ELSEVIER

Seminars in Cancer Biology 16 (2006) 333–343

seminars in
CANCER BIOLOGY

www.elsevier.com/locate/semcancer

Review

The role of the NKG2D receptor for tumor immunity

Jérôme D. Coudert, Werner Held *

Ludwig Institute for Cancer Research, Lausanne Branch and University of Lausanne, Ch. des Boveresses 155, 1066 Epalinges, Switzerland

Abstract

Natural killer (NK) cells have originally been identified based on their capacity to kill transformed cells in a seemingly non-specific fashion. Over the last 15 years, knowledge on receptor ligand systems used by NK cells to specifically detect transformed cells has been accumulating rapidly. One of these receptor ligand systems, the NKG2D pathway, has received particular attention, and now serves as a paradigm for how the immune system is able to gather information about the health status of autologous host cells. In addition to its significance on NK cells, NKG2D, as well as other NK cell receptors, play significant roles on T cells. This review aims at summarizing recent insights into the regulation of NKG2D function, the control over NKG2D ligand expression and the role of NKG2D in tumor immunity. Finally, we will discuss first attempts to exploit NKG2D function to improve immunity to tumors.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: NKG2D; Transformation; Tumor immunity; Tumor escape

Contents

* Corresponding author. Tel.: +41 21 692 5958; fax: +41 21 692 5995.
E-mail address: wheld@isrec.unil.ch (W. Held).

1044-579X/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcancer.2006.07.008

1. The NKG2D receptor

1.1. The NKG2D receptor complex

NKG2D belongs to a sub-family of C type lectin-like receptors, which have lost both calcium and carbohydrate binding. The *NKG2D* gene is located in the NK gene complex (on mouse chromosome 6 [1] or human chromosome 12 [2,3]), a locus which harbors a significant number of C-type lectin like receptors that are preferentially expressed in NK cells [4].

NKG2D is a homodimeric, type II (i.e. the C-terminus is extracellular) transmembrane glycoprotein [5]. Like most activating receptors, NKG2D is a multi-subunit receptor complex, whereby NKG2D signaling is mediated by specialized signaling adaptors. Mouse NKG2D can associate with two distinct adaptors DAP-10 and DAP-12/KARAP [6], while human NKG2D exclusively uses DAP-10 [7,8]. Association with these adaptors occurs non-covalently, via oppositely charged amino acids in the respective transmembrane domains [6,7]. Differential adaptor association with murine NKG2D occurs based on the expression of two distinct NKG2D isoforms, which arise due to alternative exon usage. The short (NKG2D-S) and long (NKG2D-L) isoforms differ by their 13 NH_2-terminal amino acids [6]. While DAP-10 associates with both NKG2D isoforms, the extended cytoplasmic domain prevents the association with DAP-12 [6,8], although this has been questioned in a recent report [9]. NKG2D-L is constitutively expressed in resting NK cells. In contrast, the abundance of NKG2D-S increases considerably upon NK cell stimulation with cytokines [6,9].

Finally, one NKG2D homodimer associates with two DAP-10 dimers to form a hexameric complex [10]. In murine NK cells it is thus possible that one NKG2D homodimer simultaneously associates with one DAP-10 and one DAP-12 homodimer. Irrespectively, the regulated association of mouse NKG2D with two distinct signaling adaptors provides a potential for diversity of NKG2D-dependent cellular responses.

1.2. NKG2D receptor expression

The NKG2D receptor is constitutively expressed on most innate immune effector cells of lymphoid origin, including NK cells [7,11–13], most TCRγδ T cells [11,13], and a large fraction of NKT cells [13,14]. In addition, functional NKG2D is found on the recently described subset of murine interferon-producing killer dendritic cells (IKDC), which are of myeloid origin [15,16]. On adaptive immune system cells, NKG2D is constitutively expressed on all human $CD8^+$ T cells and on activated and memory (but not on naive) $CD8^+$ αβ T cells in the mouse [13]. NKG2D is not normally expressed on $CD4^+$ T cells. Notwithstanding, NKG2D is expressed by subsets of circulating and synovial $CD4^+$ TCRαβ T cells in rheumatoid arthritis patients [17] (Table 1).

1.3. NKG2D function

While human NKG2D signals exclusively via DAP-10, mouse NKG2D can associate with both DAP-10 and DAP-12. DAP-12 contains an immunoreceptor tyrosine-based activation motif (ITAM) allowing the recruitment of ZAP-70 and Syk protein tyrosine kinases upon NKG2D engagement [18]. In contrast, DAP-10 lacks an ITAM but instead contains a YINM motif, which closely resembles those found in co-stimulatory molecules. These findings initially suggested that mouse and human NKG2D might mount distinct responses. However, mice deficient for DAP-12 retained significant NKG2D-dependent NK cell mediated killing [6,19]. Moreover, NK cells from Syk/ZAP-70 deficient mice retained significant lytic activity. The residual lytic activity was abrogated by pharmacological blockade of Src family kinases and phosphatidylinositol 3-kinase (PI3-K), which are acting down-stream of DAP-10 [20]. Indeed, the NKG2D–DAP-10 complex was shown to trigger granule release and cytotoxicity following NKG2D crosslinking in human NK cells [21]. Thus, ITAM-independent, DAP-10-dependent signaling is sufficient to trigger NKG2D-dependent cytotoxic function in NK cells. Thus, at least in cytokine (IL-2 or IL-15) activated NK cells and NK cell lines, NKG2D acts as a primary activation receptor. Consistent with the genetic data, the engagement of human NKG2D leads to the recruitment of the p85 subunit of PI3-K [7] and of Grb2 to DAP-10 [22]. Both p85 and Grb2 have to be recruited to DAP-10 for full calcium flux and cell-mediated cytotoxicity [23]. The recruitment of Grb2 provides a link to the phosphorylation of SLP-76, PLC-γ2 and Vav1. These data provide the biochemical basis for how NKG2D-dependent lysis can occur independently from ITAM signaling (Fig. 1).

Besides NK cells, NKG2D is constitutively expressed in human $CD8^+$ T cells and upon activation in mouse $CD8^+$ T cells. Since T cells generally lack DAP-12 expression also in

Table 1
Pattern of the NKG2D receptor expression in human and in mouse

	Human	Mouse
NK cells	All NK cells [7,11]	All NK cells [12,13]
TCRαβ T cells	Naive, activated and memory $CD8^+$ T cells [11]	Activated and memory $CD8^+$ T cells [13]
	Subpopulations of synovial and circulating $CD4^+$	Not expressed on naive $CD8^+$ T cells [13]
	T cells in rheumatoid arthritis patients [17]	Not expressed on $CD4^+$ T cells [13]
TCRγδ T cells	Most blood and IEL TCRγδ T cells [25]	25% of splenic TCRγδ T cells [13]
NKT cells	ND	Large fraction of NKT cells [13,14]
DC	ND	IKDC subset [15,16]
Macrophages	ND	Only mRNA [6,58]

IEL: intestinal intraepithelial lymphocytes, IKDC: interferon producing killer dendritic cells; ND: not determined.

