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Immunobiology and Conflicting Roles of the Human NKG2D Lymphocyte Receptor and its Ligands in Cancer

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Abstract

Cancers adopt diverse strategies to safeguard their survival, which often involve blinding or incapacitating the immune response, thereby gaining battleground advantage against the host. In immune responses against cancer, an important stimulatory lymphocyte receptor is NKG2D because the tumor-associated expression of its ligands promotes destruction of malignant cells. However, with advanced human cancers profound changes unfold, wherein NKG2D and its ligands are targeted or exploited for immune evasion and suppression. This negative imprinting on the immune system may be accompanied by another functional state wherein cancer cells co-opt expression of NKG2D to complement the presence of its ligands for self-stimulation of tumor growth and presumably malignant progression. This review emphasizes these conflicting functional dynamics at the immunity – cancer biology interface in humans, within an overview of the immunobiology of NKG2D and mechanisms underlying the regulation of its ligands in cancer, with reference to instructive clinical observations and translational approaches.

The human NKG2D receptor is normally expressed on NK cells, CD8 T cells, $\gamma\delta$ T cells, and some autoreactive and immunosuppressive CD4 T cells (1, 2). Its significance springs from the biology of its ligands, which are absent or poorly expressed on most cells but are induced in infections and malignancies by viral transactivation, and mechanisms associated with cellular stress responses and tumorigenic states, respectively (3, 4). These attributes have given rise to the concept of “induced self” serving to alert the immune system to pathological threats. Typically, engagement of NKG2D elicits protective cytolytic and cytokine responses by activation, either directly or *via* costimulation, of NK cells and CD8 T cells (5, 6). However, depending on cytokine environments together with conditions of anomalous ligand induction, NKG2D can also initiate and/or exacerbate autoimmune disease inflammatory processes such as in rheumatoid arthritis and celiac disease (5, 7).

NKG2D forms homodimers that are assembled as hexameric complexes with four chains of the DAP10 signaling adaptor (6, 8). Distinct from mice, there is no shortened human NKG2D isoform that can also bind DAP12. Upon ligation of NKG2D, cytoplasmic tail tyrosine-phosphorylated DAP10 recruits PI3K or the growth factor receptor-bound protein 2 (GRB2)/VAV1 signaling intermediate, thereby activating protein kinase B (PKB/AKT), and ERK and JNK in MAPK cascades (8). These branched signaling pathways ultimately trigger lymphocyte cytotoxic granule polarization and degranulation, cytokine production, proliferation, and survival. Stable surface expression of NKG2D is dependent on its

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association with DAP10, which is mediated by oppositely charged amino acid residues in their transmembrane domains.

NKG2D expression can be induced by several cytokines (IL-2, IL-7 and IL-15) with IL-15 being biologically most significant (7, 9). High-dose IL-15 can license NKG2D–DAP10 for direct activation of NK cell and T cell cytotoxic functions, which play a detrimental role in certain T cell-mediated pathologies such as in celiac disease (7). Moreover, IL-15 and TNF- α induce NKG2D receptors on CD28⁻ CD4 T cells, thus enabling costimulation of TCR-dependent autoreactivity against rheumatoid arthritis synoviocytes displaying inappropriately induced NKG2D ligands (5). Inhibitors of NKG2D expression include IL-21 and TGF- β , which regulates provision of DAP10 (10, 11). Macrophage migration inhibitory factor (MIF) is another negative regulator of NKG2D that is also produced by cancer cells (12). Finally, NKG2D is subject to ligand-induced down-modulation, which is especially significant in cancer settings and will be discussed further below.

