Published in final edited form as:
*Nat Rev Immunol*. 2009 August ; 9(8): 568–580. doi:10.1038/nri2604.

# Oncogenic stress sensed by the immune system: role of NK cell receptors

**David H. Raulet** and **Nadia Guerra**
Department of Molecular and Cell Biology, Cancer Research Laboratory, 485 Life Science Addition, University of California, Berkeley, Berkeley, CA 94720, USA

## Abstract

A growing body of research addresses how pathways dysregulated during tumorigenesis are linked to innate immune responses, which can contribute to immune surveillance of cancer. Innate components of the immune system localized in tissues are believed to eliminate early neoplastic cells, thus preventing or delaying the establishment of advanced tumours. This Review addresses our current understanding of the mechanisms that detect cellular stresses that are associated with tumorigenesis, and that culminate in the recognition and, in some cases, the elimination of the tumour cells by natural killer (NK) cells and other lymphocytes that express NK cell receptors.

Cancers arise in a multi-step process that involve the dysregulation of oncogenes, tumour suppressors and pro-apoptotic signals [1]. Genetic and epigenetic alterations lead to the alterations in cellular growth cycles, differentiation programmes and cell death pathways[1]. Studies of colon cancer have served as a paradigm for considering the stepwise alterations that culminate in metastatic cancer [2] (Figure 1). Initial genetic changes result in hyperproliferation of initially normal cells, and subsequent oncogene mutations and epigenetic changes underlie the development of increasingly dysplastic pre-cancerous adenomas or other precancerous lesions[2] . These initial changes activate key tumour suppressors, such as p53**[G]**, that suppress the cell cycle and may induce cellular senescence or apoptosis[2]. Mutations of the corresponding tumour suppressor genes or other genes in the relevant pathways are correlated with the appearance of cancer. Other mutations accumulate that influence angiogenesis, migration and metastasis. Although the order of occurrence of these various mutations may vary in different types of cancer or even different instances of the same type of cancer, evidence suggests that homozygous mutations in p53 usually become predominant relatively late in tumorigenesis [2], highlighting the fact that early events in tumorigenesis activate p53 and create selective pressure for loss of this key tumour suppressor.

In addition to these largely cell-intrinsic barriers to tumorigenesis, evidence has accumulated for cell extrinsic barriers to tumour development, some mediated by the immune system. Some cancers are linked to infectious agents, and in some of these cases transformation depends on direct infection of the pre-malignant cell [3]. Examples relevant to human disease include cervical carcinoma, some lymphomas and Kaposi's sarcoma**[G]**. In these instances, the transformed cell may express non-self antigens encoded by the pathogen that can be targeted by T and B cells, just as may occur in any infection. Other cancers arise by spontaneous genetic and/or epigenetic changes. In these tumours, self antigens are sometimes overexpressed and can be targeted by the adaptive immune system due to their unnaturally high abundance, but in other cases adaptive immune responses are not readily

Correspondence to D.H.R., raulet@berkeley.edu.

detected and may not have a major role in tumour suppression [4]. In those instances, tumour suppression may involve the innate immune system. In general, adaptive immune responses are initiated by signals that are associated with inflammation caused by innate immune responses[4], and this is probably also true for responses to tumours as well. Therefore, the initial innate immune response to tumours may be decisive in determining whether immune surveillance is effective. It must be emphasized that many immune responses to tumours are not only non-protective, but paradoxically promote cancer. Important research in that area has been reviewed elsewhere and will not be addressed in detail here[4, 5].

Natural killer (NK) cells are one important component of the innate immune response to tumours. NK cells are lymphocytes that differ from B and T cells in that they use numerous receptors, none of which are encoded by genes that undergo rearrangement. Each NK cell expresses several stimulatory and inhibitory NK cell receptors that can function with some independence, enabling NK cells to separately target cells that increase or decrease their expression of various ligands[6]. Many of the NK cell inhibitory receptors are specific for MHC class I molecules, which are expressed by normal cells but often lost from infected cells or tumour cells[7]. Ligands for NK cell stimulatory receptors are usually poorly expressed by healthy cells but are increased in expression on ‘unhealthy’ cells, such as transformed, infected or stressed cells [6, 7]. NK cell activation is controlled by the balance of stimulatory and inhibitory signaling incurred when target cell engagement occurs and the various receptors engage their ligands [6–8]. Hence, some normal cells display stimulatory ligands, but fail to be killed by NK cells because their MHC class I molecules engage inhibitory receptors that counteract stimulatory signaling. Increased expression of stimulatory ligands by a target cell can overcome inhibitory signaling in the NK cell, resulting in target cell lysis. Loss of inhibitory MHC ligands can also result in target cell lysis. Both occur in the context of disease, sometimes in the same cell[8] . In addition to their role in NK cells, some stimulatory NK cell receptors are also commonly expressed by subsets of T cells, where they are believed to provide an innate signal that enhances T cell activation[9].