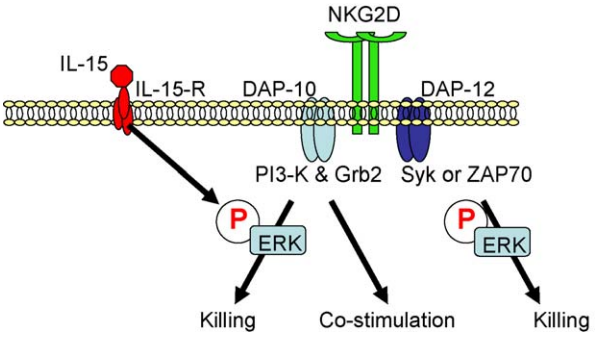

Fig. 1. NKG2D function depends on signaling adaptor usage and additional components of the signaling cascades. NKG2D signals via DAP-10 (human) or DAP-10 and DAP-12/KARAP (mouse). DAP-12 signaling is mediated by ITAMs, which can recruit ZAP-70 or Syk protein tyrosine kinases thereby providing NKG2D with primary activation function. On the other hand, DAP-10 uses a YINM motif to recruit PI3-K and Grb2. The outcome of NKG2D engagement is dependent on additional signaling inputs. For example IL-15 primes constitutive ERK phosphorylation and allows NKG2D/DAP-10 signaling to mediate primary activation function. It is possible that priming by additional signaling inputs can impact NKG2D signal transduction and consequently NKG2D function.

the mouse, NKG2D signaling is thought to occur exclusively via DAP-10. However, in contrast to cytokine activated NK cells, a number of studies have demonstrated that NKG2D engagement enhanced rather than induced $CD8^+$ T cell activation [6,11,13,24–28]. In T cells NKG2D thus serves a co-stimulatory rather than a primary activation function.

Nevertheless, there are instances where NKG2D can act as a primary activation receptor also in T cells. TCR-independent NKG2D-mediated responses can be elicited in long term cultured or cloned human $CD8^+$ T cells [26,29]. In particular, $CD8^+$ T lymphocytes from patients with celiac disease can be activated through NKG2D and mediate TCR-independent cytotoxicity [25,30]. The prolonged exposure of T cells derived from human intestinal epithelium to high amounts of IL-15 seems to be responsible for the change in NKG2D function [25,30]. Indeed, IL-15 not only up-regulated DAP-10 and NKG2D expression but also primed NKG2D signal transduction via the induction of constitutive ERK phosphorylation [30]. The importance of priming for signal transduction has been noted before. As one example, cytokine priming of neutrophils is not sufficient to increase oxidase activity, yet subsequent stimulation through cell surface receptors provokes a response that is larger than in non-primed, activated cells [31]. Thus, the outcome of NKG2D engagement is influenced by additional signaling inputs, and it will be interesting to see how exactly IL-15 primes the NKG2D pathway. Irrespectively of the precise biochemical basis, these data can account for the long known observation that T cell clones acquire NK-like activity upon prolonged culture in high doses of IL-2, a substitute of IL-15. Thus, under certain circumstances, NKG2D can act as a primary activation receptor also in T cells.

A recent report has failed to see any co-stimulatory effect of NKG2D engagement when naive or recently stimulated mouse and human $CD8^+$ T cells were (re-)stimulated *in vitro* [32]. Some of the observed differences in NKG2D function may be related to how NKG2D is triggered (i.e. ligand-tetramer [27], soluble or plate-bound antibodies [6,13,24,27,32], or target cells expressing NKG2D ligands [26,28,32]). Alternatively, as suggested above, the consequence of NKG2D stimulation may be determined to a large extent by differences in the cellular activation status. Consistent with this notion, even in NK cells NKG2D seems to have a co-stimulatory rather than an primary activating role when resting human NK cells are tested [33,34]. Collectively, the data suggest that NKG2D function is generally dependent on additional signaling inputs, such as priming NKG2D signal transduction by cytokines.

In apparent contrast to cell mediated cytotoxicity, NKG2D crosslinking using mAb induced interferon-γ (IFN-γ) secretion by mouse but failed to do so in human NK cells [13,21]. However, NKG2D crosslinking with soluble NKG2D ligand was subsequently able to trigger cytokine production in IL-2 stimulated human NK cells [21,33]. Thus, DAP-10 is also sufficient to mediate cytokine production by human NK cells. Consistent with these data, IL-2 activated NK cells from DAP-12-deficient mice produced IFN-γ, albeit at reduced levels as compared to wild type [6,19]. Yet, NK cells from DAP-12 deficient mice treated with the IFN-inducer polyI:C produced normal amounts of IFN-γ [6]. Thus, in addition to cell-mediated cytotoxicity, ITAM-signaling is also not necessary for NKG2D-dependent cytokine production.

2. NKG2D ligands

2.1. NKG2D ligands

The NKG2D receptor recognizes a significant number of cellular ligands, which belong to distinct and relatively distantly related families. The human MICA/B (MHC class I-chain-related protein A and B) are highly polymorphic molecules [35], with 60 alleles described for MICA and 25 for MICB [36]. NKG2D recognizes all expressed alleles, yet with variable affinity [37]. Like MHC class I molecules, MICA/B are type I transmembrane proteins with three immunoglobulin (Ig)-like domains (α1, α2 and α3). Yet, the overall homology to MHC class I is weak (around 25%) and MICs are not associated with β2-microglobulin or peptide [38]. The *MICA* and *MICB* genes are localized within the human HLA locus on chromosome 6, while they are absent from the mouse MHC locus and have not been found elsewhere.

ULBPs (for UL-16 binding proteins) represent a second family of human NKG2D ligands. ULBPs were recently renamed RAETs due to their homology with the murine *Rae* genes (see below). ULBPs are also distantly related HLA molecules (around 25% homology), yet they contain only two Ig-like domains [39,40]. While ULBP1-3 are inserted into the membrane by glycosylphosphatidylinositol (GPI) anchors, ULBP-4 (Letal) is a transmembrane glycoprotein [40,41]. The ULBP genes are clustered in the telomeric region of human chromosome 6; a corresponding region with NKG2D ligands is found on mouse chromosome 10. This region harbors orthologous MHC class I related *Rae* (for retinoic acid early transcripts) genes [42].

The five murine Rae-1α–ε molecules are GPI-anchored proteins containing two Ig-like domains (α1 and α2). In addition,

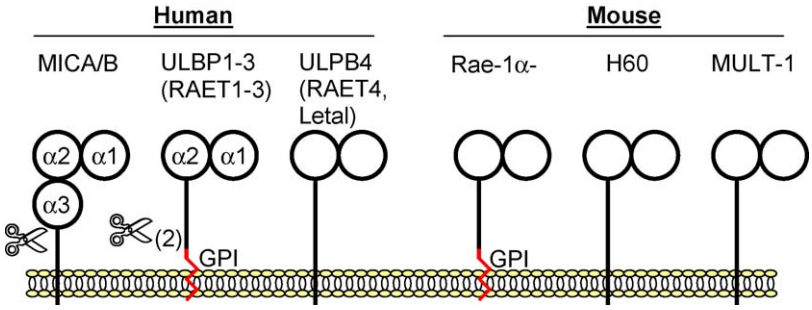

Fig. 2. Schematic representation of NKG2D ligands expressed in human (MICs, ULBPs) and in mouse (Rae-1s, H60 and MULT-1). MICs contain three Ig-like domains whereas the other ligands contain two Ig-like domains. MICs, ULPB4, H60 and MULT-1 are transmembrane proteins, while ULBP1–3 and Rae-1s are inserted into the membrane by glycosylphosphatidylinositol (GPI) anchors. MICA/B and ULBP2 can be released from the cell surface by the action of metalloproteinases.

the Rae-1 locus contains MULT-1 (Murine UL-16-binding protein like transcript 1) and H60, two type I transmembrane proteins, which share 17% and 30% with MHC class I molecules, respectively [43,44]. H60 has originally been identified as a minor histocompatibility antigen by immunizing C57BL/6 mice with MHC identical BALB.b cells [45]. Indeed, C57BL/6 mice do not express this particular NKG2D ligand (Fig. 2).

