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A potential mechanism of tumor immune escape: Regulation and application of soluble natural killer group 2 member D ligands (Review)

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Abstract. The immune system is integral to the surveillance and eradication of tumor cells. Interactions between the natural killer group 2 member D (NKG2D) receptor and its ligands (NKG2DLs) are vital for activating NKG2D receptor-positive immune cells, such as natural killer cells. This activation enables these cells to identify and destroy tumor cells presenting with NKG2DLs, which is an essential aspect of tumor immunity. However, tumor immune escape is facilitated by soluble NKG2DL (sNKG2DL) shed from the surface of tumor cells. The production of sNKG2DL is predominantly regulated by metalloproteinases [a disintegrin and metalloproteinases (ADAM) and matrix metalloproteinase (MMP) families] and exosomes. sNKG2DL not only diminish immune recognition on the tumor cell surface but also suppress the function of immune cells, such as NK cells, and reduce the expression of the NKG2D receptor. This process promotes immune evasion, progression, and metastasis of tumors. In this review, an in-depth summary of the mechanisms and factors that influence sNKG2DL production and their contribution to immune suppression within the tumor microenvironment are provided. Furthermore, due to the significant link between sNKG2DLs and tumor progression and metastasis, they have great potential as novel biomarkers. Detectable via liquid biopsies, sNKG2DLs could assess tumor malignancy and prognosis, and act as pivotal targets for immunotherapy. This could lead to the discovery of new drugs or the enhancement of existing treatments. Thus, the application of sNKG2DLs in clinical oncology was explored, offering substantial theoretical support for the development of innovative immunotherapeutic strategies for sNKG2DLs.

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1. Introduction

The immune system plays an integral role in the recognition and elimination of tumor cells, and the natural killer (NK) group 2 member D (NKG2D) receptor is a pivotal player in this intricate cellular interaction network (1,2). In humans, NKG2D is expressed on NK cells and some T cells, such as NKT cells, γδT cells, and $CD8^+$ T cells. It is a type II transmembrane receptor belonging to the C-type lectin-like dimeric family (1). Binding of the NKG2D receptor to its ligand (NKG2DL) activates NK cells, resulting in the release of cytotoxic granules containing perforin and granzymes, which induce target cell lysis (3). In addition, it induces the secretion of various cytokines and chemokines, amplifies specific immune responses, and exerts antiviral and antitumor effects (4). NKG2DL also acts as a costimulatory molecule that promotes T-cell activation.

In the human genome, NKG2DLs are major histocompatibility complex (MHC) class I polypeptide-related sequence A/B (MICA/B) and UL16 binding proteins 1-6 (5,6). These are also termed retinoic acid early transcript 1 (RAET1) and are homologous to the mouse ligands of the NKG2D receptor Raet1a-d. The correspondences are as follows: RAET1I=ULBP1,

RAET1H=ULBP2, RAET1N=ULBP3, RAET1E=ULBP4, RAET1G=ULBP5, and RAET1L=ULBP6 (5). MICA/B is highly polymorphic, with 280 MICA and 47 MICB protein sequences recorded to date [IPD-IMGT/HLA Database (ebi.ac.uk)], and these differing polymorphisms may influence their affinity for binding to NKG2D, subsequently affecting the NKG2D receptor/NKG2DL axis and altering NK cell activity. This rich MICA/B polymorphism plays a significant role in organ transplantation, immune system diseases, and tumor immunity (6). These ligands are markedly upregulated in stressed cells, tumor cells, and cells infected with viruses or bacteria, thereby facilitating immune system activation (7).

NK and $CD8^+$T cells complement each other in tumor immune responses. MHC class I molecules are often underexpressed or not expressed in tumor cells, making it impossible to activate the cytotoxic functions of $CD8^+$T cells (8,9). However, their absence can enhance NK cell activation. Nevertheless, tumor cells can simultaneously downregulate both MHC-I molecules and NKG2DL expression, thereby diminishing the immune surveillance capabilities of NK cells and leading to tumor immune evasion (10). One method to achieve this is to convert membrane-bound NKG2DL into soluble NKG2DL (sNKG2DL) molecules (5). This not only reduces the amount of NKG2DL on the tumor cell surface, competitively obstructing immune cell recognition of membrane-bound NKG2DL, but also promotes internalization of the NKG2D receptor (11). This hinders the recognition of cancer cells by NK and $CD8^+$T cells, thereby facilitating tumor progression and metastasis (11). By understanding the release mechanisms of these soluble forms in depth, new therapeutic strategies can be identified to bolster immune cell recognition and eradication of tumor cells.

Therefore, understanding the specific roles and regulatory mechanisms of sNKG2DLs in tumor immune evasion is paramount for both basic research and clinical translation. The present study aimed to elucidate the mechanisms underlying the production of sNKG2DL and its influencing factors. The role of sNKG2DL in tumorigenesis, progression, and immune evasion, with a keen focus on its mechanism of action and potential as a therapeutic target is comprehensively analyzed. In addition, its applications in oncological clinical diagnosis and immune-related treatments are explored, offering new perspectives for research on the NKG2DL family and the development of clinical treatment strategies.

2. Regulation of NKG2DL expression in tumor cells at multiple levels

Under physiological conditions, MICA/B is primarily expressed in gastrointestinal epithelial cells, potentially owing to bacterial stimulation. This is crucial for activating intestinal γδ T cells, promoting gut immunity (12). RT-PCR has revealed ULBP transcripts in various tissues, including the heart, brain, lungs, liver, testes, lymph nodes, thymus, tonsils and bone marrow (13).

Under cellular stress, NKG2DL is expressed in various tissues and tumor cells. Heat shock treatment can significantly enhance the expression of MICA and MICB on intestinal epithelial cells and can boost the lytic ability of γδ T cells (12,14).

In tumor cells, with the overactivation of oncogenes and strong proliferation signals, there can be DNA damage responses (1). Hypoxia in the tumor environment can also induce heat shock responses (11). These stress reactions can regulate NKG2DL expression at multiple levels, leading to its expression on the cell surface and mediating the killing action of NK cells (11).

Upstream of the MICA and MICB initiation codons, there are sequences that can bind to transcription factors, heat shock factor 1 and Sp1, responding to proliferative signals and participating in heat shock responses (15). DNA damage and replication stress can activate sensors, such as ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR), further activating downstream p53. Within *ULBP1* and *ULBP2* genes, there are p53 response elements (16,17) that can bind to p53 and increase transcription. ATM and ATR also promote MICA transcription by activating NF-κB (18,19). Research has revealed that although most tissue cells do not express NKG2DL under healthy conditions, most do transcribe MICA and MICB (20), indicating that post-transcriptional regulation is of significant importance for MICA/B. Various miRNAs that target NKG2DL have been identified. Stern-Ginossar *et al* (21) found constitutively expressed miRNAs in cells and proposed that when the stress-induced transcription of MICA/B surpasses the level that miRNAs can silence, MICA/B is expressed on the cell surface. There are also inducible miRNAs; for instance, interferon gamma (IFN-γ) can downregulate MICA expression by inducing miR-520b (22), and p53 can induce miR-34 a/c targeting ULBP2 (23). This inhibition may serve as a mechanism for preventing excessive NKG2DL expression. As aforementioned, while ATM and ATR can promote the expression of MICA/B and ULBP, they can also downregulate their synthesis by inducing the expression of miRNA through activating IFN-γ and p53.

