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Immunosurveillance and immunotherapy of tumors by innate immune cells

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Increasing evidence supports a role for innate immune effector cells in tumor surveillance. Natural killer (NK) cells and myeloid cells represent the two main subsets of innate immune cells possessing efficient but quite different tumor suppressive abilities. Here, we describe the germline-encoded NK cell receptors that play a role in suppressing tumor development and describe briefly the cellular pathways leading to the upregulation of their ligands in tumor cells. We also describe mechanisms underlying the elimination of tumor cells by macrophages and a recently characterized mechanism dedicated to sensing cytosolic DNA that is implicated in antitumor immune responses.

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Introduction

The innate immune system plays a significant role in recognizing and eliminating tumor cells. Innate cells and particularly NK cells express a fixed set of germline-encoded receptors, which bind tumor-specific ligands to provide tumor-suppressive functions. This review focuses on the most characterized receptor/ligand systems employed by innate immune cells to mediate innate recognition and elimination of tumor cells as well as recently discovered mechanisms of tumor sensing and immune cell activation.

NKG2D and anti-tumor immunity

NKG2D is an activating receptor expressed on NK cells, certain $CD8^+$ T cells, γδ T cells, NKT cells, and certain $CD4^+$ T cells [1]. Engagement of NKG2D upon encounters of NK cells with cells expressing ligands for NKG2D stimulates NK cell cytotoxic activity and cytokine production.

NKG2D recognizes several MHC-related ligands including three subfamilies of ligands in mice (RAE-1α-ε, MULT1, and H60a-c), and two subfamilies of ligands in humans (MICA-B and ULBP1-6) [2]. The ligands are expressed poorly by normal cells but are often induced on cancer and virus-infected cells as the result of the activation of various pathways, many associated with cell stress [2]. It is now well established that NKG2D and its ligands represent a potent and specific system that allows the recognition and elimination of unhealthy cells. NKG2D was first implicated in immune surveillance of tumors by the demonstration that many tumors, but few normal cells, express NKG2D ligands [3–5]. Subsequently, subcutaneous tumor transfer models confirmed that expression of NKG2D ligands causes tumor cell rejection [6,7] (Table 1). Further studies showed that the NKG2D receptor is important for immune surveillance of certain lymphoid and epithelial malignancies using the Eμ-Myc model of B lymphoma and the TRAMP model of prostate adenocarcinoma, respectively [8].

Understanding specific pathways that regulate NKG2D ligands has been a major effort in our laboratory for the last several years. Table 2 summarizes our current knowledge on the regulation of NKG2D ligands in mice and humans.

Many tumor cell lines release soluble NKG2D ligands through a variety of mechanisms, and ligand shedding is often considered a mechanism of immune evasion [2,9]. For instance, soluble MIC and ULBP proteins have been identified in the sera of cancer patients and their detection may in some cases serve as prognostic indicators of cancer [9]. Shedding of NKG2D ligands from tumor cells can result in dramatic reductions in the corresponding cell-surface levels, reducing the susceptibility of the tumor cells to cytolysis mediated by NK cells and T cells.

The effects of soluble NKG2D ligands likely depend on their form and specific properties. In the case of ligands cleaved from the cell surface, which are expected to be monomeric, binding to NKG2D may prevent interactions of the receptor with membrane-bound ligands [10–12]. Ligands vary in affinity, however, and some, such as MICA, may bind NKG2D with too low an affinity to have much impact in this respect. Our recent study

Table 1

NK cell activating receptors involved in tumor surveillance *in vivo*

Receptor	Ligand	Tumor type	Model	Reference
NKG2D	Transd. RAE-1/H60	Melanoma	Transferred B16	[6]
NKG2D	Transd. RAE-1/H60	T Lymphoma	Transferred RMA	[6,7]
NKG2D	Transd. MULT-1	T Lymphoma	Transferred RMA	[54]
NKG2D	RAE-1 and MULT-1	B Lymphoma	Spont. Eμ-Myc	[8]
NKG2D	RAE-1 and MULT-1	Prostate Cancer	TRAMP	[8]
DNAM-1	Transd. CD155/CD112	Melanoma	Transferred B16	[28,31]
DNAM-1	Transd. CD155/CD112	T Lymphoma	Transferred RMA	[30]
DNAM-1	CD155	Fibrosarcoma	MCA	[29]
DNAM-1	CD155	Papilloma	DMBA	[29]
DNAM-1	CD155	Multiple Myeloma	Spont. Vκ*MYC	[27]
DNAM-1	CD155/CD112	B Lymphoma	Spont. Eμ-myc	[26]
NKp30	Transd. Bat-3	B lymphoma	Transferred RPMI8226	[18]
NKp46	?	Melanoma	Transferred B16F10.9	[20]
NKp46	?	Lewis lung carcinoma	Transferred D122	[20]