Diversity and limited tissue distribution of NKG2D ligands

NKG2D ligands are distant relatives of MHC class I polypeptides but without their attributes linked to antigen presentation. There is a multitude of structurally diverse ligands including the closely related MHC class I-related chains A and B (MICA and MICB) and the more distant family of six UL16-binding proteins (ULBP1-6) (13). As with MHC class I, all NKG2D ligands have a distal $\alpha 1\alpha 2$ receptor binding platform domain. MICA and MICB also have the membrane-proximal $\alpha 3$ domain and are transmembrane-anchored. All ULBP ligands lack the $\alpha 3$ domain. ULBP1-3 and ULBP6 have GPI instead of transmembrane anchors. In outer cell membranes, GPI-anchored ULBP proteins are clustered in lipid raft microdomains, which may increase avidity of lower affinity ligands for NKG2D (14). Reflecting the evolutionary divergence of NKG2D ligands, the crystal structure of MICA shows a profoundly altered MHC class I fold with only a remnant of a shallow peptide binding groove and restructured $\alpha 1\alpha 2$ platform and $\alpha 3$ domain interfaces that preclude binding of β_2m (5). MICA and MICB are represented by numerous alleles, which differ in binding affinities for NKG2D (5, 15).

The ability of NKG2D to interact with diverse ligands seems perplexing. In a complex crystal structure, the saddle-shaped NKG2D homodimer sits astride the $\alpha 1\alpha 2$ platform domain of MICA, with each NKG2D monomer contacting either one of the subdomains. The majority of the interaction energy resides in two NKG2D binding site tyrosines that make dominant contacts at each interface in the absence of conformational plasticity (16). Complementary information from an NKG2D–ULBP3 complex structure suggests that binding of different ligands involves common interfaces and overlapping sets of amino acid side-chain interactions, thus permitting conservation of general shape complementarities and binding energies (17).

The multiplicity and diversity of NKG2D ligands apparently reflects evolutionary pressure to maintain redundancy. One explanation is based on an evolutionary arms race with viruses encoding immunoevasins, which have the capacity to obstruct the functionality of different sets of NKG2D ligands (13, 18). Another concept emanates from the function of the ligands as sensors of cellular damage cues. Raulet and colleagues proposed that NKG2D ligands may convey composite specifications, analogous to barcodes, on the surfaces of unhealthy cells, with variables including presence and relative abundancies of diverse ligands and differences in post-translational modifications (4). However, NKG2D ligands are not absent from all normal cells but presumably are insufficient, or ineffectively disposed, to stimulate an immune response when present. Some NKG2D ligand mRNAs are quite ubiquitous, which is inconsequential presumably because of their negative regulation by microRNAs

(19). Nevertheless, physiologically relevant examples for NKG2D ligand expression under normal conditions exist, including the expression of MICA/B in intestinal mucosa, thymic epithelium, and the placental syncytiotrophoblast (5).

Expression and regulation of NKG2D ligands in cancer

Expression of NKG2D ligands is pronounced in malignancies, underscoring their role in promoting tumor immune surveillance. In the most comprehensive study of hematopoietic malignancies, Hilpert and colleagues documented heterogeneous surface expression of at least one NKG2D ligand type in about 75% of altogether 205 leukemia patients (20). With solid tumors, a survey of widely scattered data suggests that MICA/B predominate alongside at least one ULBP family ligand in most epithelial cancers including head and neck, lung, breast, ovarian, cervical, prostate, gastrointestinal, pancreatic, hepatocellular, and renal cell cancer, as well as in malignant glioma, neuroblastoma, and melanoma (1, 5).

Tumor expression of NKG2D ligands is linked to generic mechanisms coupled to cellular stress, proliferation, and unfolded protein responses, as well as to signaling intermediates and checkpoint anomalies associated with oncogenic states (3, 4). Within this framework, detail knowledge of ligand regulation is fragmented. In addition to transcriptional activation, protein biosynthesis can modify ligand expression (4). Moreover, different mechanisms may activate transcription of the same ligand gene, thus expanding the range of responses to cellular damage cues.