As tumors progress, the expression of NKG2DL on the cell surface is downregulated, facilitating immune evasion. At the transcriptional level, NKG2DL mRNA synthesis is regulated by epigenetic modifications. Overexpression of histone deacetylases (HDACs) is observed in tumor tissues. The use of HDAC inhibitors enhances the transcription of NKG2DL and increases the cytotoxicity of NK cells by augmenting acetylation of histone H3 at the promoters of MICA and MICB (24). MUC1-C, a pivotal molecule in oncogenesis triggered by chronic inflammation, suppresses the transcription of MICA and MICB by promoting DNA methylation and histone H3 lysine 27 (H3K27) methylation at their promoter regions (25). Gliomas with isocitrate dehydrogenase (IDH) mutations produce the oncometabolite 2-hydroxyglutarate, which inhibits the expression of various NKG2DLs by facilitating DNA methylation (26). Additionally, transforming growth factor-beta (TGF-β) selectively reduces the mRNA levels of MICA, ULBP2 and ULBP4 in gliomas. At the post-transcriptional level, several miRNAs, including miR-34a/c, miR-10b, miR-20a, miR-93, miR-106b and miR-519a-3p, actively contribute to the downregulation of NKG2DL expression (23,27-29). IFN-γ induces miR-520b, leading to the downregulation of MICA mRNA across multiple tumor cell lines (22). Following translation, NKG2DL is transported to the cell membrane, where its soluble forms, produced by

shedding from the cell surface, contribute to immune suppression, as elaborated further below.

3. Mechanism of conversion of NKG2DL from membrane protein to a soluble form

NKG2DLs are converted into soluble molecules via three main pathways: Metalloprotease-mediated proteolytic shedding, lipid raft-mediated exosome release, and RNA selective splicing (2,5). The principal release mechanism for each NKG2DL is influenced by their molecular structure differences. MICA/B is structurally similar to human MHC class I molecules, featuring three extracellular domains, α1, α2 and α3, alongside a transmembrane region and a cytoplasmic tail (6). However, their α1 and α2 do not bind to antigenic peptides and lack the β2m microglobulin. The ULBP family, on the other hand, has only two domains, α1 and α2. ULBP1-3 and ULBP6 do not have a transmembrane domain and are anchored to the plasma membrane via a GPI-anchor, while ULBP4 and ULBP5, similar to MICA/B, possess both a transmembrane region and a cytoplasmic tail (1). MICA/B and ULBP2 are more susceptible to degradation by metalloproteases due to their molecular structures (6). ULBP3, being a GPI-anchored protein, is primarily released into exosomes through lipid rafts. Research on ULBP4 and ULBP5 has primarily focused on RNA selective splicing (30). Currently, there is no evidence indicating the primary release mode of ULBP1. Only trace amounts of soluble ULBP1 (sULBP1) have been detected. This may be due to the short half-life of ULBP1 on the cell membrane, which is rapidly internalized and degraded rather than being released via exosomes (31). Similarly, MICB has a short half-life in the plasma membrane and is rapidly internalized and degraded (32).

Proteolytic shedding of NKG2DL membrane proteins mediated by metalloproteases. ADAM and MMP are zinc-dependent metalloproteinases belonging to the metzincin superfamily. These are the main substances involved in the shedding of NKG2DL (Fig. 1). The catalytic domains of these two enzymes have features conserved within the zincin enzyme family. The first is a catalytic sequence, HEXXHXXGXXH/D, that binds zinc ions, where three histidine residues coordinate with the zinc ion, and the substrate forms a fourth coordination bond. This is followed by a 1,4-β turn containing glutamic acid (Met-turn) that helps stabilize the catalytic process (33). Humans express 21 ADAMs, of which 13 are catalytically active. ADAM 9, 10, 12, 15, 17 and 19 are widely expressed in somatic cells and hydrolyze various extracellular regions of membrane proteins (34). MMPs are mainly secreted from cells, degrade various proteins in the extracellular matrix, and participate in tissue remodeling. Membrane-bound MMPs (MT-MMP) are anchored to the plasma membrane via transmembrane or GPI-anchoring domains (35).

ADAM10 and ADAM17 [also known as tumor necrosis factor alpha (TNF-α) converting enzyme] are involved in the shedding process of MICA from the plasma membrane. Under stress conditions, such as malignancy, the expression of MICA increases and endoplasmic reticulum protein 5 (ERp5) translocates from the endoplasmic reticulum to the plasma membrane surface, thereby activating ADAM. In the α3 domain of MICA, there is a conserved 6-peptide sequence between two cysteine residues, allowing ERp5 to form a complex with MICA. Through this interaction, a transient disulfide bond is formed, leading to conformational changes (36,37). This structural change allows ADAM10 and ADAM17 to approach MICA, and catalyze the hydrolysis between the α3 domain and transmembrane region of MICA, leading to its shedding from the plasma membrane, resulting in soluble MICA (sMICA). Studies on the carboxyl-terminus of sMICA have revealed that the ADAM cleavage site is not fixed, and the efficiency of ADAM-induced shedding is positively correlated with the length of the stalk region (38).

Hydrolysis of NKG2DL by the metalloproteinases ADAM and MMP has been widely studied. Previous research indicates that ADAM10 and ADAM17 have shedding effects on various NKG2DLs, including MICA, MICB and ULBP2. By contrast, the hydrolytic activity of ULBP3 is low and there is no definitive evidence that metalloproteinases directly participate in ULBP1 hydrolysis (38-41). Numerous studies have shown that ADAM and MMP can cause NKG2DL shedding in various tumors; however, most experiments have used broad-spectrum metalloproteinase inhibitors without careful differentiation (34,38-41).

Tissue inhibitors of metalloproteinases (TIMPs) are endogenously synthesized metalloproteinase inhibitors. Of note, four TIMPs (TIMP1, TIMP2, TIMP3, and TIMP4) inhibit the activities of MMP and ADAM. Increased TIMP3 expression reduces the activity of ADAM17, thereby reducing the release of sMICA, sMICB and sULBP2 (42).

Recruitment of NKG2DL to lipid rafts and release into exosomes. The transfer of NKG2DL to exosomes and its release into the serum is another way it is shed from the plasma membrane (Fig. 1). Detergent-resistant membrane domains (DRMs) are membrane microdomains on the plasma membrane that cannot be fully dissolved using nonionic detergents, such as Triton X-100, at low temperatures. They are also rich in cholesterol and sphingolipids. GPI-anchored proteins (GPI-APs) are highly enriched in these areas. When cells are stimulated, these regions form large, stable lipid rafts that facilitate protein interactions and signal transduction on the membrane (43). the composition of exosomes is related to lipid rafts as they have similar lipid components and are enriched in GPI-APs (44). Additionally, due to mutations in the transmembrane region of MICA*008, its tail is truncated and acquires a GPI-anchoring sequence, allowing its release through exosomes (45). MICA, MICB and ULBPs have also been observed in exosomes (41,46). However, only ULBPs are GPI-anchored, whereas MICA and MICB have a transmembrane region and a cytoplasmic tail.

Transmembrane proteins, such as MICA, MICB and ADAM17, also accumulate in lipid rafts. In these areas, ADAM17 has high hydrolytic activity on various membrane proteins. Palmitoylation of cysteine residues in the cytoplasmic tails of MICA and MICB signals their location in DRMs, and inhibiting palmitoylation effectively inhibits shedding (40,47). However, this inhibitory effect was incomplete, indicating that shedding still occurred outside the DRMs.

Typically, the lipid bilayer in lipid raft regions is thicker than that in other areas of the plasma membrane. Hence, transmembrane proteins located in the lipid rafts may

