New prospects on the NKG2D/NKG2DL system for oncology

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The activating immunoreceptor NKG2D endows cytotoxic lymphocytes with the capacity to recognize and eliminate infected or malignant cells. The recognition of such harmful cells is enabled by binding of NKG2D to various MHC class I-related glycoproteins, which are upregulated in the course of viral infection or malignant transformation. The past years have witnessed substantial progress in our understanding of the mechanisms underlying the regulation of NKG2D ligands (NKG2DLs) by malignant cells, of tumor-associated countermeasures promoting escape from NKG2D-dependent immunosurveillance, and of therapeutic measures that may bolster the NKG2D/NKG2DL system against malignancies. Here, we summarize the current knowledge on the NKG2D/NKG2DL system and outline opportunities to exploit the tumoricidal function of NKG2D for anticancer immunotherapy.

Preface

Cytotoxic lymphocytes are major protagonists in concepts of immunosurveillance and immunotherapy of cancer, as they are literally able to kill malignant cells. The selective killing of transformed cells requires a precise and unerring molecular recognition of “malignant self”, which represents a quite challenging task, especially in view of the highly diverse manifestations of cellular malignancy. Different subsets of cytotoxic lymphocytes including natural killer (NK) cells, CD8+ T cells, and γδ T cells utilize distinct types of molecular recognition systems that detect malignant-self either directly (such as in the case of tumor-associated antigens) or indirectly (in the form of so-called “danger signals”). Nonetheless, all these lymphocytes (at least in humans) share the expression of NKG2D, which—upon binding to various stress-inducible NKG2D ligands (NKG2DLs)—stimulates effector responses. As the upregulation of NKG2DLs is linked to cellular processes that are associated with malignant transformation and NKG2DLs are frequently expressed on the surface of tumor cells, NKG2D has attracted vivid interest as a potential target for the development of novel immunotherapeutic anticancer regimens.

NKG2D is a Potent Activating Immunoreceptor of Immune Cells

NKG2D is composed of two disulfide-linked copies of a type II transmembrane glycoprotein that bears a single extracellular C-type lectin-like domain (CTLD), and hence is often referred to as a homodimeric C-type lectin-like receptor (CTLR). NKG2D monomers are encoded by the gene killer cell lectin-like receptor subfamily K, member 1 (KLRK1), which is embedded within a cluster of genes coding for immune-related CTLRs, called the natural killer gene complex (NKC). While NKG2D is relatively conserved among mammals, the number and types of NKG2DLs vary considerably among mammalian species. NKG2D-coding transcripts were first described and named together with the mRNAs coding for NKG2A, NKG2C, and NKG2E. However, as pointed out previously, the term NKG2D is misleading, as NKG2D is not related more closely to NKG2A, NKG2C and NKG2E, respectively, than to other NKC-encoded CTLRs. In 1999, the search for the receptor of the MHC class I-related orphan molecule MICa led to the functional characterization of NKG2D as a cell-surface receptor that stimulates the cytolytic response of human NK and T cells to stress-inducible MIC molecules. Subsequently, NKG2D was also shown to be expressed and to exert immunostimulatory functions in NK cells and activated CD8+ T lymphocytes of mice. In contrast to its mouse counterpart, human NKG2D is also expressed on the surface of naïve CD8+ T cells and even some CD4+ T cells that expand under specific pathophysiological conditions. Likewise, most γδ T cells and NKT cells express NKG2D and are activated by NKG2DLs. Very recently, the expression of NKG2D by some tumors (in situ) and malignant cell lines (in vitro) has been documented. It will be important to gain further insights into the functional relevance of NKG2D expressed by cancer cells in the context of tumor progression.

NKG2D is incapable of signal transduction by itself. Rather, the transmission of NKG2D-elicted signals requires...
the association of NKG2D with DAP10 forming together a hexameric structure containing two signaling dimers\textsuperscript{18,19} (Fig. 1). DAP10 is almost exclusively associated with NKG2D and contains motifs for the recruitment of phosphatidylinositol-3-kinase (PI3K) and GRB2/VAV1, which allow for the activation of downstream signaling by Akt and MAP kinases, respectively.\textsuperscript{18,20} Hence, the NKG2D-DAP10 receptor complex activates NK and T-cell cytotoxicity via the PI3K pathway and by recruiting VAV1.\textsuperscript{18,20}