The regulation of the genes for MICA and MICB shares similarities with *heat-shock protein 70 (HSP70)* genes (21). These genes commonly have a promoter region heat-shock response element (HSE) for conditional binding of heat shock factor 1 (HSF1), a transcriptional activator that is responsive to homeostatic changes caused by heat shock and oxidative stress. Promoter regions of MICA/B also activate proliferation-induced transcription (21). With epithelial tumor lines and presumably cancer cells as well, epigenetic derepression of promoter accessibility, exemplified by pharmacological histone deacetylase (HDAC) inhibition, plays an essential role in the transcriptional activation of NKG2D ligand genes (3). In accord with their regulation by elementary cellular processes, relevant transcription factors in addition to HSF1 are ubiquitous. They include members of the SP zinc finger transcription factor family and the CCAAT box factor (CBF) complex, which variably interact with 5'-end flanking sequences at least of *MICA/B* and *ULBP1* (3). The activator protein-1 (AP-1; Fos/Jun), which has diverse roles in inflammatory responses as well as a tumor promoter or suppressor, has been implicated in the regulation of a mouse NKG2D ligand gene (22). In humans, AP-2 α suppresses *ULBP1* transcription by interfering with SP complex binding site occupancy (3). These findings reflect the patchwork state of research with independent findings in humans and mice that are most often not mutually applicable. Along this line, E2F transcription factors involved in cell cycle entry have been implicated in mouse ligand transcriptional activation but there is no corresponding evidence in humans (4). Conversely, the p53 tumor suppressor has been implicated in transcriptional activation of *ULBP1* and *ULBP2* but not of mouse NKG2D ligand genes (4, 23). Taken together, knowledge of promoter regions and transcription factors relevant to tumor-associated expression is limited for most NKG2D ligand genes. As of yet, reprogramming events associated with malignant transformation do not appear to have a principal role in their transcriptional activation.

However, NKG2D ligands can be post-transcriptionally regulated by factors associated with oncogenic states. The *BCR-ABL* tyrosine kinase gene fusion is associated with MICA induction in chronic myeloid leukemia (CML) (24). Moreover, chronic activation of the RAS GTPase in response to extracellular signals can lead to upregulation of ULBP1-3 and

MICA *via* stimulation of MAP kinase and PI3K signaling cascades and, ultimately, by eukaryotic translation initiation factor 4E (eIF4E)-mediated increases of protein synthesis rates (4). An explanation for the near universal presence of NKG2D ligands in cancer cells was provided by Gasser and colleagues, who associated their expression with genotoxic stress and the activation of a DNA damage response owing to double-stranded DNA breaks or stalled replication forks (25). This pathway involves the ataxia telangiectasia mutated (ATM) or ATM and Rad3-related (ATR) protein kinases, which activate checkpoint kinases that ultimately effect cell cycle arrest, DNA repair, or apoptosis. Accordingly, DNA damaging agents and replication inhibitors can induce expression of ULBP1-3 and mouse NKG2D ligands (4, 25). The mechanistic coupling of ligand induction to this cellular response is in essence not understood (4).

Once induced in cancer cells, NKG2D ligands MICA/B and various ULBP are negatively regulated by cytokines including TGF- β and IL-10 (1). Carcinoembryonic antigen-related adhesion molecule 1 (CEACAM1) impedes glycosylation and hence intracellular transport of NKG2D ligands (26). Of interest is a report of *O*-glycan modification of MICA by core2 β -1,6-*N*-acetylglucosaminyltransferase (C2GnT) in bladder tumor cells. Here, poly-*N*-acetylglucosamine attached to MICA core2 *O*-glycans (*N*-acetylgalactosamine linked to branched *N*-acetylglucosamine) binds galectin-3, which impairs ligand affinity for NKG2D. This modification may explain correlations between high expression of C2GnT and poor prognosis and highly metastatic behavior of bladder cancers (27).

Regulation of NKG2D ligands by microRNAs

As with many cellular processes, immune system functions and oncogenesis are regulated by microRNAs, which assemble with partially complementary sequences in 3'-untranslated regions of target mRNAs, thus inhibiting translation or promoting degradation. Stern-Ginossar and colleagues and subsequent studies identified cellular microRNAs of various families that suppress expression of MICA and/or MICB, or ULBP2 (19, 28). Most of those microRNAs are present in steady quantities in normal human cells, consistent with their proposed role in calibrating baseline expression of NKG2D ligand mRNAs such that no lymphocyte attack ensues. The current model hence posits that the repressive capacity of the microRNAs is exceeded in pathological conditions as in malignancies when transcriptional induction of NKG2D ligand genes leads to consequential mRNA and protein expression.