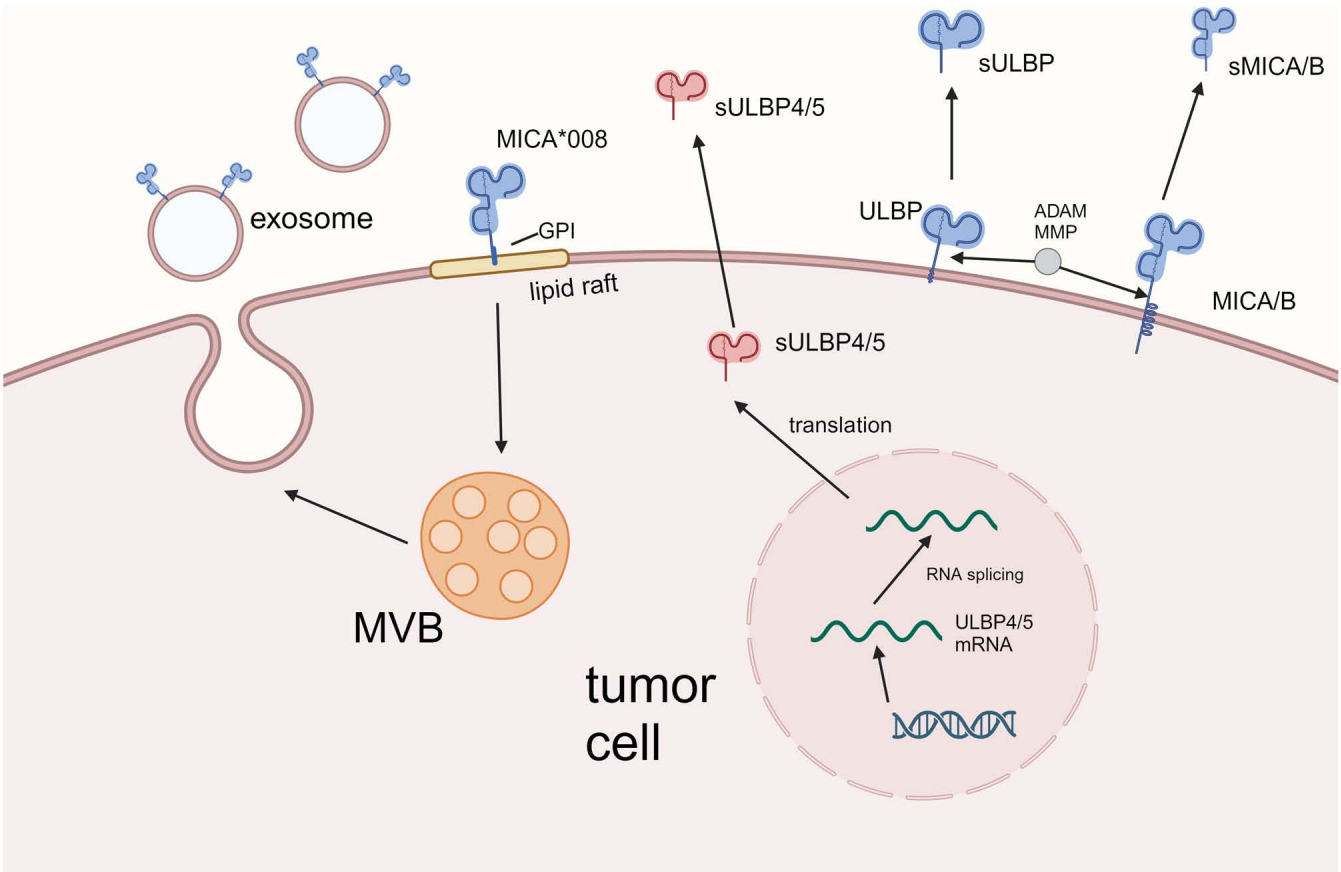

Figure 1. Mechanism for generating sNKG2DL. ADAM and MMP shed NKG2DL on the tumor cell membrane to produce sNKG2DL. The transmembrane region of MICA*008 disappears and is replaced by GPI, which has a higher affinity for lipid rafts and is released through exosomes. The mRNA of ULBP4 and ULBP5 are selectively spliced in the nucleus and translated into sULBP whose transmembrane region disappears. sNKG2DL, soluble natural killer group 2 member D ligand; ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; MICA, MHC class I polypeptide-related sequence A; GPI, glycosylphosphatidylinositol; ULBP, UL16 binding protein; sULBP, soluble ULBP; MVB, multivesicular body.

encounter hydrophobic mismatches. This phenomenon was observed experimentally. Although both MICA and ULBP1-3 co-localized with lipid raft regions, the association of MICA with DRMs was much lower than that of ULBP3 (48).

Uniquely, despite ULBP2 being a GPI-AP, it predominantly becomes soluble through metalloprotease-mediated proteolysis. ULBP2 is more susceptible to metalloprotease attack compared with ULBP3, and the presence of metalloprotease inhibitors significantly increases ULBP2 in exosomes while reducing it on the cell surface (41).

RNA selective splicing causes NKG2DL to lose its transmembrane region and be released extracellularly. ULBP4 (RAET1E) and ULBP5 (RAET1G) are members of the RAET1 family that contain transmembrane regions. In 2004, Bacon *et al* (49), while studying RAET1G, discovered alternative splicing that led to truncation of the transmembrane region, predicting that this would turn the molecule into a soluble form, and named it RAET1G2. Subsequently, in 2009, research confirmed that both RAET1G and RAET1G2 were expressed in various tumor cells. Truncated soluble RAET1G2 can reduce the expression NKG2D in NK cells, leading to immunosuppression (30). In 2007, Cao *et al* (50) discovered an alternative splice form of RAET1E and named it RAET1E2, which similarly results in the loss of the transmembrane region, becoming a soluble molecule (Fig. 1) and reducing the cytotoxicity of NK cells.

4. Main regulatory factors in the production of sNKG2DL

The transformation of NKG2DL into its soluble ligand is a complex process governed by a multitude of factors. ADAM and MMP, two key enzymes, play crucial roles in the ligand-shedding process and represent critical regulatory points. Subsequently, the factors influencing the shedding and release of NKG2DL in tumor cells are presented (Table I).

Polymorphism of MICA gene affects the production of sMICA. Being the most polymorphic member of the non-classical MHC class I gene family, the polymorphism of the *MICA* gene not only affects the quantity of MICA on the cell membrane and its affinity for the NKG2D receptor, but also influences the shedding of MICA (51). Exon 5, which encodes the MICA transmembrane region, contains short tandem repeat sequences (GCT) that encode for alanine (52). The *MICA-A5.1* gene (also known as *MICA*008*) has a purine insertion after the second GCT, leading to a frameshift mutation and a premature stop codon, giving *MICA-A5.1* a GPI anchor point and the shortest transmembrane domain compared with that of other MICA proteins (52). Although the shorter transmembrane domain

Table I. Regulatory factors and mechanisms of sNKG2DL.

	Regulatory factors	Mechanisms	Metalloproteinases	Ligands	Effects	(Refs.)
Polymorphism	MICA-A5.1	GPI anchor	-	sMICA↑	n.d.	(52,53)
	SNP rs1051792	Increased expression of MICA	-		NKG2D on NK cells↓	(51,55)
	SNP rs2596542				n.d.	(56)
Stress	Hypoxia stress	HIF	ADAM10	sMICA↑, sMICB↑	NK cytotoxicity↓, NKG2D on NK cells↓	(57,58)
	Genotoxic stress	Cellular senescence	ADAM9, ADAM10	sMICA↑, sMICB↑	NK cytotoxicity↓, NKG2D on NK cells↓	(60)
TME Cells	CAF	Secreted MMP	MMP2, MMP9	sMICA↑, sMICB↑	NK cytotoxicity↓, MICA/B on tumor cells↓	(62)
	Platelet	n.d.	ADAM10, ADAM17	sMICA↑, sMICB↑, sULBP1↑, sULBP3↑	NK cytotoxicity↓	(63)
	LN MSC	n.d.	ERp5↑, ADAM10↑	sMICA↑, sULBP3↓	T cytotoxicity↓, NKG2D on T cells↓	(64)
Molecules	IL-1β	n.d.	ADAM9	sMICA↑	NK cytotoxicity↓	(66)
	TGF-β	n.d.	ADAM17	sMICA↑, sULBP2↑	T cytotoxicity↓	(67,68)
	IDO1	IDO1-Kyn-AhR	ADAM10	sMICA↑	NK cytotoxicity↓	(69)
	MUC1-C	ERp5 RAB27A	ADAM10, ADAM17	sMICA↑, sMICB↑	NK cytotoxicity↓	(25)

sNKG2DL, soluble natural killer group 2 member D ligand; MICA, MHC class I polypeptide-related sequence A; GPI, glycosylphosphatidylinositol; sMICA, soluble MICA; n.d., not determined; ↑up; ↓down; SNP, single nucleotide polymorphism; NK, natural killer; HIF, hypoxia-inducible factor; ADAM, a disintegrin and metalloproteinase; CAF, cancer-associated fibroblast; MMP, matrix metalloproteinase; LN MSC, lymph node mesenchymal stem cells; ERp5, endoplasmic reticulum protein 5; TGF-β, transforming growth factor-beta IDO1, indoleamine 2,3-dioxygenase 1.

reduces the sensitivity of MICA-A5.1 to MMP (52), the GPI anchor prioritizes embedding into lipid rafts, thus recruiting MICA-A5.1 to exosomes and releasing more sMICA through the exosomes (53). MMP inhibitors, while inhibiting hydrolytic shedding of MICA, promote the exosome pathway, leading to the release of more sMICA (54). This indicates that drugs that suppress MICA shedding (such as MMP inhibitors) may not be as effective in patients with MICA-A5.1.