Recent evidence suggests that the host immune response to tumour cells is in some cases linked to specific events that are associated with cellular transformation and tumorigenesis. In this article, we focus on the mechanisms that link oncogenic stress to innate immune stimuli, in particular the stimuli that act through NK cell receptors and the cells that express them.

## Immune surveillance of primary tumours

Immune surveillance of cancer is a concept that was for some time considered discredited, but has received strong experimental support in the past ten years or so (Table 1). Before reviewing the evidence, it is useful to summarize the experimental systems employed in such studies.

Many past studies of the immune system’s role in cancer have relied on tumour transplant models, typically using tumour cell lines that are implanted subcutaneously in recipient animals. Although this approach is useful, it suffers from the fact that implanted tumours involve cell lines that clearly escaped immune surveillance in the animal from which they were derived and may also be aberrant due to prolonged culturing. Furthermore, they are usually implanted in ectopic sites in relatively large numbers, and fail to recapitulate the earliest stages of transformation and tumorigenesis. As a result, the physiological relevance of observations showing immune destruction of implanted tumours is often questioned. Alternative approaches include studies of fully spontaneous cancer, cancer induced by transgenic expression of oncogenes and carcinogen-induced cancer (Table I). These

approaches are considered more reliable for probing the role of the immune system, because the tumours arise in the normal tissue site, typically from a single cell, and proceed through the various stages of tumour development. Nevertheless, they vary in their difficulty of use and other shortcomings as summarized below.

## Fully spontaneous tumorigenesis

Cancer that develops in normal untreated mice is presumably the most faithful mouse model of human cancer, and has been studied in some analyses of immune surveillance. However, the very low incidence of fully spontaneous cancers in most mouse strains and the heterogeneity of cancers that arise are drawbacks for these studies. Despite the difficulties, a key report by Shankaran *et al*. showed increased incidence of spontaneous lung and intestinal carcinomas in 129/Sv mice that lacked all T and B cells due to mutations in recombination-activating gene 2 (*Rag2)*[10]**[G]**. Interestingly, mammary carcinomas were not detected in RAG2-deficient mice, but arose in mice lacking RAG2 as well as STAT1 (signal transducer and activator of transcription 1), a component of the interferon (IFN) receptor signalling complex, suggesting a role for innate immune responses in the control of mammary tumours. A general and robust role of perforin [G] in immune surveillance of cancer was demonstrated with perforin knockouts in two mouse strain backgrounds [11–14]. TRAIL (TNF-related apoptosis-inducing ligand), another mediator of cytolysis has also been implicated in responses against cancer [11–13]. These studies suggest a role for both the adaptive and innate immune responses in the control of fully spontaneous tumours.

## Transgenic models of spontaneous cancer

Genetically engineered mice that overexpress oncogenes or have altered tumour suppressor function develop spontaneous cancers that mimic the development of natural malignancies in most respects. The tumours arise and progress autochtonously (at their natural sites), including the earliest stages, and are likely to reflect the natural interactions between the tumour and the immune system in initially normal tissues. In most of these models, most or all animals develop tumours of the same type, and in some cases tumours develop with predictable kinetics, allowing systematic analysis of the various stages of cancer. A drawback of some of these models is their high penetrance, such that tumors are repeatedly initiated in the same host and can overwhelm the immune response. A few relevant models will be summarized here.

In the TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse model the rat probasin promoter directs the expression of the SV40 virus early genes**[G]** (large T and small t antigens) to the prostate epithelium of adult mice. Prostate tumours developing in these mice are infiltrated by leukocytes, including $CD8^+$ T cells specific for tumour-associated antigens [15, 16]. Other cell types that infiltrate the tumours include NK cells, γδ T cells**[G]** and NKT cells**[G]** (P. Savage, personal communication). Liu *et al*. recently demonstrated that γδ T cells could suppress high-grade prostate tumours in the TRAMP model[17]. Furthermore, another recent study demonstrated that the immunoreceptor natural killer group 2, member D (NKG2D) contributed to the control of the high-grade, aggressive form of carcinomas that develop in some of these mice [18]. As discussed later, NKG2D is a stimulatory receptor expressed by NK cells and some T cells, suggesting that one or both of these cell types is involved in the control of aggressive prostate adenocarcinomas.