Nonwithstanding the attractiveness of this model, evidence in support of the significance of microRNAs in suppressing NKG2D ligands in human cancer is scant because of insufficient or contradictory correlative data. There are no larger studies correlating clusters of relevant microRNAs in *ex vivo* cancer cells with absence or presence of surface NKG2D ligands. Moreover, unrelated studies of microRNA expression patterns in human cancers have yielded discordant results. A microRNA with regulatory activity for MICA/B (miR-106b) was found reduced in primary ovarian cancer and increased in colorectal cancer, gastric cancer, and hepatocellular carcinoma (29-31). Among other microRNAs specific for MICA/B, miR-17-5p and miR-373 were found highly expressed in multiple cancers of diverse tissue origins; however, these microRNAs have pleiotropic effects in tumor progression, which may influence NKG2D ligand expression (29, 32). Lastly, there is the conundrum that most cancers comprise substantial cell populations that are positive for NKG2D ligands, which may in fact benefit tumor progression as discussed further below.

Protective role of NKG2D and its ligands in tumor immunity

Mouse model experiments substantiated NKG2D-dependent rejection of ligand-bearing tumor lines by NK cells and/or cytotoxic CD8 T cells in syngeneic mice (1). Most decisively, a study of NKG2D-deficient mice revealed defective immune surveillance in

experimental spontaneous malignancies. Absence of NKG2D resulted in higher incidence and increased aggressiveness of early-arising tumors. Reflecting the capacity of NKG2D to trigger NK cell and CD8 T cell cytotoxicity, its functionality was associated with negative selection of cancer cells bearing its ligands (33).

In humans, evidence for a protective role of NKG2D against cancer is limited and naturally indirect. Single-nucleotide polymorphisms (SNPs) linked to *NKG2D* identified natural killer complex (NKC) haplotypes that are associated with low or high NK cell cytotoxic activity and more or less risk of colorectal and aerodigestive tract cancers in life style-adjusted control and patient cohorts (34-36). A biallelic (threonine/alanine) variant at amino acid position 72 in its transmembrane segment may implicate NKG2D more directly. Threonine⁷² may alter binding of DAP10 thereby increasing signaling strength. This variant was significantly less frequent in a study of patients with cervical carcinoma than in the healthy controls (37). Perhaps more instructive are correlative studies of NKG2D ligand expression in cancers and clinical outcomes. In a colorectal cancer study, MICA was an independent marker of good prognosis for stage I and stage II but not later stage cancers (38). A similar relationship was observed in pancreatic cancer (39). In a breast cancer study, MICA/B and ULBP2 were associated with decreased relapse in early-stage cancer (40). These clinical data support a consensus that NKG2D and its ligands are protective at early but not advanced tumor stages, reflecting the progressive immune system failure that allows unchecked cancer growth. Consequently, tumor progression coupled to persistent expression of NKG2D ligands points to changes in functional dynamics that may increasingly favor host tumor susceptibility instead of resistance.

Exploitation of NKG2D ligands as tumor survival assets enabling immune evasion

NKG2D is subject to ligand-induced downmodulation by endocytosis and partial degradation. This is most evident in patients with cancers positive for MICA/B, in which large proportions of CD8 T cells and NK cells among infiltrating lymphocytes have diminished surface NKG2D. Moreover, NKG2D is systemically reduced on peripheral blood CD8 T cells and NK cells. This deficiency is caused by circulating tumor-derived soluble ligands and associated with impaired immune responses (41, 42). Shedding of soluble MICA and/or MICB and ULBP2 has been reported for most cancers and some hematopoietic malignancies. Tumor-derived exosomes may also contribute to effects initially attributed to soluble ligands alone (43). Experimental support for a critical role of soluble ligands in promoting tumor immune evasion was provided by a mouse model study comparing tumor formation by implants expressing either wild-type or a shedding-resistant form of MICB, or just the secreted soluble ligand. The results confirmed the capacity of soluble ligand to foster tumor growth, wherein impairment of NK cell responses was noted as the probable cause (44). This relationship is highlighted by beneficial effects of neutralizing anti-MICA autoantibodies that were induced as a result of anti-CTLA-4 immunotherapy in a clinical trial with melanoma patients (45). By and large, the observed negative imprints on the immune response are corroborated by clinical data, as tumor-associated or soluble NKG2D ligands are associated with parameters of disease progression and poor prognosis in pancreatic and prostate cancer (MICA/B; 39, 46), ovarian cancer (ULBP2 and ULBP4; 47), melanoma (ULBP2; 48), and multiple myeloma (MICA; 49).

