	Lytic viral factors	Upregulation or absence of NKG2DL expression	Increased or decreased cytotoxicity	[79] Azzi (2014); [80] Desimio (2024); [81] Pappworth (2007); [82] Williams (2015)
RSV SARS-CoV-2		Production of sMICA	Decreased susceptibility to NK cell-mediated lysis	[76] Zdrenghea (2012)
		Production of sULBPs	Decreased susceptibility to NK cell-mediated lysis	[78] Fernàndez-Soto (2024)
KSHV	IL-6-mediated effect	Down-modulation of NKG2D expression	Hyperactivated yet functionally impaired	[85] Osman (2020)
HSV		Reduction of NKG2D expression	Disturbed development and functions	[84] Lenart (2021)

HIV-1: Human Immunodeficiency Virus-1; HCMV: Human CytoMegaloVirus; EBV: Epstein–Barr Virus; RSV: Respiratory Syncytial Virus; SARS-CoV-2: Severe Acute Respiratory Syndrome CoronaVirus 2; KSHV: Kaposi's sarcoma-associated HerpesVirus; HSV: Herpes Simplex Virus.

3.2. Regulation of NKG2D expression and function during tumor transformation

During tumor progression, the chronic exposure of NK cells to NKG2DL-expressing cells induces receptor down-modulation with the consequent impairment of NKG2D-mediated cytotoxic cell functions both on human and murine NK cells [41,44–46,87–91].

In humans, accumulating data have evidenced NKG2D reduced expression on circulating NK cells of patients affected by different kinds of solid cancers including colon adenocarcinomas, breast cancer, pancreatic cancer, and gastric cancer [87,92–94]. In breast cancer patients, analyses of both peripheral blood and tumor-infiltrating NK cells revealed a strong down-modulation of NKG2D expression accompanied by a compromised cytotoxic activity, particularly evident on tumor-infiltrating NK cells, that correlates with an invasive phenotype and poorer prognoses [92]. Additionally, NKG2D down-modulation is evident in haematological malignancies, which also result in impaired NK cell cytotoxic functions [95–97]. This functional impairment is achieved by NKG2D internalization from the plasma membrane and sorting along the endocytic compartments until it reaches lysosomes, where internalized receptor complexes are degraded [42–44].

NK cell activation can also be impaired by a strong reduction of NKG2DLs from the surface of tumor cells. Indeed, during tumor progression, the amount of a determined ligand can be controlled by

various processes responsible for its release as a soluble form, including the protease-mediated cleavage and the exosome secretion [88,90,98–101].

Interestingly, several lines of evidence have demonstrated that the presence of soluble ligands in the sera of patients with different epithelial cancers correlate with disease progression [102–104] and a reduced NKG2D surface expression [87,89,91,95,96], which suggests that soluble NKG2DLs share the ability to regulate receptor expression with their respective membrane-bound counterpart. To this regard, ligands on exosomal membranes show a great ability to induce receptor down-modulation [90,91,101], likely because they can multimerize and bind NKG2D with a high avidity. For instance, exosome-released ULBP3 molecules reduce NKG2D surface expression and compromise NKG2D-mediated NK cell cytotoxic function with a higher efficiency than the metalloproteinase-shed ULBP2 ligands [105]. The ability of exosomal multimeric NKG2DLs to efficiently down-modulate receptor expression can also reflect their ability to induce intracellular signals and elicit selective functional responses [91].

All together, these data confirm the prominent role for NKG2D in cancer immune surveillance and demonstrate that tumors often exploit NKG2D down-modulation to evade NK cell surveillance.

Of note, prolonged NKG2D stimulation and down-modulation can lead to a more general NK cell dysfunction, as depicted in Figure 1; this is characterized by the upregulation of inhibitory receptors and reduced levels of other unrelated activating receptors and effector functions collectively known as NK cell exhaustion (NCE) [106–110].

In mice lacking NKG2D, NK cell prolonged stimulation results in minimal NCE compared with the control mice, thus identifying NKG2D as a crucial mediator of NCE [109]. Moreover, prolonged stimulation of murine NK cells with cells overexpressing the NKG2D ligand H60 impairs NKG2D-dependent cytotoxicity but also affects the functionality of CD3ζ chain-containing receptors CD16, NK1.1, and NKp46 [46]. Similarly, a downregulation of CD3ζ was observed when human NK cells were chronically exposed to MICA, thus rendering them hypo-responsive to CD16, NKp46, and NKp30, but not to 2B4 stimulation [111].

Moreover, *in vitro* experiments showed that the sustained stimulation of NK cells to MICA leads to a defective activation of DNAM-1-triggered signaling and a concomitant increased expression of the inhibitory receptor TIGIT [110].