The Eμ-Myc transgene model consists of the *Myc* oncogene expressed under the control of the Eμ immunoglobulin heavy chain enhancer and the *Myc* promoter. Eμ-Myc transgenic mice that lack T and B cells due to a mutation in *Rag1* succumb to preB lymphomas a few weeks earlier than their $Rag1^+$ littermates, suggesting that mature T (or B) cells may limit the development of these tumours[19]. Furthermore, lymphomagenesis was also accelerated in

Eμ-Myc mice that lacked the gene encoding NKG2D, suggesting a role for NKG2D either in promoting NK- or T cell-dependent elimination of lymphomas[18]. All Eμ-Myc transgenic mice eventually develop lymphomas, perhaps because the initiation of new tumours finally overwhelms immune system control [18, 20].

Mutations of the gene encoding the tumour suppressor p53 occur spontaneously in most naturally arising cancers. p53 deficiency removes a key barrier to tumorigenesis and contributes to genomic instability [21]. Mice with homozygous germline mutations in *p53* ($p53^{-/-}$ mice) develop mainly lymphomas of thymic origin, whereas $p53^{+/-}$ mice develop both disseminated lymphoma and non-lymphoid tumours, mainly sarcomas. Studies demonstrate that tumour incidence is higher in *p53*-deficient mice that are also deficient in IFNγ, the IFNγ receptor and/or STAT1 [22]. IFNγ-insensitive $p53^{-/-}$ mice developed a broader spectrum of tumours compared to mice lacking p53 alone [22]. In addition, iNKT cells have been shown to contribute to the control of tumour development in $p53^{+/-}$ mice[23] and both perforin- and TRAIL-mediated apoptotic pathways participate in this response. [12, 13].

### Carcinogen models

Tumours arising as a result of chemical carcinogenesis arise autochtonously and proceed through the stages of tumorigenesis. Carcinogen-based models, although useful, have been criticized because the potent carcinogens may induce local tissue inflammation that has the potential to alter experimental outcomes [24]. On the other hand, an advantage to these models compared to some transgenic models is that the carcinogen can be titrated so that tumor incidence is limiting.

Among the most commonly studied models of mouse tumorigenesis is the induction of fibrosarcomas in mice treated subcutaneously or intradermally with the carcinogen methyl-cholanthrene (MCA). Genetic studies showed an increased incidence of MCA-induced fibrosarcomas in mice with defects in perforin [25, 26], the IFN signalling pathway [10, 22, 27], or the TRAIL mediated cytotoxicity pathway [28, 29]. Both αβ and γδ T cells were implicated in the surveillance of MCA-induced fibrosarcomas [30, 31]. Among αβ T cells, NKT cells[32] and possibly $CD4^+$ T cells [10] were found to be most important. Additionally, NK cells play a critical role as direct effectors of tumor lysis and possibly as collaborators with iNKT cells [33, 34] . By contrast, CD8-deficient mice exhibited no defect in the control of MCA-induced tumours [25].

Another widely used carcinogen model is the induction of skin cancer involving the tumour initiator and promoter combination DMBA (dimethylbenz(a)anthracene) and TPA (12-O-tetradecanoyl phorbol 13-acetate). Mice deficient in skin-associated γδ T cells exhibited a higher incidence of DMBA/TPA-induced tumours [30]. $CD8^+$ T cells, by contrast, may promote tumorigenesis in this model under some conditions [35].

## Receptors mediating immune surveillance

As reviewed elsewhere[36], evidence supports a role for the adaptive immune system in immune surveillance, indicating a role for T or B cell receptors. Here we focus on receptors used by NK cells and in some cases shared by T cells that have defined antigen specificities and have been implicated in tumour surveillance. Among these receptors are NKG2D, NKp46, NKp30, NKp44, NKp80, 2B4 and DNAM1 (DNAX accessory molecule 1). Several of the receptors seem to mediate “induced self recognition”, meaning that the ligands are encoded by the host’s genome, are poorly expressed by normal cells, and are upregulated in stressed or diseased cells[8]. This phenomenon is best characterized in the case of the NKG2D receptor. For this reason, and because its role in tumour surveillance has been examined

extensively, we focus our discussion on NKG2D and its ligands, after introducing some of the other key NK cell receptors.