The association between MICA single nucleotide polymorphisms (SNPs) and sMICA levels has also garnered attention. SNPs in MICA can regulate its expression, and the degree of MICA expression is often correlated with sMICA levels. For instance, the SNP rs1051792 causes the 129th amino acid site in the MICA α2 domain to change from valine (Val) to methionine (55). In patients with multiple myeloma (51), those with the MICA-129Val/Val genotype release more sMICA, which is linked to the increased expression and quantity of MICA. In rs2596542 (A/G), the G allele promotes MICA expression, leading to an increase in sMICA generation (56).

Stress and elevated levels of sNKG2DL. NKG2DL is rarely expressed in normal tissues. When cells are subjected to various stress stimuli, such as oncogenic factors, infections, physical injuries, or inflammatory responses, they enhance NKG2DL expression through various mechanisms (1). For instance, when cells undergo heat stress, heat shock factor 1 binds to the heat shock element in the MIC promoter sequence, thereby promoting the expression of MICA and MICB through the heat shock response. This leads to increased expression of MICA/B membrane proteins in tumor cells (15). However, in leukemia and lymphoma, heat and oxidative stress promote the secretion of MICA, ULBP1 and ULBP2 in exosomes, thus reducing NK cell cytotoxicity (46).

Contrary to most stress stimuli that elevate NKG2DL membrane protein levels and boost tumor immunity, hypoxia induced by the tumor microenvironment promotes the production of sNKG2DL, exacerbating immune suppression. Under hypoxic conditions, hydroxylation of hypoxia-inducible factor (HIF)-α in tumor cells is inhibited. The non-hydroxylated HIF-α accumulates and binds with HIF-β, collectively acting on the hypoxia-responsive element of the *ADAM10* gene, elevating *ADAM10* expression and promoting MICA shedding (57). Upregulation of circ_0000977 modulates this process during hypoxia (58). Circ_0000977, acting as a miRNA sponge,

binds to miR-153, reducing the inhibitory effect of miR-153 on HIF-α and ADAM10 (58). Targeting this pathway, nitric oxide (NO) mimetics, such as glyceryl trinitrate and DETA-NO, can promote the degradation of HIF-α, reducing MICA shedding induced by hypoxia. They activate the NO-cyclic guanosine monophosphate-protein kinase G pathway, enhancing prolyl hydroxylase-mediated degradation of HIF1-α, interfering with the accumulation of HIF-α under hypoxic conditions, thereby decreasing ADAM expression (58).

Genotoxic stress, such as that caused by chemotherapeutic drugs and ionizing radiation, can produce reactive oxygen species, leading to DNA damage, protein misfolding, and inactivation. This overactivates the extracellular signal-regulated kinase pathway, triggering cellular senescence (59). During cell cycle arrest, senescent cells activate and secrete a senescence-associated secretory phenotype, including metalloproteinases (59), which can stimulate tumor progression. In a neuroblastoma model, low doses of chemotherapeutic drugs induced cellular senescence and stimulated lncRNA MALAT1 to bind to miR-92a-3p. This reduces the inhibitory effect of miR-92a-3p on ADAM10 and promotes the shedding of MICA and MICB (60). However, drugs, such as sorafenib (61), have the opposite effect in hepatocellular carcinoma, inhibiting ADAM9 and ADAM10 and reducing MICA shedding. Therefore, it is crucial to assess the effects of drugs on NKG2DL shedding carefully.

Production of sNKG2DL is regulated by the tumor microenvironment. The tumor microenvironment plays an important role in the development and metastasis of tumors, and the generation of sNKG2DL is regulated by cells and molecules in the tumor microenvironment. Cancer-associated fibroblasts promote tumor cell migration by reshaping the microenvironment. Research has shown that cancer-associated fibroblasts secrete high levels of active MMPs, causing MICA/B shedding from the surfaces of melanoma cells (62). Platelets release exosomes containing soluble ADAM10 and ADAM17 into tumor cells, facilitating the shedding of NKG2DL from the tumor cell surface (63). In lymph node mesenchymal stromal cells, both transcription and expression levels of ERp5 and ADAM10 were revealed to be elevated, promoting the shedding of MICA and ULBP3 from the surface of lymph node mesenchymal stromal cells and Reed-Sternberg cells (64).

Metalloproteinases, which are essential enzymes for the generation of sNKG2DL, influence sNKG2DL production based on changes in their expression and content. Cytokines in the TME affect the expression of metalloproteinases through various signaling pathways and transcription factors. IFN-γ affects MMP expression via STAT, TGF-β through Smad, and IL-1β and TNF-α through the mitogen-activated protein kinase pathway (65). Thus, cytokines may influence the shedding of NKG2DL through these pathways. IL-1β facilitates ADAM9-mediated NKG2DL cleavage (66). TGF-β induces ADAM17 mRNA expression, promoting sMICA and sULBP2 production (67,68). Furthermore, indoleamine 2,3-dioxygenase 1 regulates ADAM10 expression via the indoleamine 2,3-dioxygenase 1-Kyn-AhR pathway, thereby inducing sMICA shedding and immune evasion (69).

Morimoto *et al* (25) found that MUC1-C suppresses MICA/B expression and promotes sMICA/B production through various pathways. MUC1-C can activate NF-κB, promoting H3K27 trimethylation mediated by EZH2 and methylation of the MICA/B promoter region by DNA methyltransferase, thus suppressing MICA/B expression. Another possible mechanism is that MUC1-C facilitates MICA/B shedding mediated by ERp5. Notably, RAB27A plays an important role in exosome secretion. MUC1-C also forms a complex with RAB27A, which effectively promotes the release of exosomes containing MICA/B.

In summary, ADAM and MMP are the core enzymes responsible for producing sNKG2DL and are critical targets for regulating sNKG2DL. Their roles in tumor development, invasion, and related regulatory factors have been extensively studied. Although the precise mechanisms by which certain regulatory factors affect sNKG2DL are yet to be elucidated, their potential impact on sNKG2DL production cannot be overlooked. Considering the multifaceted roles of ADAM and MMP in regulating tumor growth, inducing apoptosis, and promoting invasion, targeted interventions against these proteinases may yield more pronounced antitumor effects.

5. sNKG2DL is a key molecule in tumor immune evasion

NKG2D is an activating receptor of NK cells and a coactivating receptor of $CD8^+$T cells (1). Thus, within tumor tissues, activation of the NKG2D-NKG2DL axis is crucial for maintaining immune surveillance and inhibiting tumor development. In addition to suppressing NKG2DL expression, tumor cells evade the immune system by producing or secreting sNKG2DL.