Transd: transduced ligand, Spont: spontaneous model, TRAMP: transgenic adenocarcinoma mouse prostate, MCA: 3-methylcholanthrene, DMBA: 7,12-dimethylbenz(a)anthracene.

showed that in mice, a shed monomeric form of a high-affinity NKG2D ligand, MULT1, caused NK cell activation and tumor rejection [13••]. We demonstrated that soluble MULT1 inhibited the engagement of NKG2D with other membrane NKG2D ligands expressed on non-tumor cells in tumor-bearing mice, thus preventing global desensitization of NK cells. These results challenge the conventional thought that soluble NKG2D ligands generally act as inhibitory molecules.

Some forms of ligands may impair immune surveillance by modulating NKG2D expression, but this may be more likely in the case of multimeric ligands, such as ligands on exosomes, which can crosslink the receptor and modulate it from the cell surface. NKG2D ligand-containing exosomes derived from human DCs were reported to directly activate human NK cells *ex vivo* [14], but reduced levels of NKG2D on immune cells *in vivo* could also reduce tumor killing.

NCRs and anti-tumor immunity

Natural cytotoxicity receptors (NCRs) such as NKp46, NKp44, and NKp30 play roles in tumor cell recognition. NKp46 and NKp30 are expressed on both resting and activated human NK cells, whereas NKp44 is expressed only on activated human NK cells. Recognition of tumor

Table 2

Regulation of NKG2D ligands

Transcriptional regulation

- Proliferative conditions induce expression of *Raet1* family genes and the *MICA* and *ULBP2* genes. E2F transcription factors transactivate *Raet1* family genes [55].
- Heat shock and the heat shock factor 1 (HSF1) regulate the *MICA* and *MICB* genes [56,57].
- The p53 transcription factor amplifies transcription of *ULBP1* and *ULBP2* genes [58,59].
- NF-κB and Sp family transcription factors regulate the transcriptional activation of human NKG2D ligands [60,61].
- The ATF4 transcription factor induces *ULBP1* gene expression [62].

Regulation at the mRNA level

- The DNA damage response (DDR) pathway is an important mode of regulation of NKG2D ligands in both mouse and human cells and appears to act largely post-transcriptionally [32,63,64].
- AID deregulation in Abelson murine leukemia virus-infected cells induced the DDR and the expression of NKG2D ligands [65].
- The HIV Vpr protein activates the ATR kinase and the DDR leading to the expression of NKG2D ligands [66].
- The HIV Vif protein degrades the antiviral host protein APOBEC3G, preventing the deamination of cytosine residues, the DDR and the expression of NKG2D ligands [67].
- Many different microRNAs have been implicated in NKG2D ligands regulation, including miR-17-5p, miR-20a, miR-34a, miR-34c, miR-93, miR-106b, miR-373, and miR-520 [68].
- PI3K signaling was implicated in the induction of RAE-1 [69].
- The oncogene RAS induces the expression of RAE-1α and RAE-1β in mouse cells as well as ULBP1-3 in human cells [70].
- The adenovirus E1A oncogene protein induces *Raet1* mRNAs and the RAE-1 protein [71].
- The RNA-binding protein RBM4 supports ULBP1 expression by facilitating proper splicing of the first two exons of the primary transcript [62].

Regulation at the protein level

- UV irradiation or heat shock reduces the poly-ubiquitination of MULT1 protein resulting in its stabilization and induction at the cell surface. MULT1 degradation was in part mediated by two ubiquitin ligases, MARCH4 and MARCH9, which regulate turnover of the ligand cell-surface induction [72,73].