Biological precedent for tumor shedding of NKG2D ligands exists in pregnancy where immunosuppressive factors must prevent rejection of the semiallogeneic fetus. Placental syncytiotrophoblast cells express NKG2D ligands including MICA/B and ULBP family members. Both shedding of soluble ligands and release of exosomes may contribute to

NKG2D downmodulation on NK cells and CD8 T cells and thereby to maintenance of an immune tolerogenic environment (5).

Regulation of NKG2D ligand shedding

Shedding of MICA and presumably MICB requires domain-specific deconstruction by an accessory protein before proteolytic cleavage can ensue. On the surface of tumor lines and *ex vivo* cancer cells, MICA interacts with endoplasmic reticulum protein 5 (ERp5), a disulfide isomerase that typically assists in the folding of nascent proteins inside cells. ERp5 and membrane-anchored MICA form transitory mixed-disulfide complexes from which soluble MICA is released after proteolytic cleavage near the cell membrane (50). ERp5 reduces a deeply buried disulfide bond in the membrane-proximal MICA $\alpha 3$ domain, thereby likely generating a conformational change that enables proteolytic cleavage of MICA. This physical relationship is reflected by positive correlations between ERp5 and MICA/B on the surface of leukemic cells and soluble ligands in multiple myeloma and CLL (51, 52). Recruitment of MICA into cholesterol-enriched membrane lipid microdomains by palmitoylation of cysteine residues in its cytoplasmic tail may promote shedding although there is no confirmatory evidence in cancer cells (53). NKG2D ligands are cleaved near the cell membrane by ADAM family of cell surface disintegrins and metalloproteases, among which members 9, 10, 14, and 17 have been variously implicated (54).

Persistent NKG2D ligand expression may cause systemic immune suppression

Chronic expression of NKG2D ligands, as in advanced cancer, may have broader immunosuppressive effects. Peripheral blood NK cells from patients with colorectal or cervical cancer lack functional natural cytotoxicity receptors (NCRs) NKp30 and/or NKp46, which has been associated with soluble MICA or diminished NKG2D (42, 55). Moreover, loss or diminished expression of the CD3 ζ signaling adaptor is well documented in T cells and NK cells from patients with cancer (56). CD3 ζ is associated with CD16 (Fc γ RIII), and is essential for NKp30/46 signaling and TCR complex expression and function. Experimentally, sustained ligand stimulation of signaling-competent NKG2D on activated human CD8 T cells or NK cells results in loss of CD3 ζ concomitant with the gradual decrease of surface NKG2D (57). Mechanistically, these seemingly unrelated events are linked in a causal chain wherein NKG2D signaling initiates FasL/Fas receptor-mediated activation of caspases-3/7 resulting in CD3 ζ cleavage and subsequent degradation (57). Hence, cancer expression of NKG2D ligands may lead to impairment of multiple CD3 ζ -dependent receptor functions, thus promoting far-reaching lymphocyte tolerization.

Other immunosuppressive effects are associated with a small subset of normally occurring CD4 T cells with activation-independent, constitutive expression of NKG2D. These T cells lack proinflammatory cytokine and cytolytic signatures and play no discernible role in anti-microbial recall responses. Instead, they are autoreactive and biased towards an immunosuppressive IL-10- and TGF- β -dominated cytokine profile (2, 58). For reasons unknown, the NKG2D⁺CD4⁺ T cells are non-susceptible to ligand-induced NKG2D downmodulation, thus maintaining NKG2D functionality under conditions of persistent ligand exposure. Hence, in environments providing stimulation of both TCR and NKG2D, the NKG2D⁺CD4⁺ T cells proliferate and may expand in numbers relative to other T cells, thereby causing imbalances in the lymphocyte pool and imposing an immunosuppressive cytokine milieu. This homeostatic adjustment may normally serve to dampen chronic immune activation. In advanced cancer patients, however, tumor expression and shedding mainly of soluble MICA/B can drive substantial proliferative expansions of the NKG2D⁺CD4⁺ T cells. Casting light on the physiological significance of these T cells, their

frequencies are inversely correlated with disease activity in SLE, suggesting that they participate in suppression of effector responses (2).