The reduction of membrane-bound NKG2DL on the surface of tumor cells diminishes the recognition and attack of these cells by NK cells (70). Tumor cells employ mechanisms such as proteolysis to convert membrane-bound NKG2DL into sNKG2DL, which is then released into the tumor microenvironment, directly resulting in a decrease in membrane-bound NKG2DL levels (71). Studies have demonstrated that inhibiting MMPs can reduce the release of sMICA and promote the accumulation of MICA on the tumor cell surface (72). Additionally, sNKG2DL exerts immunosuppressive effects in other ways (Fig. 2). sNKG2DL can inhibit the interaction between NKG2D on the tumor cell membrane and NKG2DL on immune cells, inducing the internalization of the NKG2D receptor and impairing the immunological function of NK cells (73). Beyond its effects on NK cells, sNKG2DL also targets other immune cells within the tumor microenvironment, further promoting immunosuppression.

sMICA competitively binds to the NKG2D receptor, blocking the binding of MICA on the tumor cell membrane to NKG2D. sNKG2DL consistently binds to NKG2D, prompting the internalization of NKG2D on the surface of NK cells (73) or $CD8^+$T cells (74), leading to a decrease in cell surface NKG2D content (Fig. 2) and weakening the immune rejection effect against tumors. After binding to NKG2D, sMICA activates the ubiquitin ligase c-Cbl, promotes DAP10 ubiquitination, directs the internalization of NKG2D, and sends it to lysosomes for NKG2D degradation (73). This process results in a 50% reduction in cytolytic activity of NK cells relative to those not exposed to sMICA (73,75). Compared to culturing NK cells from AML patients without

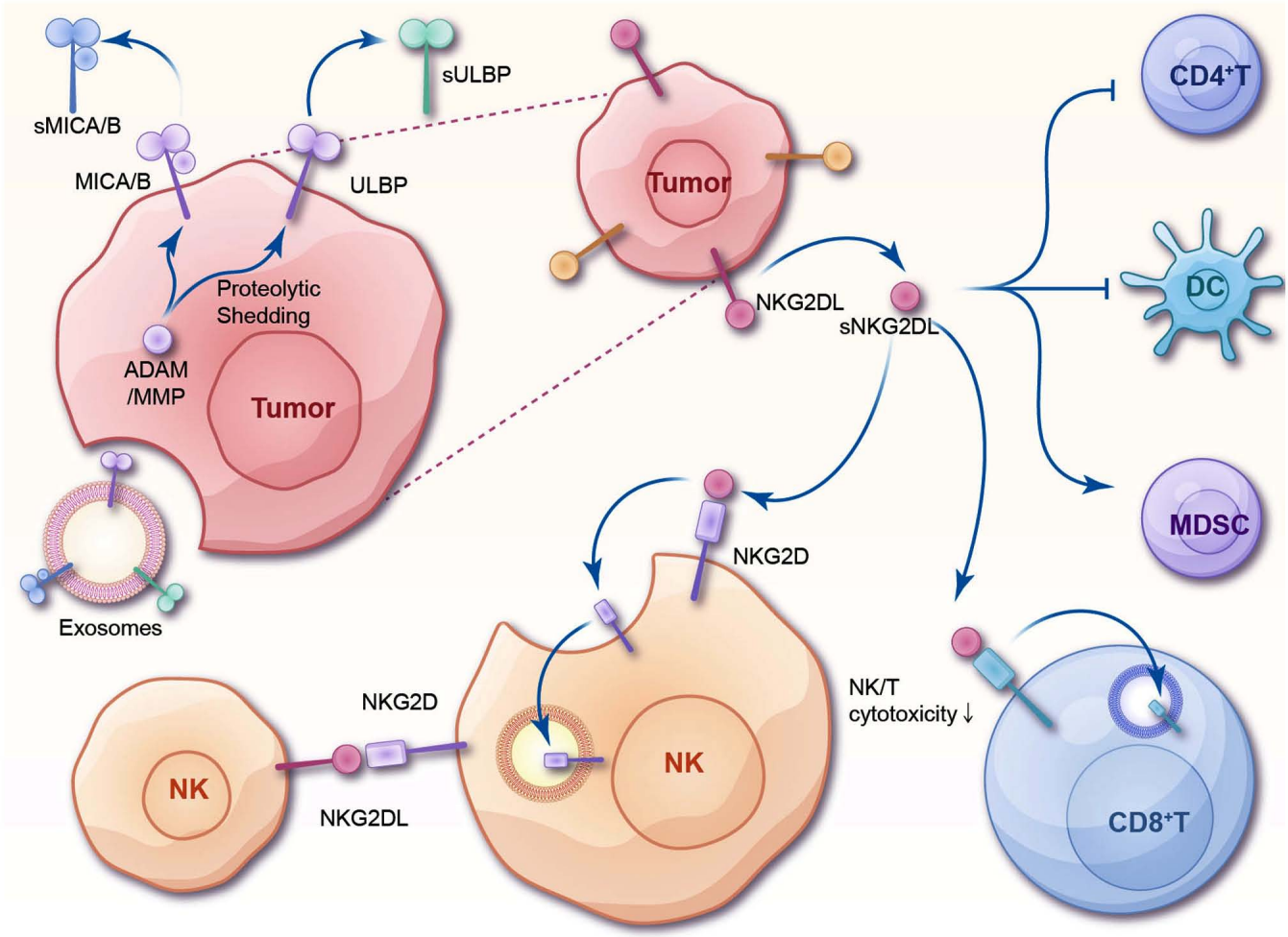

Figure 2. Potential mechanism of sNKG2DL-induced immunosuppression. Tumor cells generate sNKG2DL, including sMICA/B and sULBP, through the ADAM/MMP-mediated proteolytic cleavage of NKG2DL or by secreting sNKG2DL via exosomes. The binding of sNKG2DL to NKG2D receptors on the surface of NK cells and CD8$^+$T cells induces the internalization and downregulation of NKG2D, leading to a decrease in cytotoxicity. Additionally, sNKG2DL present on exosomes can be transferred to the membrane of NK cells, prompting these cells to attack each other. sNKG2DL also inhibits DC and CD4$^+$T cells, while promoting the activation of myeloid-derived suppressor cells. sNKG2DL, soluble natural killer group 2 member D ligand; sMICA/B, soluble MHC class I polypeptide-related sequence A/B; sULBP, soluble UL16 binding protein; ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; NK, natural killer.

sULBP1, the presence of sULBP1 leads to a 40% reduction in NKG2D expression on the surface of NK cells (71). However, high-affinity sNKG2DL, similar to sMULT1 in mice, can reduce the internalization of cell membrane NKG2D due to persistent binding to tumor cell NKG2DL, thereby increasing the content of NKG2D on the NK cell membrane and enhancing the function of NK cells (76). In humans, both MICA/B are low-affinity receptors, suggesting that affinity may influence whether sNKG2DL induces NKG2D internalization. In addition, the production mode of sNKG2DL also affects the internalization of NKG2D, and sNKG2DL secreted by exosomes can cause more NKG2D internalization (41). sULBP3 secreted by exosomes can cause ~15% more downregulation of NKG2D than sULBP2 produced by proteolytic cleavage ($P<0.05$), resulting in more severe immunosuppression (41). Apart from the differences in the ligands themselves, the reason for this may be that sNKG2DL on the exosome membrane is multimeric, with a higher affinity and longer binding time with NKG2D.

A recent study has found that MICA*008 secreted by exosomes can be transferred to the surface of NK cells, inducing mutual attacks between NK cells, leading to an increase in NK cell death, and providing new insights into the mechanisms by which sNKG2DL causes immune evasion (77).

Innate and specific immunity continuously regulates the intensity of the immune system through different cytokines and chemokines. sNKG2DL influences other immune cells in the tumor microenvironment, resulting in severe immunosuppression. sNKG2DL inhibits the activation of CD4$^+$T cells and DC cells by NK cells (Fig. 2) (78). The sMICA/B-neutralizing antibody B10G5 stimulates the expression of DC co-stimulatory molecules CD80 and CD86 (79). After binding with NKG2D, sMICB can activate the STAT signaling pathway of MDSC and promote the proliferation and accumulation of MDSC (80). For CD8$^+$T cells, sMICA/B activates caspase3/7 through the FasL/Fas pathway, leading to the degradation of CD3ζ, destroying the stability of CD3ζ, and thus damaging the signal transmission of the TCR/CD3 complex (79).

Table II. Clinical significance of soluble ligands.