NKG2D has the ability to interact with a significant number of distinct ligands with affinities ranging from 4 to 800 nM [46–48]. Both chains of the NKG2D homodimer contribute to the interaction with the different monomeric ligands, making contacts with either the α1 or α2 domain of the ligand. Thus, the symmetric, homodimeric NKG2D receptor binds asymmetric ligands, consequently the contribution of the individual NKG2D chains is unequal. Indeed, the resolution of NKG2D as well as NKG2D/ligand crystal structures revealed that NKG2D uses a relatively large, flat surface for ligand binding. Upon complex formation with ligand, NKG2D seems to undergo conformational adjustments, whereby identical NKG2D residues may adopt different conformations in the receptor subunits. This structural plasticity seems to enable the binding of distinct ligands using common elements of the NKG2D binding platform [49,50]. These elements are conserved to the extent that mouse and human NKG2D, which are only 69% identical in their ectodomains [50], can recognize most ligands of the other species [37]. Indeed, the conserved ligand binding modality of NKG2D allows xeno-recognition, with implications for xenotransplantation [51].

2.2. Developmental regulation of NKG2D ligands

NKG2D ligands, like Rae-1 or MICA/B are not expressed in most tissues in healthy adult mice and human [52,53]. ULBP1-3 mRNA is expressed in various healthy tissues [39] and ULBP4 mRNA expression is detected in the skin [40]. Likewise, MULT-1 mRNA is expressed in a wide variety of tissues [43,44]. Rae-1β and Rae-1δ mRNA expression is detected in the early embryo, particularly in the brain [52]. However, it is currently unclear to what extent ULBP4 and MULT-1 are present at the cell surface.

However, NKG2D ligands are expressed at the cell surface during normal hematopoiesis in the adult. In human, MICA and ULBP3 are expressed on bone marrow stem cells [54]. In mice, bone marrow cells express low levels of Rae-1 and H60 but not MULT-1 [55]. Interestingly, the levels of NKG2D ligands on bone marrow cells vary in different mouse strains [55]. Indeed, these differences seem to play a role for the rejection of bone marrow grafts by recipient NK cells, which target NKG2D ligand-expressing myeloid progenitors in the spleen of recipient mice [55]. H60 is clearly detected on immature $CD4^+8^+$ thymocytes from BALB/c mice [56]. Embryonic and developmentally regulated expression of NKG2D ligand does not seem to pose a problem with regard to autoimmunity. The immune system was either not yet developed or the relevant tissues do not contain functionally mature NK cells.

2.3. Defined signals that induce NKG2D ligand expression

The first insights into the regulation of NKG2D ligands stemmed from the finding that the MICA/B promoters resembled those of heat-shock proteins, such as HSP70 [53]. Indeed, heat shock was sufficient to induce MICA expression on epithelial cells. Thus, the finding that MICA was recognized by the NKG2D receptor [11] provided the first evidence that NK cells may be able to detect stressed cells.

Similarly, the identification of Rae-1 as NKG2D ligands [57,58] immediately provided information regarding the regulation of these ligands. Retinoic acid, an agent used to differentiate cells, rapidly induced Rae-1α, β, γ expression in embryocarcinoma cells *in vitro* [52]. Retinoic acid also induces MICA/B expression in a hepatoma cell line [59]. Thus, consistent with the developmentally regulated expression, cellular differentiation can be associated with NKG2D ligand expression.

T cell activation *in vitro* using peptide antigen pulsed APC or ConcanavalinA (ConA) also leads to NKG2D ligand up-regulation [58,60]. This may provide NK cells with an opportunity to regulate adaptive immune responses.

Finally, NKG2D ligand induction is detectable in the context of infection. Infection of epithelial cell lines with enteric bacteria resulted in a marked and rapid increase of MICA expression. This was dependent on the interaction of bacterial adhesin with its cellular receptor CD55 [61]. These findings provide a likely basis for the prominent MICA/B expression on intestinal epithelial cells in healthy humans [53].

Human macrophages infected with either Influenza or Sendai virus up-regulate MICB. This stimulates NKG2D-dependent

IFN-γ release by NK cells [62]. In fact, NKG2D ligands can be induced on murine macrophages using various Toll-like receptor (TLR) agonists [63]. Similarly, mouse cytomegalovirus (MCMV) infected DC and natural interferon-producing cells transiently express Rae-1, which is dependent on TLR9 [64]. The coculture of NK cells with infected DC enhanced the lysis of YAC-1 cells [65], which is in part dependent of NKG2D engagement [13].

The importance of the NKG2D pathway in conferring a protective effect against viral infections is further highlighted by the fact that viruses have evolved ways to interfere with the induction of NKG2D ligands (for review see [66]). Thus, the NKG2D receptor system is capable of detecting various types of infections by exploiting the capacity of intracellular sensing mechanisms to induce cell surface expression of NKG2D ligands. Similar mechanisms seem to be used to detect non-infectious cellular stress.

2.4. NKG2D ligand expression on tumor cells

In contrast to the transient expression of NKG2D ligands during development or in normal cells due to exogenous or endogenous stimuli, a large fraction of tumor cells express NKG2D ligands constitutively. MICA/B expression is detected on many types of epithelial tumor cell lines of different tissue origins [11,53,59,67–69] but less in myeloid and lymphoblastic leukemia [70–73]. In contrast, ULBPs are rarely found on epithelial tumors but are preferentially expressed on T cell leukemia cell lines [74] as well as on freshly isolated lymphoid leukemia cells [70]. Rae-1 and H60 are up-regulated in skin treated with carcinogens [75] and are found on skin, renal and lung carcinoma cell lines [75,76]. The murine NKG2D ligands H60 and Rae-1 are also found on numerous hematopoietic tumor cell lines [57,58,77].

In general, the basis for NKG2D ligand expression during transformation is poorly understood. However, in methylcholanthrene (MCA)-induced fibrosarcoma or human breast cancer cells, the adenovirus E1A oncogene, was shown to induce Rae-1 expression [78]. Along the same lines, in chronic myeloid leukemia (CML), BCR/ABL oncogene also controls MICA cell surface expression on primary leukemic bone marrow cells [79]. Further, embryonic fibroblasts deficient for JunB exhibit an enhanced expression of Rae-1ε and MULT-1 [80]. JunB exerts tumor suppressor activity through the negative regulation of c-jun function [81]. Thus, the data indicate that NKG2D ligand up-regulation is associated with transformation, with a role for both oncogenes and tumor suppressors.

However, the expression of several oncogenes (including K-ras, Akt, c-myc, E6, E7, E1A or Ras V12) or the lack of a tumor suppressor (p53) was not sufficient to induce NKG2D ligand expression in primary ovarian cells *in vitro* [82]. Transformation of primary cells is thus not sufficient for NKG2D ligand induction. Rather, DNA damage, induced by ionizing radiation or alkylating agents, up-regulated NKG2D ligands in primary mouse lymphoid cells (Rae-1, MULT-1) and human fibroblasts (MICA, ULBPs) [82]. Double strand DNA breaks induce the phosphorylation of ATM and ATR. These kinases subsequently phosphorylate effector kinases, which induce cell cycle arrest and apoptosis. At the same time, this pathway is also used to up-regulate NKG2D ligands. Thus, damaged cells are subjected to an additional level of quality control, which involves immune recognition. The chronic activity of the DNA damage response pathway may account for the constitutive expression of NKG2D ligands in tumor cell lines [82].

Notwithstanding, the control over NKG2D ligand expression is still incompletely understood. It seems likely that besides ATM/ATR additional sensing mechanisms exist, which detect alterations in the intracellular physiology and are capable of reporting such changes to the cell surface. Undoubtedly, the underlying sensing and signaling pathways will be an area of intense future research.

3. Role of NKG2D in cancer

3.1. NKG2D-dependent tumor cell graft rejection

Soon after the discovery of murine NKG2D ligands, two groups reported experimental evidence that recognition by NKG2D played an important role in tumor graft rejection *in vivo*. NKG2D ligand-expressing tumor cell grafts were efficiently rejected, while the parental, NKG2D-ligand negative tumor cell lines formed tumors [83,84]. Depending on the tumor cell line used, rejection was mediated by NK cells or the combined action of NK cells and $CD8^+$ T cells [83]. Moreover, NKG2D-dependent tumor graft rejection required perforin [85].