Co-option of NKG2D as a potential tumor growth factor receptor

In addition to promoting tumor immune evasion and suppression, NKG2D ligands may constitute yet another tumor survival asset. In a conceptual twist, variable proportions of *ex vivo* breast, ovarian, prostate, and colon cancer cells express signaling proficient NKG2D, thereby complementing the presence of its ligands for self-stimulation of tumor growth and possibly malignant progression (59). Triggering of cancer cell NKG2D by antibody crosslinking or engagement of ligands on adjacent cancer cells activates the oncogenic PI3K–AKT–mammalian target of rapamycin (mTOR) signaling axis, which is commonly hyperactive in cancer, and downstream effectors controlling protein synthesis and cell growth. In addition, as in lymphocytes, engagement of NKG2D stimulates phosphorylation of ERK and JNK in MAPK cascades (59). These kinases are activation targets of receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR), which, because of mutation of aberrant expression, frequently cause excessive cancer cell proliferation and increased motility and survival (60).

Consistent with these signaling activities, above-threshold expression of NKG2D–DAP10 in ligand-bearing tumor lines increases their bioenergetic metabolism and proliferation, thus implying similarities with oncogenic growth factor receptors such as the EGFR and the insulin-like growth factor-1 receptor (IGF-1R). Thus, cancers may co-opt expression of NKG2D–DAP10 for their own benefit to promote tumorigenesis. In a preliminary assessment, this role is supported by significant correlations between percentages of *ex vivo* cancer cells that are positive for surface NKG2D and criteria of tumor progression (59). Hence, NKG2D itself might in fact represent the main factor underlying the typically poor clinical outcomes that have been associated with cancer cell expression of its ligands (39, 46, 47, 49). Notably, tumor cell expression of NKG2D was not detected in several experimental models of spontaneous cancers in mice (59).

Targeting of NKG2D or its ligands for cancer therapy

Therapy approaches are tailored to enhance or redirect NK cell and/or T cell-mediated cytotoxicity by utilizing agents that may increase NKG2D ligand expression or bifunctional fusion proteins including an NKG2D ligand and a single-chain antibody fragment (scFv) targeting a specific tumor cell surface marker. Among pharmaceuticals tested for upregulation of NKG2D ligands on tumor lines *in vitro* are demethylating agents, proteasomal inhibitors, and genotoxic drugs used for chemotherapy such as HDAC inhibitors (61). Objectives for the bifunctional fusion proteins are to target NK cells and T cells onto tumor cells and engage NKG2D. Examples are ULBP2–anti-prostate-specific membrane antigen (PSMA) scFv and ULBP2–anti-syndecan-1 (CD138) scFv (62). CD138 is highly expressed in some hematopoietic malignancies such as multiple myeloma. Other ligand fusion proteins incorporating anti-HER2 (EGFR-2) antibody fragments have been developed for preliminary testing in mice (63).

Another type of bispecific T cell and NK cell engager, thus far tested in mice only, combines the extracellular domain of NKG2D with either CD3e or the Fc portion of IgG2a for binding to CD16 (64, 65). In an *in vivo* tumor model, administration of the NKG2D–CD3e fusion protein reduced growth of tumors bearing NKG2D ligands and promoted T cell infiltration. Likewise, the NKG2D–Fc region fusion protein triggered lysis and reduced growth of ligand-positive lymphomas (64, 65).