In mice, NKG2D exists in two distinct splice variants, a short (NKG2D-S) and a long (NKG2D-L) isoform, the latter of which contains 13 additional amino acids at the N-terminus.\textsuperscript{21,22} In activated mouse NK cells, NKG2D-S recruits immunoreceptor tyrosine-based activation motif (ITAM)-bearing DAP12, which enhances the reactivity mediated by NKG2D.\textsuperscript{21,22} In particular, the signaling cascade elicited by NKG2D-S and DAP12, which operates via the spleen tyrosine kinase (SYK) and \( \zeta \) chain (TCR)-associated protein kinase 70kDa (ZAP70), not only triggers cytotoxicity, but also stimulates cytokine secretion.\textsuperscript{21-23} (Fig. 1).

**E Pluribus Ad Unum: Multiple NKG2DLs Bind One Receptor**

The realm of NKG2DLs is surprisingly varied with regard to number, structure, and expression pattern. All NKG2DLs share an MHC class I-related \( \alpha \)1\( \alpha \)2 superdomain that—in spite of a substantial sequence diversity—constitutes the common site for interaction with NKG2D.\textsuperscript{24} Comparative structural analyses of several NKG2D/NKG2DL complexes revealed that promiscuous NKG2D binding is enabled by an interaction mode of rigid adaptation to structurally conserved patches in the highly diversified \( \alpha \)1\( \alpha \)2 superdomains.\textsuperscript{25,26} In humans, there are eight NKG2DLs, belonging to the MIC (i.e., MICA, MICB) and UL16-binding protein (i.e., ULBP1–6) families of MHC class I-related glycoproteins.\textsuperscript{4,27-29} Similarly, the mouse genome encodes multiple NKG2DLs belonging to various subfamilies of MHC class I-related molecules. These include retinoic acid early inducible gene 1 (Rae1)-like proteins (i.e., Rae1\( \alpha \), \( \beta \), \( \gamma \), \( \delta \), \( \epsilon \)), members of the H60 protein family (i.e., H60a, b, c) as well as Mult1.\textsuperscript{4,30,31} (Fig. 1).

In general, NKG2DLs - unlike MHC class I molecules - do not bind \( \beta \)2-microglobulin or low molecular weight ligands, and are associated with the plasma membrane via transmembrane domains (such in the case of MICs, ULBP4, ULBP5, Mult1, H60a and H60b) or glycosylphosphatidylinositol (GPI) anchors (such in the case of ULBP1, ULBP2, ULBP3, ULBP6, Rae1 and H60c)\textsuperscript{4,29} (Fig. 1). The complexity of NKG2DLs is further increased by their polymorphic nature (particularly polymorphic are human MICA and MICB), their widely varying affinities for NKG2D, and their heterogeneous expression pattern.\textsuperscript{4,5,29}

Owing to the variety and complexity of NKG2DLs, a comprehensive knowledge of the expression and regulation of these ligands is critical for a complete understanding of NKG2D functions.

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**Figure 1.** Diversity of NKG2D ligands and NKG2D signaling in mice and humans. Numerous NKG2D ligands (NKG2DLs) can be expressed on the surface of mouse (left) or human (right) malignant cells. In mice, NKG2D exists as a short (NKG2D-S) or a long (NKG2D-L) splice isoform. The activation of NKG2D-S promotes the cytotoxic function of natural killer (NK) cells as well as their capacity to secrete immunostimulatory cytokines. Upon engagement of NKG2D-S, SYK or ZAP70 are recruited through the ITAM-bearing adaptor DAP12. NKG2D-L and human NKG2D signal upon the recruitment/activation of DAP10, PI3K, AKT1, and GRB2/VAV1.
Regulation of NKG2DLs