Importantly, the recognition of NKG2D ligand-expressing tumor cells induced T cell memory capable of controlling a subsequent challenge with the respective NKG2D ligand-negative tumor cells [83,86]. A possible scenario is that recognition via NKG2D mediates NK cell-dependent killing of tumor cells and cytokine release. The presence of tumor cell debris together with inflammatory cytokines may improve the activation of DC, thereby mounting a relatively efficient adaptive immune response directed against various tumor cell-associated antigens. This may improve the generation of immunological memory and allow the subsequent targeting of NKG2D-negative tumor cells [83,86].

3.2. NKG2D and tumor immunosurveillance

Recent experiments have provided evidence that NKG2D played a significant role for immunosurveillance against chemically induced tumors. The intradermal administration of the carcinogen MCA induces fibrosarcomas in mice. Alternatively, skin painting with dimethyl-benzanthracene (DMBA) and the phorbol ester TPA induces papillomas and sarcomas. During the development of such chemically induced skin tumors NKG2D ligands are induced in transformed cells, while they are absent from normal skin cells. Importantly, the tumor incidence and severity was enhanced in the absence of TCRγδ T cells [75], consistent with a protective role for the NKG2D pathway. Similarly, *in vivo* blockade of NKG2D using mAbs significantly exacerbated tumor development upon MCA treatment. Such MCA-induced fibrosarcomas, which expressed Rae-1, failed to

grow in wild type mice due to NKG2D-dependent NK cell-mediated rejection [87].

Consistent with such mouse data, MIC over-expressing tumors seem to contain larger lymphocytic infiltrates as compared to MIC-negative tumors [67,88]. Moreover, high level of NKG2D ligand expression is correlated with a favorable prognosis in colorectal cancer [89].

Despite the evidence that NKG2D engagement plays a key role in tumor immuno-surveillance in a number of model systems, it is important to note that direct evidence for a role of the NKG2D pathway in the control of spontaneously arising tumors is currently lacking [90].

4. Escape from NKG2D-mediated immunosurveillance

Despite the likely induction of NKG2D ligands at some point during the development of most types of cancers, NKG2D-dependent immune reactions may eventually fail and overt tumors develop. Thus, many NKG2D-ligand positive tumors progress, providing circumstantial evidence that *in vivo* NKG2D function may be impaired at some stage during tumor progression. Similar to viruses, tumor cells seem to have means to counteract NKG2D-dependent attack.

4.1. Escape mechanisms acting on NKG2D ligands

During cancer progression, immune pressure on the tumor may lead to the selection of cells devoid of NKG2D ligands. Consistent with this view, in cancer patients, most primary tumors seem to express NKG2D ligands, whereas more advanced tumors and metastasis express low levels, if any, ligand [91–93]. Thus, variants expressing low levels of NKG2D ligand may be selected during cancer progression.

Metalloproteinases have been shown to cleave MICA/B off the cell surface of tumor cells [94,95]. This reduces MICA/B cell surface levels and limits recognition by NKG2D-expressing effector cells. In addition, soluble NKG2D ligands are detected at high titers in the serum. Upon binding to NKG2D, soluble MICA induces the internalization and lysosomal degradation of the NKG2D receptor on $CD8^+$ T cells and NK cells [72,95–97], further reducing the efficacy of NKG2D recognition. The serum titer of MICA/B is now used as a prognostic factor in cancer patients. High MICA/B titers correlate with an elevated tumor burden and the presence of metastasis [98]. Besides MICA/B, the GPI-anchored ULBP2 can also be cleaved by metalloproteinases and soluble ULBP2 is detected in the serum of some patients with hematopoietic malignancies [99]. Thus, NKG2D ligand cleavage impairs tumor cell recognition by reducing the level of the NKG2D ligand as well as that of their receptor.

Additional, soluble factors negatively regulate NKG2D ligand density. TGF-β represents a major immunosuppressive cytokine produced by tumor cells. With regard to the NKG2D pathway, MICA transcription was low in a human glioma cell line secreting TGF-β. Reducing TGF-β production by RNA interference strongly enhanced MICA expression and promoted tumor cell recognition by $CD8^+$ T and NK cells [100]. Thus, TGF-β directly impacts NKG2D ligand expression. Additional roles of TGF-β will be discussed below. IFN-γ is well known for its capacity to up-regulate MHC class I expression and consequently to improve T cell responses against tumor cells. At the same time, IFN-γ can render certain susceptible target cells resistant to NK cell responses *in vitro* and *in vivo* [101]. This was attributed to an up-regulation of MHC class I molecules, which are recognized by inhibitory NK cell receptors. It now turns out that the treatment of tumor cells with IFN-γ down-regulates the NKG2D ligand H60 (and to a lesser extent MULT-1 mRNA)[102]. Thus, the combination of increased MHC class I expression with decreased NKG2D ligand may be responsible for the resistance of IFN-γ treated tumor cells to NK cells.

4.2. Mechanisms acting on the NKG2D receptor

Soluble NKG2D ligands induce the down-regulation of the NKG2D receptor. This results in a moderate reduction of NKG2D function [103]. In contrast, the chronic engagement of NKG2D with cell-bound NKG2D ligand can have profound effects on NKG2D function [104]. The effect actually exceeds that predicted based on the reduction of NKG2D levels since NKG2D-dependent NK cell-mediated cytotoxicity was completely abolished [104]. In addition, certain NKG2D-independent NK cell activation pathways were also rendered dysfunctional (JDC and WH, unpublished results). Deficient NKG2D function was accounted for by the near complete absence of the signaling adaptors DAP-10 and DAP-12 from NKG2D receptor complexes. In addition to DAP-12 and DAP-10, CD3ζ was also absent in NK cells chronically stimulated via tumor cell bound NKG2D ligands [104]. Interestingly, CD3ζ is not associated with NKG2D, therefore the precise connection between CD3ζ loss and chronic NKG2D engagement is currently unknown. However, it is interesting to note that the loss of CD3ζ has also been observed in chronically activated NK cells and/or T cells in cancer patients [105]. It is thus tempting to speculate that CD3ζ loss and NK cell (and/or T cell) dysfunction reflects the chronic exposure to cells expressing NKG2D ligands. In our model, NK cell dysfunction was not permanent, as NK cells recovered within a few hours of their separation from NKG2D ligand-positive tumor cells [104], suggesting that NK cell inactivation may be overt only at or within an NKG2D ligand expressing tumor.

The enforced constitutive expression of NKG2D ligands (Rae-1β, Rae-1ε or MICA) as transgenes in mice has confirmed that sustained encounter of NKG2D ligands impaired NKG2D functions *in vivo* [55,106,107]. NKG2D-dependent NK cell inactivation occurred irrespectively of which NKG2D ligand was expressed or in which tissue it was expressed. Thus, sustained NKG2D ligand encounter can promote NK cell dysfunction *in vitro* and *in vivo*.

NKG2D dysfunction was also observed when mouse NK cells lacked DAP-12, suggesting that signaling via DAP-10 is sufficient for NK cell inactivation [104]. This raises the possibility that $CD8^+$ T cells and human NK cells may similarly be susceptible to the inactivation via the NKG2D pathway. Indeed, human NK cells infiltrating a lung adenocarninoma showed

impaired cytotoxic activity associated with low NKG2D levels [93].

These findings obviously contrast with the above cited data highlighting a prominent role of NKG2D ligands for the induction of anti-tumor immunity. It will thus be crucial to precisely define the factors which determine the consequences of NKG2D engagement, i.e. reactivity versus inactivity. The outcome may be determined by numerous factors including the growth rate of the tumor cells, the levels, timing and nature of the expressed NKG2D ligands and the site of tumor formation [108,109] and perhaps also to what extent the NKG2D pathway is primed by additional signaling inputs.