Transduction of chimeric activating lymphocyte receptors consisting of human NKG2D, CD3 ζ and either costimulatory CD28 or 4-1BB (CD137), a tumor necrosis factor receptor (TNFR) family member, represents a strategy for adoptive T cell therapy (66, 67). Finally, another approach aims at induction or administration of antibodies for neutralization of soluble NKG2D ligands. It remains to be seen how these or other therapeutic approaches impact the dynamic functionality of NKG2D and its ligands at the interface between the immune system and cancer as there may be unintended consequences.

Conclusion

Although best known for their protective role in tumor immune surveillance, NKG2D and its ligands are also exploited as tumor survival assets, enabling immune evasion and suppression, and quite possibly stimulation of tumor growth and malignant progression (Fig. 1). The tentative role of NKG2D as an oncoprotein still requires *in vivo* experimental substantiation in an animal model. Cancer biology questions of further relevance are whether NKG2D imparts cellular changes in cancer environments that are distinct or merely synergistic to those conferred by prototypic growth factor receptors, and whether its activities impact other aspects of tumorigenesis. These issues broadly reflect an increasing appreciation of a need of an integrated approach that considers tumor and host factors together in the evaluation and targeting of tumor progression.

Acknowledgments

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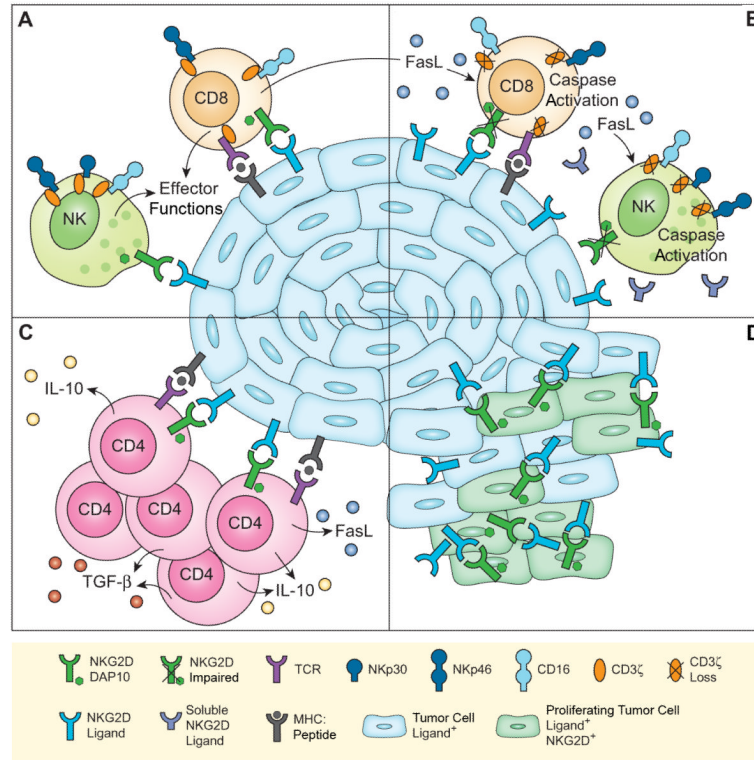


FIGURE 1. Schematic representation of NKG2D/ligand-mediated effects in cancer settings. (A) Tumor-associated NKG2D ligands trigger effector functions of NK cells and, *via* costimulation, of CD8 T cells. NKG2D-mediated CD8 T cell stimulation triggers FasL release. At this stage, the TCR, CD16, and the NKp30 and NKp46 NCRs are functionally expressed in complex with CD3ζ. (B) Persistence of tumor cell membrane-bound and soluble NKG2D ligands cause NKG2D downmodulation and functional impairment. Still functional NKG2D (see A and C) initiates FasL/Fas-mediated caspase activation and resultant CD3ζ loss. As a consequence, the functional capacities of the TCR, CD16, and NKp30 and NKp46, which all signal through CD3ζ, are impaired. (C) NKG2D costimulates proliferation, and IL-10, TGFβ and FasL release by immunosuppressive NKG2D⁺CD4⁺ T cells. (D) NKG2D⁺ cancer cells proliferate in response to autocrine NKG2D-mediated stimulation. See text for further explanations.