The expression of NKG2DLs is considered as an indicator of a state of cellular stress, for instance as it occurs in the course of infection or malignant transformation. In line with this notion, NKG2DLs are rarely found on the surface of healthy cells. Still, transcripts coding for MICS and some members of the ULBP family have been detected in several healthy tissues. An early report described the expression of MICA by cells of the gastrointestinal epithelium, but a comprehensive knowledge of NKG2DL expression at the protein level in healthy tissues is still missing. A recent report by the Raulet laboratory shows that E2F transcription factors, which play a major role in cell cycle entry, regulate the expression of mouse NKG2DLs. High rates of proliferation are not a prerogative of malignant cells but also occur during the development of normal tissues and in the course of tissue renewal. In this respect, Rael was shown to be expressed in the developing embryonic brain as well as in healing wounds. It can be assumed that, in the absence of cell stress or other pathological conditions, the elimination of NKG2DL-expressing cells is avoided as either NKG2DLs are expressed concomitantly with high levels of inhibitory ligands such as MHC class I molecules or as these cells are inaccessible to NK cells. The regulation of NKG2DL expression is a field of intense research that covers various transcriptional and post-transcriptional mechanisms affecting not only mRNA and protein stability, but also regulation of NKG2DL density on the cell surface. (Fig. 2). Besides being regulated by cell-intrinsic mechanisms, the expression of NKG2DLs is greatly influenced by factors released in the tumor microenvironment, such as interferons. Here, we will focus on the mechanisms of NKG2DL regulation that are of clinical relevance for anticancer immunotherapy.

In 2005, Raulet and colleagues identified the DNA damage response mediated by the ataxia telangiectasia mutated (ATM) and (ataxia telangiectasia- and Rad3-related) ATR protein kinases as a key mechanism for the upregulation of NKG2DL on mouse and human tumor cells. Subsequently, human NKG2DLs were detected on cells that underwent senescence upon the activation of the DNA damage response. ATM as well as ATR are capable of activating the transcription factor TP53 (commonly known as p53), which is mutated or inactivated in a large fraction of human tumors. Using cellular systems of p53 induction, Textor et al. demonstrated that p53 can transactivate ULBP1 and ULBP2, but not the genes coding for other human NKG2DLs. Of importance, small p53-stabilizing compounds that are currently tested in clinical trials, such as ‘reactivation of p53 and induction of tumor cell apoptosis’ (RITA), have been shown to upregulate the expression of ULBP2 on the surface of some malignant cell lines, resulting in enhanced killing by NK cells. Accordingly, in a murine tumor model, the inducible genetic reactivation of p53 in malignant cells drove not only cellular senescence but also disease regression, which was partially dependent on the presence of NK cells. This study did not specifically address whether the NKG2D/NKG2DL system was involved in anticancer immunity, and presumably other factors produced by senescent cells were involved. Importantly, the upregulation of NKG2DLs by DNA damage can also occur in the absence of p53, pointing to the existence of p53-independent mechanisms of NKG2DL regulation. Low doses of DNA-damaging chemotherapeutic or radiotherapeutic regimens were shown to increase the expression of NKG2DLs on the surface of different cancer cell lines. Similarly, low doses of proteasome inhibitors such as bortezomib induced the expression of NKG2DLs in an ATM- and ATR-dependent fashion. The activation of NK cells might therefore contribute to the clinical benefit provided by these therapeutic regimens. Thus, combinatorial regimens incorporating low-dose chemo- or radiotherapy together with interventions that promote the activation of NK cells via NKG2D might improve current protocols of anticancer immunotherapy.

The expression of NKG2DLs is also regulated by histone deacetylases (HDACs), a class of enzymes that control key cellular processes including proliferation, survival and motility. HDAC inhibitors (HDACis) were shown to upregulate the expression of NKG2DLs on the surface of some cancer cells, promoting their NKG2D-dependent killing. Of note, we have evidence that HDACis also downregulate expression of B7-H6, a ligand of the activating NK-cell receptor NKp30. Thus, the net response of NK cells to HDACi-treated targets depends on the relative contribution of NKG2D vs. NKp30-dependent signaling pathways. Thus, potential combinatorial regimens involving HDACis and NK cell-based therapies should take such a differential regulation of activating NK-cell ligands by tumor cells into consideration. There is increasing evidence that the expression of some NKG2DLs is under the control of cancer-relevant microRNAs. In this respect, it has been reported that the metastasis-associated microRNA (metastamir) miR-10b directly downregulates MICB, linking metastatic dissemination with the escape of malignant cells from NK-mediated immunosurveillance. In addition, the oncosuppressive microRNAs miR-34a and miR-34c repress ULBP2 in a p53-dependent manner, as recently documented in some human cancer cell lines. Thus, the microRNA expression pattern might greatly influence the recognition of malignant cells by NK cells via the NKG2D/NKG2DL signaling axis.