Accordingly, NK cell exhaustion is evident in post-transplant lymphoproliferative disorder (PTLD), where a reduced expression of NKG2D and NKp46, coupled with elevated PD-1 levels, results in a decreased cytolytic activity and a diminished IFN-γ production [97]. These data suggest a pivotal role for NKG2D in promoting a dysfunctional phenotype that characterizes tumor-infiltrating NK cells (Figure 1).

In conclusion, these studies collectively emphasize the critical role of NK cells in the immune response against cancer; however, their effectiveness can be compromised by various factors within the TME, where NKG2D appears to be particularly susceptible to this dysfunction. Understanding these mechanisms is crucial to develop novel therapeutic strategies to enhance NK cell function and improve cancer immunotherapy. By targeting the factors that contribute to NK cell exhaustion, such as NKG2D signaling and soluble factors in the TME, it may be possible to restore NK cell function and improve the patient outcomes.

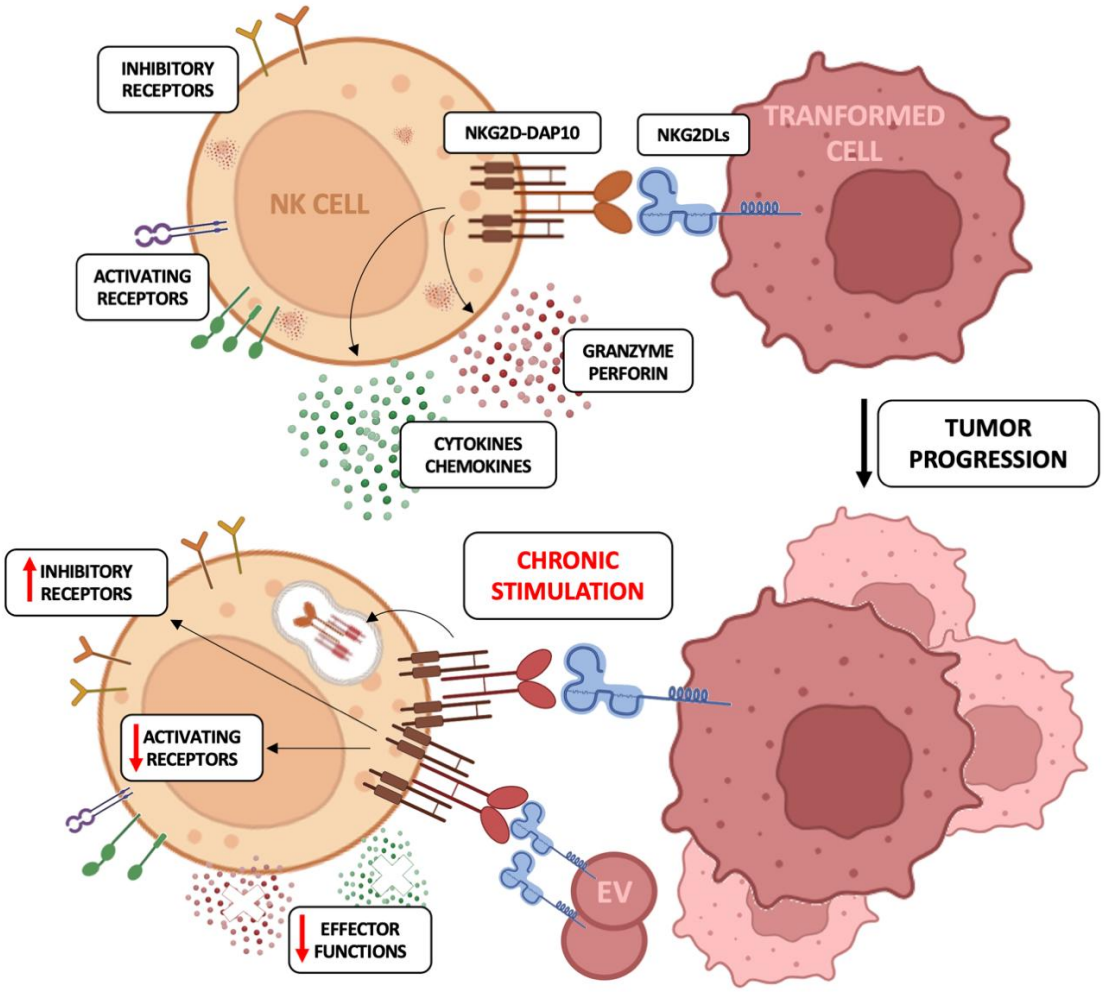

Figure 1. The dual role of NKG2D/NKG2DL in NK cell tumor immunity. Expression of NKG2DLs on tumor cells induces immune surveillance via binding to NKG2D receptors on NK cells. During tumor progression, the chronic exposure to NKG2DLs on tumor cells and on exosomal membrane induces NKG2D down-modulation with a consequent acquisition of a dysfunctional NK cell phenotype. NKG2D, Natural killer group 2, member D; NKG2DLs, NKG2D ligands. This figure was created using https://BioRender.com.