4.3. Role of soluble factors for escape from NKG2D-mediated immunosurveillance

In addition to the cognate interaction with NKG2D ligands, tumor-derived or micro-environmental soluble factors are able to suppress immunosurveillance against cancer. Cancer patients often present an increased concentration of TGF-β in their serum. In addition to an effect on NKG2D ligands [100], *in vitro* experiments showed that the culture of NK cells in the presence of TGF-β down-regulated NKG2D receptor expression [110,111]. The decreased expression was associated with impaired NKG2D-dependent killing of tumor cells expressing NKG2D ligands [100,110]. NKG2D down-modulation was also observed in an *in vivo* model, in which a TGF-β secreting carcinoma cell line was grafted into mice. This reduced NKG2D cell surface expression was accompanied with an impairment of NKG2D-dependent killing [112]. The molecular mechanism for the TGF-β-dependent NKG2D loss has not been clarified, even though reductions in NKG2D mRNA amounts have been noted in some cases [100,110,112].

In addition to tumor cells, TGF-β may also derive from tumor-infiltrating cells such as regulatory T cells (Treg). In fact, Treg are able to suppress NK cell-mediated tumor rejection [113,114]. This may explain the inverse correlation between NK cell function and patient survival with Treg accumulation in the tumor [115].

Besides TGF-β, IFN-γ was also shown to down-regulate NKG2D expression and dampen NK cell-mediated lysis of MICA-expressing tumor cells [116,117]. Since IFN-γ is a major product of NK cells and $CD8^+$ T cells, these cells may limit NKG2D-dependent effector function via a negative regulatory loop. In this context, it is interesting to note that chronic NKG2D engagement actually stimulates sustained IFN-γ while cytotoxicity is abrogated [104], which may further amplify NKG2D dysfunction.

5. NKG2D-dependent immunotherapy of cancer

5.1. NKG2D-dependent tumor targeting

NKG2D recognizes conserved ligands, which are constitutively expressed on many transformed but not on most normal cells, providing opportunities for immunotherapy of cancer. To begin to exploit NKG2D recognition, chimeric anti-tumor mAb/NKG2D-ligand reagents have been generated. The antibody portion is used for specific tumor cell targeting, while the NKG2D ligand re-directs NKG2D-expressing effector cells. An anti-CEA (carcinoembryonic antigen)/MICA chimera did specifically bind CEA^+ human tumor cells and enhanced the *in vitro* lysis by NK cells in a NKG2D-dependent manner [118]. Similarly, H60/anti-CEA or H60/anti-survivin chimeric proteins improved NK cell and $CD8^+$ T cell-mediated tumor elimination *in vivo* [119,120]. Such approaches may be of particular interest to target tumors with very low or deficient expression of endogenous NKG2D ligands, such as acute myeloid leukemia [121].

5.2. Modified NKG2D receptors in T cells

In the mouse, $CD8^+$ T cells express NKG2D only upon activation through the TCR. As discussed above, in these cells NKG2D seems to play a costimulatory rather than a primary activating function. An NKG2D receptor fused to the cytoplasmic portion of CD3ζ conferred primary activation function to splenic T cells in response to NKG2D ligand-bearing tumor cells *in vitro*. In addition, upon adoptive transfer, the genetically modified T cells protected mice from transplanted NKG2D ligand-expressing tumor cells. Importantly, it also induced a memory response, which was able to reject parental NKG2D ligand-negative tumor cells [122]. Along the same lines, the transgenic expression of DAP-12 allows mouse T cells to kill Rae-1β expressing tumor cells in a TCR-independent but NKG2D-dependent fashion [123]. These data provide an entry point to try to exploit NKG2D expression by T cells in adoptive immunotherapy of cancer.

5.3. NKG2D and cytokine-dependent tumor therapy

It is known that certain cytokines exert anti-tumor effects raising the possibility that some of the beneficial effects are mediated by NKG2D. For instance, mice treated with IL-21, a cytokine produced by activated T cells, reject tumor cells more efficiently than control mice. Indeed, IL-21 up-regulated NK cell mediated NKG2D-dependent tumor cell lysis *in vitro* and the rejection of grafted tumor cells *in vivo* [124]. Similarly, mice receiving repeated doses of IL-12 develop significantly fewer MCA-induced sarcomas as compared to controls. Neutralization of NKG2D reversed the efficacy of IL-12 therapy, as the mice displayed a higher incidence of sarcomas [87]. Finally, IFN-α as been known for a long time to improve NK cell function (for review see Ref. [125]), which is mediated at least in part by increased NKG2D function [117]. IFN-α up-regulates NKG2D cell surface expression (A. Chalifour, W. Held, unpublished observation). Moreover, it is possible that some of the above cytokines improve NKG2D function by acting on NKG2D signal transduction.

5.4. Induction of NKG2D ligands

The activation of the DNA damage response pathway is able to induce the expression of NKG2D ligands in mouse and

human cells. Indeed, DNA damage induced by irradiation or alkylating compounds commonly used in chemotherapy of cancer were both effective [82]. Similarly, ULBP3 and MICA are up-regulated by transretinoic acid in patients with chronic B cell lymphocytic leukemia (B-CLL) [126]. These treatments rendered cells susceptible to killing by autologous NKG2D-expressing effector cells [82]. These data raise the interesting issue of whether the effectiveness of conventional anti-cancer therapies is mediated in part through the up-regulation of NKG2D ligands, rather than their direct cytotoxic or cytostatic effects on tumor cells. In addition, the data suggest that anti-cancer drugs should routinely be tested for their capacity to induce NKG2D ligands on tumor cells.

Finally, treatment modalities, which induce NKG2D ligands combined with immunotherapy approaches involving NKG2D-based recognition may represent a powerful way to improve cancer treatment. Indeed, the effectiveness of combined tumor targeting using oncolytic viruses and immunotherapy has just been reported [127].

6. Concluding remarks

For a number of reasons, the NKG2D recognition system represents a promising entry point to induce and/or improve immune responses to cancer. First, NKG2D ligands are generally poorly and only transiently expressed on healthy tissues, while they are constitutively expressed at significant levels on tumor cells. Second, NKG2D ligands are expressed on a broad variety of tumor cells of distinct tissue origins. Third, in situations where NKG2D ligands are poorly expressed, it may be possible to enhance their expression using radiations and/or chemotherapies. Fourth, NKG2D is expressed on all NK cells and also on a substantial fraction of T lymphocytes, providing a large number of potential effector cells. Fifth, cytokines may be used to improve NKG2D function. Finally, NKG2D-mediated adoptive immunotherapy should in principal be applicable to all individuals as the NKG2D receptor is monomorphic. Therefore, it may be possible in the future to fully exploit the fact that diseased cells have the capacity to alert the immune system, in order to boost immune recognition of cancer.

Acknowledgements

We thank Jonathan Back and Anick Chalifour for critical reading of the manuscript. Research in this laboratory is supported in part by grants from Oncosuisse and the Swiss National Science Foundation to W.H.

References

[1] Ho EL, Heusel JW, Brown MG, Matsumoto K, Scalzo AA, Yokoyama WM. Murine Nkg2d and Cd94 are clustered within the natural killer complex and are expressed independently in natural killer cells. Proc Natl Acad Sci USA 1998;95(11):6320–5.

[2] Glienke J, Sobanov Y, Brostjan C, Steffens C, Nguyen C, Lehrach H, et al. The genomic organization of NKG2C, E, F, and D receptor genes in the human natural killer gene complex. Immunogenetics 1998;48(3):163–73.

[3] Renedo M, Arce I, Montgomery K, Roda-Navarro P, Lee E, Kucherlapati R, et al. A sequence-ready physical map of the region containing the human natural killer gene complex on chromosome 12p12.3–p13.2. Genomics 2000;65(2):129–36.