Tumor	Type of sNKG2DL	Level	Diagnostic value	Assessing tumor malignancy	Progression and prognosis	(Refs.)
Lung cancer	sMICA	High level	+	-	+	(97,98)
	sULBP2	High level	+	-	+	(81)
Pancreatic ductal adenocarcinoma	sMICA/B	High level	+	-	+	(83)
Head and neck squamous cell	sNKG2DLs	High level	+	-	+	(99)
carcinoma	sMICA	High level	-	-	+	(100)
Hepatocellular carcinoma	sMICA	High level	-	+	+	(89,101, 102)
	sMICA/B	High level	-	-	+	(92)
Breast cancer	sMICA	High level	-	+	+	(103,104)
Renal cell carcinoma	sMICA	High level	-	+	+	(85,105)
Cervical adenocarcinoma	sMICA	High level	-	+	+	(106)
Prostate cancer	sMICA	High level	-	+	+	(88)
Pancreatic cancer	sMICA/B	High level	-	+	-	(107)
	sMICA	High level	-	+	+	(108,109)
Nasopharyngeal carcinoma	sMICA	High level	-	+	-	(110)
Oral squamous cell carcinoma	sMICA	High level	-	+	+	(111)
Cervical cancer	sMICA	High level	-	-	+	(112)
Chronic lymphocytic leukemia	sULBP2	High level	-	-	+	(113)
Melanoma	sULBP1	High level	-	-	+	(114)
	sULBP2	High level	-	-	+	(94)
Multiple myeloma	sMICA	High level	-	-	+	(115)

sNKG2DL, soluble natural killer group 2 member D ligand; MIC, MHC class I polypeptide-related sequence; sMIC, soluble MIC; -, not discussed; +, clinically significant; ULBP, UL16 binding protein; sULBP, soluble ULBP.

6. Clinical significance of soluble ligands

This section discusses the role of sNKG2DLs as biomarkers across various cancers. Elevated levels of sNKG2DL, including sMICA/B and sULBP variants, have shown diagnostic and prognostic relevance. As detailed in Table II, these biomarkers contribute to tumor diagnosis, malignancy assessment, and prognosis prediction, enhancing clinical outcomes in oncology.

sNKG2DL as a tumor-related biomarker. Immunological molecular markers play a vital role in tumor diagnosis and are increasingly used in clinical practice. Numerous studies have shown significant elevations in sNKG2DL levels in the sera of various patients with cancer, suggesting its potential as an auxiliary diagnostic biomarker for tumors. It has been proven to be effective in some cancers (81). Particularly in pancreatic ductal adenocarcinoma, CA19-9 (a commonly used clinical biomarker for pancreatic cancer diagnosis) levels can increase under several benign conditions, affecting its specificity (82). However, using sMICA and sMICB as biomarkers has the potential to overcome the diagnostic limitations of CA19-9 with higher specificity and sensitivity (83,84). Nevertheless, distinguishing between malignant and benign kidney tumors using sMICA alone has limited applicability (85). Moreover, a study with 512 participants with various cancers concluded that when potential confounding factors were considered, the sMICA level was a valuable tool for differential diagnosis and treatment efficacy evaluation (86). However, the same conclusion has not been drawn for sMICB (87).

Whether the level of sNKG2DL has diagnostic value depends on the tumor type. However, despite the limited number of studies, sNKG2DL has demonstrated great potential for auxiliary or early diagnosis of some cancers and can be used to differentiate between benign and malignant tumors (81-87). Additionally, multiple experiments have suggested combining detection with other biomarkers to improve results.

sNKG2DL levels for assessing tumor malignancy. Serum sNKGDL levels have been observed to be closely associated with higher tumor grading and staging (86,87). It has now been observed in various cancers and serves as a biomarker to assist in determining tumor malignancy or in early screening (88,89). Notably, a report on liver cancer observed that the concentration of sMICA/B decreased in the T4 stage or poorly differentiated tumors (G3). This outcome might be due to severe impairment of liver cell function in patients with end-stage cancer, such as protein synthesis disorders, or it might be due to post-transcriptional regulation, such as proteasomal degradation (90).

Although no studies have explicitly indicated that serum sNKG2DL levels can be used clinically for tumor grading and staging, the significant correlation between them suggests great potential in this regard. However, a study has noted that serum-sNKG2DL is a superior indicator of systemic tumor manifestations than local tumor differentiation (91). Their roles in grading and staging, however, require further evaluation.

sNKG2DL levels for predicting tumor progression and prognosis. sNKG2DL reflects biological behaviors centered

on cancer cells and the state of tumor immune surveillance, potentially serving as a predictive factor for rapid tumor development, lymph node metastasis, or distant metastasis. sNKGDLs have been found to correlate with tumor size, progression, and/or metastasis (84,88). For instance, Qiu *et al* (85) found that sMICA concentration was significantly associated with lymph node metastasis, distant metastasis, and vascular invasion in renal cell carcinoma. Given the current absence of reliable noninvasive serum biomarkers for the diagnosis and monitoring of patients with renal cell carcinoma, this noninvasive detection method appears to have an advantage (85). Additionally, sNKG2DL may also be related to the transformation of precancerous lesions into tumors, as it has been observed to increase under various precancerous conditions (92,93). For example, MICA shedding is a crucial determinant of host immunity in the progression from monoclonal gammopathy of undetermined significance (a precancerous condition) to mature multiple myeloma (93).

Furthermore, sNKGDLs can be used to predict tumor prognosis by correlating them with overall survival, recurrence rates, treatment outcomes, and drug resistance, among other indicators. Strong correlations have already been observed in numerous cancers (81,94), with some being considered independent indicators of poor prognosis. Notably, Paschen *et al* (94) observed that sULBP could serve as a prognostic biomarker, outperforming the already established melanoma serum marker B100P. However, comparisons are still lacking in a number of cancers, and the broader implications of sNKG2DL require further evaluation. In a meta-analysis, Zhao *et al* concluded that sMICA/B is a marker of tumor prognosis and immunotherapy efficacy, corroborating the views expressed in the present review (95). However, some studies have indicated that sNKGDLs are not related to tumor size, metastasis, or poor prognosis (86,96). This may be due to factors, such as detection methods or sample sources, suggesting the need for a standardized detection criterion to make results comparable across different laboratories (8).

7. sNKG2DLs in drug development and treatment

The development of therapies targeting sNKG2DLs represents a dynamic frontier in cancer treatment. Advances in NKG2D-CAR-T and CAR-NK cell therapies, alongside monoclonal antibodies and targeted therapeutic approaches, exemplify innovative strategies to enhance immune response against tumors. These approaches focus on overcoming the immunosuppressive effects of sNKG2DLs and enhancing the cytotoxic activity of immune cells. An overview of various drugs targeting sNKG2DLs, their mechanisms, and clinical indications, illustrating the breadth of current and potential applications in hematological and solid tumor malignancies is presented in Table III.

Immunotherapeutic approaches

Association between NKG2D-CAR-T cell therapy and sNKG2DL. NKG2D-CAR-T cell therapy is gaining increasing attention. While NK cells are not restricted by MHC and have relatively weak antitumor capabilities due to insufficient migration to tumor cells, T cells can effectively kill tumor cells; however, their recognition is limited by T-cell receptor (TCR). NKG2D-CAR-T cells effectively combine the advantages of both NK and T cells, exhibiting strong targeted cytotoxicity against various tumors (Fig. 3) (116). As NKG2DL is rarely expressed in normal tissues, the likelihood of autoimmune reactions or long-term toxicity is low (117). The efficacy of NKG2D-CAR-T cells depends on the expression level of NKG2DL. Therefore, they may be less effective against tumor cells that express low levels of NKG2DL, or may shed them as soluble ligands. However, Zhang *et al* (116) observed that sMICA, even at concentrations markedly higher than those of serum, maintains full functionality, suggesting that NKG2D-CAR-T cell therapy can, to some extent, overcome the immunosuppressive effects of soluble ligands.

Nevertheless, both CAR-T and CAR-NK cell therapies unavoidably face challenges, such as the need for *ex vivo* expansion and modification, and relatively high costs. Recently, Liu *et al* (118) developed a new soluble NK-CAR for hematological malignancies, termed MS-Ig. One end can bind to CD20 on tumor cells and the other end can bind to the NKG2D receptor on NK cells, thus mediating targeted cytotoxicity. This approach does not require personalized treatment and reduces toxicity caused by immune cell infusion (118).