Post-transcriptional mechanisms of regulation, including those mediated by miRNAs, are likely to account for the discrepancy between the levels of NKG2DLs transcripts and NKG2DL cell surface expression. Another of such mechanisms is the shedding of NKG2DLs by malignant cells that contributes to reduced cell surface expression, as originally reported for MICA. Tumor cells shed some NKG2DLs from the cell surface owing to the activity of metalloproteases such as ADAM metallopeptidase domain 10 (ADAM10) and ADAM17, while other NKG2DLs are released in exosomes. Accordingly, many cancer patients exhibit increased circulating levels of soluble NKG2DLs. Some studies have shown that soluble NKG2DLs downregulate NKG2D expression, thereby impairing the NKG2D-mediated recognition of tumor cells by cytotoxic lymphocytes. The potential prognostic value of the circulating levels of soluble NKG2DL ligands is discussed below.
Up to now, numerous studies have addressed the function of NKG2D/NKG2DLs in vitro and in vivo, supporting the initial notion that this constitutes a peculiar immunosurveillance system for the recognition and elimination of potentially harmful (i.e., stressed, infected or malignant) cells by cytotoxic lymphocytes. Particularly suggestive are the numerous viral glycoproteins specifically dedicated to the retention or degradation of NKG2DLs, representing a countermeasure against the induction of NKG2DLs upon viral infection.\textsuperscript{56,57} Such an interplay between so-called “viral immunoevasins” and NKG2DLs provides a conceptual framework for explaining the diversity of NKG2DLs in the context of the evolutionary selection pressure exerted by viruses. In addition, the complexity of the NKG2DL system may have evolved by the necessity for tissue-specific or insult-specific immune responses.

The relevance of the NKG2D/NKG2DL system for cancer immunosurveillance has first been hypothesized when the expression of NKG2DLs was found to be associated with malignancy.\textsuperscript{58} Subsequently, the spontaneous or therapy-elicited expression of NKG2DLs on tumor cells, triggering their recognition and elimination by NK cells, has been documented in vitro and in animal studies.\textsuperscript{59-61} These studies also demonstrated that overexpression of NKG2DLs on cancer cells

![Figure 2. Upregulation of NKG2D ligands in transformed cells. Healthy cells generally do not express NKG2D ligands (NKG2DLs) and suppress the reactivity of natural killer (NK) cells by delivering inhibitory signals. In contrast, various stress conditions promote the upregulation of NKG2DLs in malignant or infected cells. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia- and Rad3-related protein kinases; CHK-1, checkpoint kinase 1.](image-url)
leads to tumor rejection irrespective of MHC class I molecules, but in a perforin-dependent manner.\textsuperscript{59-61} Importantly, the NKG2D-dependent cytotoxic activity of NK cells turned out not to be restricted to transformed cells, as NKG2DL-expressing splenocytes could be equally eliminated.\textsuperscript{62,63}

Mice treated with neutralizing anti-NKG2D antibodies exhibited a significantly increased incidence of carcinogen-induced fibrosarcoma.\textsuperscript{64} Sarcoma cell lines derived from control mice expressed low (if not undetectable) levels of NKG2DLs such as Rael, whereas sarcomas derived from animals treated with anti-NKG2D antibodies exhibited medium to high levels of Rael, a phenomenon that was ascribed to tumor editing by NKG2D-expressing lymphocytes.\textsuperscript{65} Along similar lines, sarcoma cells from perforin-deficient mice also exhibited elevated expression levels of Rael. These observations not only underline the impact of the perforin system in NKG2D-mediated cytotoxicity, but also lend further support to the tumor editing concept.\textsuperscript{65}

Further evidence for a role of NKG2D in cancer immunosurveillance was obtained from NKG2D-deficient (\textit{Klrk1}\textsuperscript{−/−}) mice, which turned out to be more prone to develop various types of spontaneous cancer than NKG2D-sufficient mice.\textsuperscript{66} A detailed analysis of these mice revealed subtle changes in the receptor repertoire and maturation profile of NK cells, while the overall development and NKG2D-independent functions of NK cells appeared unaffected.\textsuperscript{67} Using another NKG2D-deficient mouse model, Polic and coworkers observed a decreased number of splenocytes, relatively pronounced alterations in NK cell subpopulations and an increased NK cell proliferation rate, suggesting a regulatory role for NKG2D in NK-cell development. These NKG2D-deficient mice were also more resistant to infection with a murine cytomegalovirus strain lacking the dominant NK cell antigen m157, presumably as a consequence of perturbed NK cell maturation.\textsuperscript{68} Future studies will have to elucidate the reasons for the discrepancies between these NKG2D-deficient mouse models, which may be due to differences in the gene-targeting strategy or housing conditions.