[4] Yokoyama WM, Plougastel BF. Immune functions encoded by the natural killer gene complex. Nat Rev Immunol 2003;3(4):304–16.

[5] Wolan DW, Teyton L, Rudolph MG, Villmow B, Bauer S, Busch DH, et al. Crystal structure of the murine NK cell-activating receptor NKG2D at 1.95 A. Nat Immunol 2001;2(3):248–54.

[6] Diefenbach A, Tomasello E, Lucas M, Jamieson AM, Hsia JK, Vivier E, et al. Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D. Nat Immunol 2002;3(12):1142–9.

[7] Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, et al. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 1999;285(5428):730–2.

[8] Rosen DB, Araki M, Hamerman JA, Chen T, Yamamura T, Lanier LL. A Structural basis for the association of DAP12 with mouse, but not human, NKG2D. J Immunol 2004;173(4):2470–8.

[9] Rabinovich B, Li J, Wolfson M, Lawrence W, Beers C, Chalupny J, et al. NKG2D splice variants: a reexamination of adaptor molecule associations. Immunogenetics 2006;58(2/3):81–8.

[10] Garrity D, Call ME, Feng J, Wucherpfennig KW. The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure. Proc Natl Acad Sci USA 2005;102(21):7641–6.

[11] Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999;285(5428):727–9.

[12] Vance RE, Tanamachi DM, Hanke T, Raulet DH. Cloning of a mouse homolog of CD94 extends the family of C-type lectins on murine natural killer cells. Eur J Immunol 1997;27(12):3236–41.

[13] Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity 2002;17(1):19–29.

[14] Gumperz JE, Miyake S, Yamamura T, Brenner MB. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. J Exp Med 2002;195(5):625–36.

[15] Taieb J, Chaput N, Menard C, Apetoh L, Ullrich E, Bonmort M, et al. A novel dendritic cell subset involved in tumor immunosurveillance. Nat Med 2006;12(2):214–9.

[16] Chan CW, Crafton E, Fan HN, Flook J, Yoshimura K, Skarica M, et al. Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. Nat Med 2006;12(2):207–13.

[17] Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci USA 2003;100(16):9452–7.

[18] Lanier LL, Corliss BC, Wu J, Leong C, Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature 1998;391(6668):703–7.

[19] Zompi S, Hamerman JA, Ogasawara K, Schweighoffer E, Tybulewicz VL, Di JP, et al. NKG2D triggers cytotoxicity in mouse NK cells lacking DAP12 or Syk family kinases. Nat Immunol 2003;4(6):565–72.

[20] Colucci F, Schweighoffer E, Tomasello E, Turner M, Ortaldo JR, Vivier E, et al. Natural cytotoxicity uncoupled from the Syk and ZAP-70 intracellular kinases. Nat Immunol 2002;3(3):288–94.

[21] Billadeau DD, Upshaw JL, Schoon RA, Dick CJ, Leibson PJ. NKG2D-DAP10 triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. Nat Immunol 2003;4(6):557–64.

[22] Chang C, Dietrich J, Harpur AG, Lindquist JA, Haude A, Loke YW, et al. Cutting edge: KAP10, a novel transmembrane adapter protein genetically linked to DAP12 but with unique signaling properties. J Immunol 1999;163(9):4651–4.

[23] Upshaw JL, Arneson LN, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ. NKG2D-mediated signaling requires a DAP10-bound Grb2–Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. Nat Immunol 2006;7(5):524–32.

[24] Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T. Costimulation of CD8alphabeta T cells by NKG2D via engagement

by MIC induced on virus-infected cells. Nat Immunol 2001;2(3):255–60.
[25] Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC, et al. NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. J Immunol 2001;167(10):5527–30.
[26] Verneris MR, Karami M, Baker J, Jayaswal A, Negrin RS. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8+ T cells. Blood 2004;103(8):3065–72.
[27] Maasho K, Opoku-Anane J, Marusina AI, Coligan JE, Borrego F. NKG2D is a costimulatory receptor for human naive CD8+ T cells. J Immunol 2005;174(8):4480–4.
[28] Markiewicz MA, Carayannopoulos LN, Naidenko OV, Matsui K, Burack WR, Wise EL, et al. Costimulation through NKG2D enhances murine CD8+ CTL function: similarities and differences between NKG2D and CD28 costimulation. J Immunol 2005;175(5):2825–33.
[29] Maccalli C, Pende D, Castelli C, Mingari MC, Robbins PF, Parmiani G. NKG2D engagement of colorectal cancer-specific T cells strengthens TCR-mediated antigen stimulation and elicits TCR independent anti-tumor activity. Eur J Immunol 2003;33(7):2033–43.
[30] Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity 2004;21(3):357–66.
[31] Hallett MB, Lloyds D. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. Immunol Today 1995;16(6):264–8.
[32] Ehrlich LI, Ogasawara K, Hamerman JA, Takaki R, Zingoni A, Allison JP, et al. Engagement of NKG2D by cognate ligand or antibody alone is insufficient to mediate costimulation of human and mouse CD8+ T cells. J Immunol 2005;174(4):1922–31.
[33] Andre P, Castriconi R, Espeli M, Anfossi N, Juarez T, Hue S, et al. Comparative analysis of human NK cell activation induced by NKG2D and natural cytotoxicity receptors. Eur J Immunol 2004;34(4):961–71.
[34] Bryceson YT, March ME, Ljunggren HG, Long EO. Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion. Blood 2006;107(1):159–66.
[35] Elsner HA, Schroeder M, Blasczyk R. The nucleotide diversity of MICA and MICB suggests the effect of overdominant selection. Tissue Antigens 2001;58(6):419–21.
[36] Bahram S, Inoko H, Shiina T, Radosavljevic M. MIC and other NKG2D ligands: from none to too many. Curr Opin Immunol 2005;17(5):505–9.
[37] Steinle A, Li P, Morris DL, Groh V, Lanier LL, Strong RK, et al. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics 2001;53(4):279–87.
[38] Bahram S, Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci USA 1994;91(14):6259–63.
[39] Cosman D, Mullberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. Immunity 2001;14(2):123–33.
[40] Jan Chalupny N, Sutherland CL, Lawrence WA, Rein-Weston A, Cosman D. ULBP4 is a novel ligand for human NKG2D. Biochem Biophys Res Commun 2003;305(1):129–35.
[41] Conejo-Garcia JR, Benencia F, Courreges MC, Khang E, Zhang L, Mohamed-Hadley A, et al. Letal, A tumor-associated NKG2D immunoreceptor ligand, induces activation and expansion of effector immune cells. Cancer Biol Ther 2003;2(4):446–51.
[42] Radosavljevic M, Cuillerier B, Wilson MJ, Clement O, Wicker S, Gilfillan S, et al. A cluster of ten novel MHC class I related genes on human chromosome 6q24.2–q25.3. Genomics 2002;79(1):114–23.
[43] Carayannopoulos LN, Naidenko OV, Fremont DH, Yokoyama WM. Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D. J Immunol 2002;169(8):4079–83.
[44] Diefenbach A, Hsia JK, Hsiung MY, Raulet DH. A novel ligand for the NKG2D receptor activates NK cells and macrophages and induces tumor immunity. Eur J Immunol 2003;33(2):381–91.
[45] Malarkannan S, Shih PP, Eden PA, Horng T, Zuberi AR, Christianson G, et al. The molecular and functional characterization of a dominant minor H antigen, H60. J Immunol 1998;161(7):3501–9.
[46] Carayannopoulos LN, Naidenko OV, Kinder J, Ho EL, Fremont DH, Yokoyama WM. Ligands for murine NKG2D display heterogeneous binding behavior. Eur J Immunol 2002;32(3):597–605.
[47] O'Callaghan CA, Cerwenka A, Willcox BE, Lanier LL, Bjorkman PJ. Molecular competition for NKG2D: H60 and RAE1 compete unequally for NKG2D with dominance of H60. Immunity 2001;15(2):201–11.
[48] Li P, Morris DL, Willcox BE, Steinle A, Spies T, Strong RK. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. Nat Immunol 2001;2(5):443–51.
[49] Radaev S, Kattah M, Zou Z, Colonna M, Sun PD. Making sense of the diverse ligand recognition by NKG2D. J Immunol 2002;169(11):6279–85.
[50] McFarland BJ, Kortemme T, Yu SF, Baker D, Strong RK. Symmetry recognizing asymmetry: analysis of the interactions between the C-type lectin-like immunoreceptor NKG2D and MHC class I-like ligands. Structure 2003;11(4):411–22.
[51] Forte P, Lilienfeld BG, Baumann BC, Seebach JD. Human NK cytotoxicity against porcine cells is triggered by NKp44 and NKG2D. J Immunol 2005;175(8):5463–70.
[52] Nomura M, Zou Z, Joh T, Takihara Y, Matsuda Y, Shimada K. Genomic structures and characterization of Rae1 family members encoding GPI-anchored cell surface proteins and expressed predominantly in embryonic mouse brain. J Biochem (Tokyo) 1996;120(5):987–95.
[53] Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc Natl Acad Sci USA 1996;93(22):12445–50.
[54] Poggi A, Prevosto C, Massaro AM, Negrini S, Urbani S, Pierri I, et al. Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol 2005;175(10):6352–60.
[55] Ogasawara K, Benjamin J, Takaki R, Phillips JH, Lanier LL. Function of NKG2D in natural killer cell-mediated rejection of mouse bone marrow grafts. Nat Immunol 2005;6(9):938–45.
[56] Li J, Rabinovich BA, Hurren R, Cosman D, Miller RG. Survival versus neglect: redefining thymocyte subsets based on expression of NKG2D ligand(s) and MHC class I. Eur J Immunol 2005;35(2):439–48.
[57] Cerwenka A, Bakker AB, McClanahan T, Wagner J, Wu J, Phillips JH, et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. Immunity 2000;12(6):721–7.
[58] Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol 2000;1(2):119–26.
[59] Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, et al. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int J Cancer 2003;104(3):354–61.
[60] Rabinovich BA, Li J, Shannon J, Hurren R, Chalupny J, Cosman D, et al. Activated, but not resting, T cells can be recognized and killed by syngeneic NK cells. J Immunol 2003;170(7):3572–6.
[61] Tieng V, Le Bouguenec C, du Merle L, Bertheau P, Desreumaux P, Janin A, et al. Binding of *Escherichia coli* adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. Proc Natl Acad Sci USA 2002;99(5):2977–82.
[62] Siren J, Sareneva T, Pirhonen J, Strengell M, Veckman V, Julkunen I, et al. Cytokine and contact-dependent activation of natural killer cells by influenza A or Sendai virus-infected macrophages. J Gen Virol 2004;85(Pt 8):2357–64.
[63] Hamerman JA, Ogasawara K, Lanier LL. Cutting edge: Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. J Immunol 2001-;172(4):5.
[64] Krug A, French AR, Barchet W, Fischer JA, Dzionek A, Pingel JT, et al. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. Immunity 2004;21(1):107–19.