Targeting membrane-bound MICA/B with mAbs to prevent its shedding enhances the cytotoxic effect of NK cells. mAbs targeting the MICA α3 domain, such as 7C6 and 6E1, can inhibit the shedding of MICA/B and increase its surface expression, suppressing tumor metastasis (Fig. 3) (119-121). When used in conjunction with HDAC inhibitors, HDAC inhibitors enhance the expression of the *MICA/B* gene, whereas MICA/B antibodies stabilize proteins synthesized on the cell surface, thereby achieving improved antitumor effects (121,122). Additionally, the Fcγ segment of the mAb can mediate the antibody-dependent cellular cytotoxicity effect, triggering immune cells to kill target cells (121). Since the α3 domain is relatively conserved, it can overcome the effects of MICA/B polymorphism (119). Recently, Badrinath *et al* (123) designed a tumor vaccine targeting the MICA α3 domain, which can induce the body to produce antibodies against the MICA α3 domain, inhibiting the proteolytic shedding of MICA/B from tumor cells, enhancing the cytotoxic function of NK cells, and increasing the cDC1-mediated cross-presentation of tumor antigens to $CD8^+$T cells, inducing responses against MICA/B in both $CD4^+$ and $CD8^+$ T cells (123).

mAbs targeting sNKG2DL neutralize and reduce immunosuppressive effects. The mAb targeting sMICA, B10G5, is considered to neutralize free sMIC in the plasma, thus alleviating the immunosuppressive effect of sMIC (Fig. 3). Although B10G5 can also recognize membrane-bound MIC, it binds at a site different from that of NKG2D, thus it does not interfere with the function of NKG2D. On the contrary, owing to its antibody-dependent cellular cytotoxicity action, it may enhance the reactivity of NK cells toward tumor cells (124). Basher *et al* (125) observed that the monotherapy with B10G5 to clear sMIC effectively controlled tumor growth and eliminated lung metastasis. Additionally, since the membrane-bound MIC is already low in patients in the late stages of cancer, the effect of inhibiting its shedding is minimal; thus, using the neutralizing antibody B10G5 can have a superior effect at this stage (124).

Table III. Drugs targeting sNKG2DL.

Category	Drug	Mechanism	Indication	(Refs.)
NKG2D CAR-T	CM-CS1 T-cell	NKG2D-modified autologous CAR-T cells	Acute myeloid leukemia, myelodysplastic syndrome, multiple myeloma	(117)
NCT02203825	KD-025	NKG2DL-targeting CAR-T	Colorectal cancer cells	NCT05382377 Early Phase1
	IMC008	NKG2D-modified autologous CAR-T cells targeting CLDN18.2	Advanced solid tumor	NCT05837299 Phase 1
NKG2D CAR-NK	MS-Ig	NKG2D-modified autologous CAR NK cells targeting CD20	CD20(+) lymphoma cells	(118)
	NKG2D CAR-NK	NKG2DL-targeting CAR NK cells	Refractory metastatic colorectal cancer	NCT05213195 Phase 1
	NKG2D CAR-NK	NKG2DL-targeting CAR NK cells	Multiple myeloma	NCT06379451 Early Phase 1
Monocloning Antibody	7C6	Targeting MICA α3 domain to prevent its shedding	Melanoma, multiple myeloma, cholangiocarcinoma	(119,121,122)
	5C6 and 1D11	Targeting MICA α3 domain to prevent its shedding	Lymphoma	(120)
	B10G5	Targeting soluble NKG2DL	Prostate cancer, melanoma	(124,125)
	CLN-619	Targeting MICA/MICB	Multiple myeloma	NCT06381141 phase 1
	DM919	Targeting MICA/MICB	Solid tumors	NCT06328673 Phase 1
HDACi	Valproic acid	Inhibition of histone deacetylase	Pancreatic cancer, Acute myeloid leukemia	(128,129)
	Sodium butyrate	Inhibition of histone deacetylase	Multiple myeloma	(135)
Metalloproteinase Inhibitor	ADAMi	Inhibition of ADAM10 expression	Hodgkin lymphoma	(131)
	MMPi	Inhibition of MMP expression	Multiple myeloma	(135)
Other drugs	Gemcitabine	Inhibition of ADAM10 expression	Hepatocellular carcinoma, Pancreatic cancer	(137)
	phenylarsine oxide	Inhibition of the protein disulfide isomerase ERp5	Multiple myeloma	(135)
	Disulfiram	Inhibition of ADAM10 expression	Hepatocellular carcinoma	(138)

sNKG2DL, soluble natural killer group 2 member D ligand; sNKG2D, soluble natural killer group 2 member D; MMP, matrix metalloproteinase; ADAM, a disintegrin and metalloproteinase; CAR, chimeric antigen receptor; MICA, MHC class I polypeptide-related sequence A; MICB, MHC class I polypeptide-related sequence B; HDACi, histone deacetylase inhibitor.

High-affinity soluble ligands upregulate membrane NKG2D expression and affect NK cell activity. MULT1 is a ligand for NKG2D in mice, and compared with MICA/B, it is a high-affinity, soluble ligand. It can upregulate the expression of membrane NKG2D and prevent NK cells from being desensitized by sNKG2DL, thus emerging as a potential mechanism to enhance antitumor immunity. In an experimental design developed by Deng *et al* (76), cells were generated to induce the production of secMULT1 (an extracellular fragment of HA-MULT1 secreted by induced fibroblasts). It was found to be resistant in various mouse models, in which secMULT1 mobilized or activated antitumor effector cells. As the adjustment of NK cell reactivity by sNKG2DLs depends on their affinity to NKG2D, the preclinical development of this new class of candidate drugs may reveal new pharmacokinetics and pharmacodynamic guidelines (126).

Targeted therapeutic approaches

Reducing sNKG2DL production using HDAC inhibitors. HDAC inhibitors have been approved by the FDA for the