4. Is NKG2D a novel pattern recognition receptor?

NKG2D's function is largely dictated by the nature of its ligands, yet its full repertoire remains incompletely understood. Although established ligands such as MICA, MICB, and ULBPs have been extensively studied in humans, emerging evidence suggests the existence of unidentified ligands that could broaden the scope of NKG2D-mediated immune responses.

In certain noncanonical contexts, particularly in fungal infections, NKG2D has been proposed to act as a pattern recognition receptor (PRR) [24]. In this capacity, it directly recognizes fungal cells, most likely through conserved fungal carbohydrates, initiates immune activation, and facilitates fungal killing by both human and murine NK cells. Through this mechanism, NKG2D can target a broad range of fungal pathogens, including clinically significant species such as *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.

However, despite the receptor's activation during fungal infections, no specific fungal-derived ligands for NKG2D have been conclusively identified, thus leaving a critical gap in understanding how NKG2D recognizes and responds to fungal pathogens. This lack of knowledge highlights the possibility that unknown host-encoded ligands might be induced or modified in response to fungal infections. Alternatively, fungal pathogens might trigger atypical activation of NKG2D through indirect mechanisms, such as stress-related signals or the release of damage-associated molecular patterns from host cells.

The interaction between NKG2D and fungal ligands can have different consequences on NK cells functions. Santiago et al. showed that NK cells form strong adhesions with *Aspergillus fumigatus*, which is accompanied by F-actin accumulation at the immune synapse and granule polarization [112]. Despite these signals leading to activation, the contact with *Aspergillus fumigatus* ultimately promotes an exhausted NK cell phenotype marked by the diminished expression of activating receptors including NKG2D [112]. Moreover, *Aspergillus fumigatus* compromises NK cell responses against leukemic cells, thus potentially exacerbating immune surveillance deficiencies in leukemia patients with pulmonary aspergillosis [112]. The induction of NKG2D dysfunction may vary depending on the specific pathogen. Indeed, a previous study has indicated that the expression of NKG2D remains unchanged during *Candida albicans* infections [113].

The dual roles of NKG2D as a fungal PRR and an activator of NK cell-mediated cytotoxicity against compromised cells highlight the critical function for NKG2D in innate immunity. However, NK cells dysfunction in consequence of persistent fungal infections, such as those caused by *Aspergillus fumigatus*, underscores the complexity of host-pathogen interactions. Utilizing these findings to create therapeutic approaches aimed at enhancing NKG2D activity could provide significant advancements in controlling fungal infections and related immune dysfunctions.

5. Conclusions

The intricate regulation of NKG2D underscores its pivotal role in the immune system. NKG2D activation is indispensable for targeting infected or transformed cells; however, its chronic engagement could lead to NKG2D downregulation and dysfunction.

In viral infections, pathogens could exploit the NKG2D axis to escape immune detection. On the other hand, in cancer, the TME profoundly affects NKG2D expression and NK cells, often inducing a dysfunctional phenotype which hampers the NK cell ability to effectively target the tumor cells.

Moreover, emerging research revealed NKG2D's role as a fungal PRR, thus enabling NK cells to recognize and eliminate fungal pathogens. This function is vital for immunocompromised individuals susceptible to invasive fungal infections. However, a chronic exposure to fungal ligands can induce NK cell exhaustion, thus reducing antifungal efficacy.

A deeper understanding of NKG2D biology could provide a foundation for innovative therapeutic strategies. These approaches might include fine-tuning the receptor signaling pathways, developing drugs to prevent exhaustion, or engineering ligands to enhance recognition and response. However, several critical questions remain: How can NKG2D signaling be modulated without disrupting its roles in immune responses? What are the precise mechanisms by which chronic stimulation leads to exhaustion, and can these be reversed or prevented? Additionally, could engineering NKG2D ligands improve specificity and minimize off-target effects? Transforming these insights into therapies could unlock the full potential of NKG2D, thus ensuring a robust NK cell functionality across diverse

diseases. Such advancements hold promises for more effective treatments in cancer, infectious diseases, and immune disorders, thus positioning NKG2D as a pivotal target in the future of immunotherapies.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Author contributions

All authors contributed to write the manuscript and prepared the figures.
All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

All authors declare that they have no conflict of interest in this paper.

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