The upregulation of NKG2DLs stimulates anticancer surveillance via NKG2D-dependent pathways that lead to NK and T cell activation. However, the prolonged expression of NKG2DLs induces the downregulation of NKG2D, in vitro and in vivo, hence promoting immune evasion, as documented in transgenic mice that constitutively and ubiquitously express MICA or Rael.\textsuperscript{69} The ubiquitous expression of human MICA appeared to have no influence on lymphocyte subset distribution, but led to reduced levels of NKG2D on the surface of NK and activated CD8\textsuperscript{+} T cells. This resulted in impaired immune responses against MICA-overexpressing RMA cells as well as against \textit{Listeria}-infected cells.\textsuperscript{62,63,69} Furthermore, a constitutive overexpression of Rael1 in the normal epithelium caused both local and systemic defects in NKG2D-dependent NK cell cytotoxicity. Remarkably, in this model, cutaneous carcinogenesis correlated with the extent of NKG2D downregulation.\textsuperscript{62} Of note, transgenic mice genetically modified to express Rael in the dermal compartment under the control of a doxycycline-inducible promoter exhibit important changes in cellular infiltration during the early phase of local immune responses, resulting in multiple effects on carcinogenesis in the absence of additional pro-inflammatory stimuli.\textsuperscript{70} A more recent study using MICA-transgenic mice evaluated the function of NKG2D in the memory response of CD8\textsuperscript{+} T cells, demonstrating that NKG2D malfunction critically impairs the effector response of tumor-specific memory T cells to NKG2DL-expressing tumors.\textsuperscript{71}

Whereas many cancer patients exhibit increased circulating levels of soluble NKG2DLs, which have been correlated in some studies with disease progression, the shedding of NKG2DLs from mouse cancer cells has not been investigated extensively apart from reports in Rael-transgenic mice.\textsuperscript{66} To gain insights into the functional impact of soluble NKG2DLs in vivo, the mouse prostate cancer TRAMP-C2 cells overexpressing wild-type MICB, soluble MICB (rsMICB) or a shedding-resistant (non-cleaveable) form of MICB (MICB.A2), were implanted in severe combined immunodeficient (SCID) mice. This study revealed that MIC shedding significantly contributes to oncogenesis, as mice receiving cancer cells expressing the shedding-resistant MICB variant did not develop any manifestations of prostate cancer.\textsuperscript{72} In conclusion, multiple studies in animal models confirmed that NKG2D acts as a central mediator of cancer immunosurveillance by promoting the cytotoxic functions of NK and T lymphocytes against malignant cells. This function is compromised by the persistent overexpression of NKG2DLs as well as by their shedding from transformed cells, resulting in decreased amounts of NKG2DLs, and NKG2D downregulation.\textsuperscript{62,72} These studies nurture the promise that immunotherapeutic interventions aimed at restoring the functionality of NKG2D combined with the targeted inhibition of NKG2DL shedding will be of benefit for cancer patients.

**Relevance of the NKG2D/NKG2DL System for Immunotherapy**

Malignant transformation induces the expression of NKG2DLs, as documented in a variety of human and mouse tumors including multiple types of leukemia, multiple myeloma, neuroblastoma, glioblastoma, as well as head and neck, hepatocellular, colorectal and cervical carcinoma.\textsuperscript{36,73} Presumably depending on the specific scenario, NKG2DLs appear to play quite different roles. As mentioned above, the expression of NKG2DLs on the surface of malignant cells enables their immunological clearance, thus preventing oncogenesis and tumor progression. In this respect, high levels of MICA were associated with a good prognosis among colorectal\textsuperscript{74} and breast carcinoma\textsuperscript{75} patients, while reduced NKG2DL expression reportedly correlates with early recurrence among hepatocellular carcinoma patients.\textsuperscript{76} Notably, the interactions of NKG2D with ULBP6 also appear to determine the clinical outcome of allogeneic stem cell transplantation, as a strong association between five distinct single nucleotide polymorphisms (SNPs)
affecting the ULBP6-coding gene (*RAET1L*) and relapse-free survival has been reported.77