[65] Andoniou CE, van Dommelen SL, Voigt V, Andrews DM, Brizard G, Asselin-Paturel C, et al. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. Nat Immunol 2005;6(10):1011–9.
[66] Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nat Rev Immunol 2001;1(1):41–9.
[67] Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc Natl Acad Sci USA 1999;96(12):6879–84.
[68] Groh V, Steinle A, Bauer S, Spies T. Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science 1998;279(5357):1737–40.
[69] Busche A, Goldmann T, Naumann U, Steinle A, Brandau S. Natural killer cell-mediated rejection of experimental human lung cancer by genetic overexpression of major histocompatibility complex class I chain-related gene A. Hum Gene Ther 2006;17(2):135–46.
[70] Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003;102(4):1389–96.
[71] Carbone E, Neri P, Mesuraca M, Fulciniti MT, Otsuki T, Pende D, et al. HLA class I, NKG2D, and natural cytotoxicity receptors regulate multiple myeloma cell recognition by natural killer cells. Blood 2005;105(1):251–8.
[72] Sconocchia G, Lau M, Provenzano M, Rezvani K, Wongsena W, Fujiwara H, et al. The antileukemia effect of HLA-matched NK and NK-T cells in chronic myelogenous leukemia involves NKG2D-target-cell interactions. Blood 2005;106(10):3666–72.
[73] Romanski A, Bug G, Becker S, Kampfmann M, Seifried E, Hoelzer D, et al. Mechanisms of resistance to natural killer cell-mediated cytotoxicity in acute lymphoblastic leukemia. Exp Hematol 2005;33(3):344–52.
[74] Pende D, Rivera P, Marcenaro S, Chang CC, Biassoni R, Conte R, et al. Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. Cancer Res 2002;62(21):6178–86.
[75] Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of cutaneous malignancy by gammadelta T cells. Science 2001;294(5542):605–9.
[76] Smyth MJ, Swann J, Kelly JM, Cretney E, Yokoyama WM, Diefenbach A, et al. NKG2D recognition and perforin effector function mediate effective cytokine immunotherapy of cancer. J Exp Med 2004;200(10):1325–35.
[77] Lowin-Kropf B, Kunz B, Schneider P, Held W. A role for the src family kinase Fyn in NK cell activation and the formation of the repertoire of Ly49 receptors. Eur J Immunol 2002;32(3):773–82.
[78] Routes JM, Ryan S, Morris K, Takaki R, Cerwenka A, Lanier LL. Adenovirus serotype 5 E1A sensitizes tumor cells to NKG2D-dependent NK cell lysis and tumor rejection. J Exp Med 2005;202(11): 1477–82.
[79] Boissel N, Rea D, Tieng V, Dulphy N, Brun M, Cayuela JM, et al. BCR/ABL oncogene directly controls MHC class I chain-related molecule A expression in chronic myelogenous leukemia. J Immunol 2006;176(8):5108–16.
[80] Nausch N, Florin L, Hartenstein B, Angel P, Schorpp-Kistner M, Cerwenka A. Cutting edge: the AP-1 subunit JunB determines NK cell-mediated target cell killing by regulation of the NKG2D-ligand RAE-1epsilon. J Immunol 2006;176(1):7–11.
[81] Deng T, Karin M. JunB differs from c-Jun in its DNA-binding and dimerization domains, and represses c-Jun by formation of inactive heterodimers. Genes Dev 1993;7(3):479–90.
[82] Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005;436(7054):1186–90.
[83] Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001;413(6852):165–71.
[84] Cerwenka A, Baron JL, Lanier LL. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. Proc Natl Acad Sci USA 2001;98(20):11521–6.
[85] Hayakawa Y, Kelly JM, Westwood JA, Darcy PK, Diefenbach A, Raulet D, Smyth MJ. Cutting edge: tumor rejection mediated by NKG2D receptor–ligand interaction is dependent upon perforin. J Immunol 2002;169(10):5377–81.
[86] Westwood JA, Kelly JM, Tanner JE, Kershaw MH, Smyth MJ, Hayakawa Y. Cutting edge: novel priming of tumor-specific immunity by NKG2D-triggered NK cell-mediated tumor rejection and Th1-independent CD4+ T cell pathway. J Immunol 2004;172(2):757–61.
[87] Smyth MJ, Swann J, Cretney E, Zerafa N, Yokoyama WM, Hayakawa Y. NKG2D function protects the host from tumor initiation. J Exp Med 2005;202(5):583–8.
[88] Vetter CS, Groh V, thor Straten P, Spies T, Brocker EB, Becker JC. Expression of stress-induced MHC class I related chain molecules on human melanoma. J Invest Dermatol 2002;118(4):600–5.
[89] Watson NF, Spendlove I, Madjd Z, McGilvray R, Green AR, Ellis IO, et al. Expression of the stress-related MHC class I chain-related protein MICA is an indicator of good prognosis in colorectal cancer patients. Int J Cancer 2006;118(6):1445–52.
[90] Street SE, Hayakawa Y, Zhan Y, Lew AM, MacGregor D, Jamieson AM, et al. Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. J Exp Med 2004;199(6):879–84.
[91] Vetter CS, Lieb W, Brocker EB, Becker JC. Loss of nonclassical MHC molecules MIC-A/B expression during progression of uveal melanoma. Br J Cancer 2004;91(8):1495–9.
[92] Raffaghello L, Prigione I, Airoldi I, Camoriano M, Morandi F, Bocca P, et al. Mechanisms of immune evasion of human neuroblastoma. Cancer Lett 2005;228(1/2):155–61.
[93] Le Maux Chansac B, Moretta A, Vergnon I, Opolon P, Lecluse Y, Grunenwald D, et al. NK cells infiltrating a MHC class I-deficient lung adenocarcinoma display impaired cytotoxic activity toward autologous tumor cells associated with altered NK cell-triggering receptors. J Immunol 2005;175(9):5790–8.
[94] Salih HR, Rammensee HG, Steinle A. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. J Immunol 2002;169(8):4098–102.
[95] Doubrovina ES, Doubrovin MM, Vider E, Sisson RB, O'Reilly RJ, Dupont B, et al. Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. J Immunol 2003;171(12):6891–9.
[96] Wu JD, Higgins LM, Steinle A, Cosman D, Haugk K, Plymate SR. Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. J Clin Invest 2004;114(4):560–8.
[97] Jinushi M, Takehara T, Tatsumi T, Hiramatsu N, Sakamori R, Yamaguchi S, et al. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. J Hepatol 2005;43(6): 1013–20.
[98] Holdenrieder S, Stieber P, Peterfi A, Nagel D, Steinle A, Salih HR. Soluble MICA in malignant diseases. Int J Cancer 2006;118(3):684–7.
[99] Waldhauer I, Steinle A. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. Cancer Res 2006;66(5):2520–6.
[100] Friese MA, Wischhusen J, Wick W, Weiler M, Eisele G, Steinle A, et al. RNA interference targeting transforming growth factor-beta enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res 2004;64(20):7596–603.
[101] Welsh RM, Karre K, Hansson M, Kunkel LA, Kiessling RW. Interferon-mediated protection of normal and tumor target cells against lysis by mouse natural killer cells. J Immunol 1981;126(1):219–25.
[102] Bui JD, Carayannopoulos LN, Lanier LL, Yokoyama WM, Schreiber RD. IFN-dependent down-regulation of the NKG2D ligand H60 on tumors. J Immunol 2006;176(2):905–13.
[103] Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 2002;419(6908):734–8.