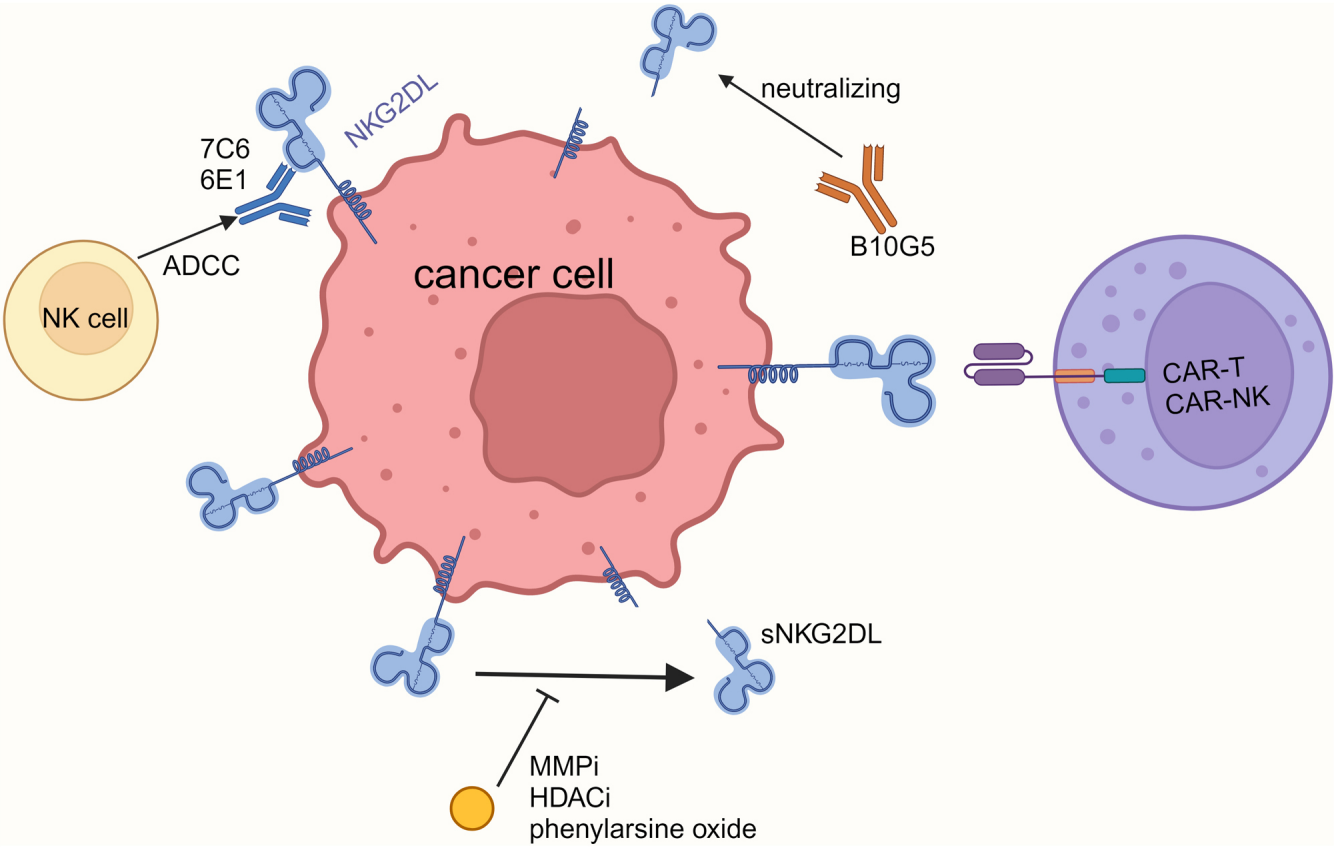

Figure 3. Treatment targeting sNKG2DL. mAb 7C6 and 6E1 can bind to MICA α3 domain, which inhibits the shedding of MICA, and triggers immune cells to kill tumor cells through ADCC. mAb B10G5 neutralizes sMICA. MMPi, HDACi and disulfiram can inhibit the shedding of MICA. NKG2D-CAR-T/NK targets NKG2DL and kills tumor cells. sNKG2DL, soluble natural killer group 2 member D ligand; mAb, monoclonal antibody; MICA, MHC class I polypeptide-related sequence A; ADCC, antibody-dependent cellular cytotoxicity; sMICA, soluble MICA; MMPi, matrix metalloproteinase inhibitor; HDACi, histone deacetylase inhibitor; CAR, chimeric antigen receptor; NK, natural killer; NKG2DL, natural killer group 2 member D ligand.

treatment of hematological malignancies and play a role in reducing the generation of sNKG2DL (Fig. 3). When used alone or in combination with mAbs and cytotoxic chemotherapy drugs, HDCA inhibitors may exert antitumor effects by increasing MICA expression and downregulating metalloproteinase activity, thereby inhibiting sNKG2DL production (127-129). However, due to their significant side effects, the use of HDAC inhibitors is limited. Enhancing selectivity might improve their clinical value (130).

Reducing sNKG2DL production by inhibiting metalloproteinase activity. Given the critical role of ADAM and MMP in the release of sNKG2DL, specific inhibition of ADAM and MMP may enhance the recognition of tumor cells by the immune system. Multiple studies have observed a reduction in sNKG2DL content in supernatants after treatment with metalloproteinase inhibitors (72,119,122), with some studies concurrently observing elevated levels of membrane-bound NKG2DL (Fig. 3) (60,131). However, most experiments remain at the preclinical cell-level stage, and studies on the *in vivo* efficacy and potential side effects are either insufficient or absent. Furthermore, TIMPs, which are endogenous proteinase inhibitors, inhibit MMPs when their expression is upregulated. Fatty acid amide hydrolase inhibitors (132) and demethylating agents (azacytidine and decitabine) (42) inhibited sNKG2DL shedding by upregulating expression of TIMPs. Targeted therapies that inhibit multiple enzymes and thus reduce sNKG2DL production are feasible approaches. However, some inhibitors have been reported to have side effects or toxicity, which limit the application of this method (133).

Reducing sNKG2DL production by inhibiting protein disulfide isomerase. Experiments have observed that phenylarsine oxide, an inhibitor of the protein disulfide isomerase ERp5, can inhibit the release of sNKG2DL (Fig. 3). However, its effective concentrations are toxic. The combination of phenylarsine oxide with HDAC inhibitors and MMP inhibitors may have a synergistic effect (134,135).

8. Discussion

The present review examined the soluble forms of NKG2DLs released from the cell membrane into the extracellular environment. Their generation mechanisms, such as cleavage by MMPs and ADAMs, and their release via exosomes were explored, and the factors influencing their formation, such as cellular stress and the tumor microenvironment were outlined. Studies have shown that sNKG2DLs can inhibit NKG2D receptors, diminish NK cell activity, and affect their ability to monitor tumors (1,5). Clinically, their increase correlates with higher tumor staging and poor prognosis, whereas some drugs or treatments can decrease their production,

alleviate the immunosuppressive effects, and promote anti-tumor responses (95,136).

Tumors and their microenvironments are intricate, and the impact of various cells and cytokines on the production of soluble ligands remains unclear. The extensive polymorphisms of the MICA family and their implications for tumor immunity require detailed analysis. The complexity of extracellular vesicles makes research particularly challenging, especially because the ligands on exosomes may have even more crucial immunosuppressive roles in the tumor microenvironment (41). From a mechanism standpoint, the generation of these soluble ligands appears more as 'byproducts' caused by tumor progression, such as the activation of MICA/B and expression of ULBPs, activation of MMPs and ADAMs, and exosome release. Thus, it remains unclear whether these ligands play pivotal roles in tumor progression.

Circulating NK cells and the NKG2D-NKG2DL axis play vital roles in the surveillance and suppression of tumor metastasis. Elevated sNKG2DL levels can be observed in the serum of most patients with cancer, and those with higher levels usually exhibit higher malignancy and worse prognosis. Hence, serum sNKG2DL levels have the potential to be used as immunological molecular markers for tumor diagnosis, malignancy evaluation, and prognostic prediction. Monitoring serum sNKG2DL levels, especially during the early tumor stages, can be invaluable. As a non-invasive, cost-effective, and repeatable circulating biomarker, serum sNKG2DL levels overcome the limitations of other tumor markers, especially when tumors are deep-seated or when patients cannot undergo biopsies (85,95). Additionally, as aforementioned, various genotypes influence sMICA production, potentially affecting tumor progression and prognosis. Evaluating the MICA genotypes of patients may offer new insights (51,56).

However, serum sNKG2DL levels are significantly elevated across various tumors, resulting in low specificity. Their primary value may be in assessing tumor malignancy and predicting metastasis, or perhaps in combination with other tumor markers. Questions regarding the detection efficiency, inter-individual variability, and appropriate threshold values remain unresolved. Additionally, there is a scarcity of related clinical studies, and most have issues, such as small sample sizes or singular sample origins, indicating a gap before wide clinical application.

The effects of immunotherapy on soluble ligand production have opened new avenues for therapeutic interventions targeting these ligands. Numerous immunotherapies targeting the NKG2D-NKG2DL axis are currently being developed. These treatments enhance cellular recognition, inhibit ligand shedding, and remove existing soluble ligands that exert anti-tumor effects. This offers a novel approach for determining whether existing immunotherapies can be adapted to target the NKG2D-NKG2DL axis for broader recognition and fewer side effects. However, many of these therapies are in the early stages of development, and their efficacy and safety in humans remain unclear. Further clinical trials are required to determine their roles in cancer treatment.

In summary, this review thoroughly examined the production and multifaceted roles of sNKG2DL in the tumor microenvironment. This underscores the immunosuppressive ability of sNKG2DL, its potential as a diagnostic and prognostic marker, and its emerging role as a therapeutic target. Despite the existing limitations and areas needing further exploration, the evidence strongly attests to the significance of sNKG2DL in tumor immunity. Future research should probe the potential of targeting sNKG2DL in tumor immunotherapy, providing a scientific foundation for integrated tumor immune treatments.

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Authors' contributions

SH, ZQ, FW contributed to the drafting and editing of the manuscript. YK contributed to drawing the figures and editing the manuscript. BR designed, revised and finalized the manuscript. All authors contributed toward the drafting and revising, and agreed to submit the present study. All authors read and approved the final manuscript. Data authentication is not applicable.

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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