Conversely, NKG2DLs released from cancer cells by shedding, exosomal excretion or secretion are responsible for the escape of malignant cells from immune recognition, as they impair NKG2D function by blockade and internalization.54,73 Accumulating evidence indicates that the serum levels of soluble NKG2DLs increase with tumor progression.78 In this context, soluble ULBP2 has been proposed to constitute an indicator of poor prognosis in melanoma patients.79 Moreover, the accumulation of soluble NKG2DLs in the serum of neuroblastoma patients was associated with a drastic reduction in the cytotoxicity of infused donor NK cells upon haploidentical stem cell transplantation.80 Along these lines, Huang and colleagues suggested that screening cancer patients for the levels of MICA or MICB on the surface of transformed cells and the rate of MICA and MICB shedding might be useful to decide on treatment regimens boosting NKG2D expression prior to the adoptive transfer of NKG2D-expressing cells.81

Strategies to overcome the escape of malignant cells from NKG2D-dependent immunosurveillance are of interest for the clinic and include the administration of substances such as HDACis, to upregulate MICA and/or MICB expression,44,46,47 as well as compounds that prevent the shedding of NKG2DLs from tumor cells, hence limiting the inhibition of NKG2D-dependent cytotoxicity in immune effector cells.44,73 For example, the HDACi valproic acid was reported to cause the upregulation of MICA, MICB and ULBP2 by an extracellular signal-regulated kinase (ERK)-dependent signaling cascade, increasing the NKG2D-dependent elimination of myeloma cells by NK cells.82 However, valproic acid has also been described to have negative effects on NK cell functions such as the downregulation of NKG2D and other activating receptors, resulting in the suppression of cytotoxicity and cytokine secretion.53,84 These data indicate that the pleiotropic effects of these compounds, which may vary with concentration and clinical setting, must be carefully considered for the development of immunotherapeutic regimens.

Recent data from a mouse model of breast carcinoma suggest that NKG2D reinforces the antitumor activity of antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4) by suppressing the increased motility of tumor-infiltrating CD8+ T cells normally developing in this context.85 In this setting, the antitumor activity of CD8+ T cells was improved by combining anti-CTLA4 antibodies with ionizing radiation, promoting the expression of NKG2DLs on the surface of transformed cells. These data suggest that anti-CTLA4 immunotherapy which is currently approved by FDA for use in cancer patients86 can be complemented with strategies that enhance NKG2D function, including interventions that promote the expression of NKG2DLs on the surface of cancer cells, neutralize soluble MICA/MICB or prevent MICA/MICB shedding from tumor cells. The activation of NKG2D has also been reported to enhance the antitumor effects of various tumor-targeting antibodies.87,88 Furthermore, substantial antitumor effects have been reported for bispecific fusion proteins that are capable of eliciting NKG2D signaling.89,90 Similarly, the use of cytotoxic lymphocytes targeted to malignant cells with chimeric antigen receptors (CARs) that recognize tumor-associated NKG2DLs provides an appealing strategy for the development of novel immunotherapeutic anticancer interventions.91 Sentman and colleagues have shown that the adoptive transfer of T cells genetically modified to express a NKG2D-targeting CAR exert therapeutic effects in mice bearing various types of cancer.92,93 More recently, T cells have been successfully redirected to Ewing's sarcoma cells with a chimeric NKG2D receptor.94 Still, the antitumor activity of these strategies critically depends on the robust expression of NKG2D by malignant cells as well as on the aversion of tumor-derived factors that promote NKG2D downregulation.

**Outlook**

NKG2D is among the best characterized activating NK receptors with a major relevance for anticancer therapy. Although profound insights into the molecular events that regulate the expression of NKG2DLs on the surface of cancer cells have already been obtained, many questions remain open. For clinical purposes, it will be important to determine the heterogeneity of NKG2D expression within a given tumor as well as the main factors that drive the expression of specific NKG2DLs and their release. Most studies employing small compounds to upregulate the expression of NKG2D that have been performed so far relied on cultured cancer cells, and hence need to be validated in appropriate mouse models. Moreover, the role of soluble NKG2DLs in tumor progression has not yet been clearly defined. Therefore, it will be important to include the quantification of soluble NKG2DLs in the serum of large cohorts of cancer patients and healthy individuals, allowing for the assessment of the prognostic value of circulating NKG2DLs alone or in combination with established biomarkers. In conclusion, harnessing the natural capacity of cytotoxic lymphocytes to eliminate cancer cells in a NKG2D-dependent manner and exploiting the inducible NKG2D expression by malignant cells offer attractive prospects for the development of novel immunotherapeutic anticancer regimens.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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