[104] Coudert JD, Zimmer J, Tomasello E, Cebecauer M, Colonna M, Vivier E, et al. Altered NKG2D function in NK cells induced by chronic exposure to NKG2D ligand-expressing tumor cells. Blood 2005;106(5):1711–7.
[105] Horiguchi S, Petersson M, Nakazawa T, Kanda M, Zea AH, Ochoa AC, et al. Primary chemically induced tumors induce profound immunosuppression concomitant with apoptosis and alterations in signal transduction in T cells and NK cells. Cancer Res 1999;59(12):2950–6.
[106] Oppenheim DE, Roberts SJ, Clarke SL, Filler R, Lewis JM, Tigelaar RE, et al. Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. Nat Immunol 2005;6(9):928–37.
[107] Wiemann K, Mittrucker HW, Feger U, Welte SA, Yokoyama WM, Spies T, et al. Systemic NKG2D down-regulation impairs NK and CD8 T cell responses in vivo. J Immunol 2005;175(2):720–9.
[108] Das S, Khar A. Regulation of NK cell function in vivo by the dose of tumour transplanted in the peritoneum. Immunol Lett 2002;83(2):133–42.
[109] Baniyash M. TCR zeta-chain downregulation: curtailing an excessive inflammatory immune response. Nat Rev Immunol 2004;4(9):675–87.
[110] Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, et al. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci USA 2003;100(7):4120–5.
[111] Lee JC, Lee KM, Kim DW, Heo DS. Elevated TGF-beta1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. J Immunol 2004;172(12):7335–40.
[112] Dasgupta S, Bhattacharya-Chatterjee M, O'Malley Jr BW, Chatterjee SK. Inhibition of NK cell activity through TGF-beta 1 by down-regulation of NKG2D in a murine model of head and neck cancer. J Immunol 2005;175(8):5541–50.
[113] Smyth MJ, Teng MW, Swann J, Kyparissoudis K, Godfrey DI, Hayakawa Y, et al. CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. J Immunol 2006;176(3):1582–7.
[114] Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, et al. CD4+ CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med 2005;202(8):1075–85.
[115] Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10(9):942–9.
[116] Zhang C, Tian ZG, Zhang J, Feng JB, Zhang JH, Xu XQ. The negative regulatory effect of IFN-gamma on cognitive function of human natural killer cells. Zhonghua Zhong Liu Za Zhi 2004;26(6):324–7.
[117] Zhang C, Zhang J, Sun R, Feng J, Wei H, Tian Z. Opposing effect of IFNgamma and IFNalpha on expression of NKG2 receptors: negative regulation of IFNgamma on NK cells. Int Immunopharmacol 2005;5(6):1057–67.
[118] Germain C, Larbouret C, Cesson V, Donda A, Held W, Mach JP, et al. MHC class I-related chain A conjugated to antitumor antibodies can sensitize tumor cells to specific lysis by natural killer cells. Clin Cancer Res 2005;11(20):7516–22.
[119] Zhou H, Luo Y, Kaplan CD, Kruger JA, Lee SH, Xiang R, et al. A DNA-based cancer vaccine enhances lymphocyte crosstalk by engaging the NKG2D receptor. Blood 2005;107(8):3251–7.
[120] Zhou H, Luo Y, Lo JF, Kaplan CD, Mizutani M, Mizutani N, et al. DNA-based vaccines activate innate and adaptive antitumor immunity by engaging the NKG2D receptor. Proc Natl Acad Sci USA 2005;102(31):10846–51.
[121] Nowbakht P, Ionescu MC, Rohner A, Kalberer CP, Rossy E, Mori L, et al. Ligands for natural killer cell-activating receptors are expressed upon the maturation of normal myelomonocytic cells but at low levels in acute myeloid leukemias. Blood 2005;105(9):3615–22.
[122] Zhang T, Lemoi BA, Sentman CL. Chimeric NK-receptor-bearing T cells mediate antitumor immunotherapy. Blood 2005;106(5):1544–51.
[123] Teng MW, Kershaw MH, Hayakawa Y, Cerutti L, Jane SM, Darcy PK, et al. T cells gene-engineered with DAP12 mediate effector function in an NKG2D-dependent and major histocompatibility complex-independent manner. J Biol Chem 2005;280(46):38235–41.
[124] Takaki R, Hayakawa Y, Nelson A, Sivakumar PV, Hughes S, Smyth MJ, et al. IL-21 enhances tumor rejection through a NKG2D-dependent mechanism. J Immunol 2005;175(4):2167–73.
[125] Reiter Z. Interferon—a major regulator of natural killer cell-mediated cytotoxicity. J Interferon Res 1993;13(4):247–57.
[126] Poggi A, Venturino C, Catellani S, Clavio M, Miglino M, Gobbi M, et al. Vdelta1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by trans-retinoic acid. Cancer Res 2004;64(24):9172–9.
[127] Thorne SH, Negrin RS, Contag CH. Synergistic antitumor effects of immune cell-viral biotherapy. Science 2006;311(5768):1780–4.