### NKp46, NKp30, NKp44 and NKp80

These four stimulatory receptors are collectively called natural cytotoxicity receptors (NCR) [37–40] although NKG2D and other receptors also have important roles in the recognition and cytotoxicity of tumour cells by NK cells as discussed below. The four receptors have been well studied in human NK cells, but only one of them, NKp46, has been characterized in mice. In humans, NKp46, NKp30 and NKp80 are expressed by all NK cells, whereas NKp44 is only expressed by activated NK cells [41, 42]. Blocking one or more of these receptors with antibodies often inhibits killing of tumour cell lines by human NK cells in vitro [37–39]. Furthermore, mice with a targeted mutation in *NKp46* were impaired in their ability to reject a transferred lymphoma cell line that expressed ligands for that receptor [43].

A surprising diversity of unrelated ligands has been reported for NCRs, including viral haemagglutinins (NKp46 and NKp44) [44, 45], heparan sulphate proteoglycans (NKp30 and NKp46) [46] and the nuclear factor HLA-B-associated transcript 3 (BAT3) (NKp30) [47] and the activation-induced C-type lectin (AICL) (NKp80) [48]. Additional studies will be needed to determine the importance of these various ligands for NCRs.

### 2B4

2B4 is a member of the SLAM (signalling lymphocyte activation molecule)-related family of receptors, which is expressed by all NK cells, γδ T cells, a subset of $CD8^+$ T cells and all human $CD14^+$ monocytes [49]. The unique ligand for 2B4 is CD48, also a SLAM-related receptor, which is expressed by all haematopoietic cells. 2B4 can reportedly function as either a stimulatory or inhibitory receptor, depending on the splice isoform that is expressed, the identity of the signalling adaptor molecule it associates with, and the extent of cross-linking of the receptor [50–52]. 2B4 has a role in rejecting tumours that express its ligand, CD48 [53].

### DNAM1

DNAM1 is an adhesion molecule that is constitutively expressed by most NK cells, T cells, macrophages and dendritic cells [54, 55]. Ligands identified for DNAM1 include nectin 2 (also known as CD112) protein and CD155 (also known as the poliovirus receptor, PVR) [56, 57]. These ligands are often expressed by tumour cells and can activate or enhance tumour cell lysis *in vitro* [58, 59]. Recent studies showed that DNAM1-deficient mice have reduced capacity to reject certain tumour cells *in vivo* and to limit formation of carcinogen-induced tumours *in vivo* [34, 60].

### NKG2D

NKG2D is a lectin-like type II transmembrane homodimer that has received considerable attention in light of evidence for its role in immune responses in the context of cancer, infection and autoimmunity. NKG2D is expressed by virtually all NK cells and activated $CD8^+$ T cells, and subsets of γδ T cells and NKT cells [61, 62]. In certain conditions, NKG2D is also expressed by $CD4^+$ T cells, at least in humans[63–67].

A surprisingly large number of different NKG2D ligands have been identified, all of which are related self proteins that are similar to MHC class I molecules (Box 1). However, normal cells typically do not express the ligands well, whereas they are often specifically upregulated in cancerous or stressed tissues (Table 2).

**Box 1**

### The ligands for NKG2D

NKG2D ligands are self proteins that are related to MHC class I molecules, although they differ from MHC class I molecules in that they do not present molecular cargo and fail to bind $\beta_2$-microglobulin [134]. The ligands include MHC class I polypeptide-related sequence A (MICA) and MICB in humans [62], which have no mouse homologues, and the ULBP (cytomegalovirus UL16-binding protein) or RAET1 (retinoic acid early transcript 1) ligand families that exist in both humans and mice[135–138]. Each human or mouse strain has the capacity to express approximately 5–10 different NKG2D ligands. Most normal cells, however, do not express significant levels of NKG2D ligands on the cell surface. By contrast, most tumour cell lines express one or more NKG2D ligand [39, 62, 135, 136]. Furthermore, many primary human tumours express NKG2D ligands [117, 139, 140]. Similarly, expression of the NKG2D ligands RAE1 or MULT1 (murine ULBP-like transcript 1) was observed on primary lymphomas generated in Eμ-Myc mice [18, 141] and on primary adenocarcinomas generated in TRAMP mice [18]. Ligand expression is also induced in cells infected with certain pathogens [142].

Evidence has accumulated showing that NKG2D has an important role in immune surveillance of tumours (Figure 2). NKG2D-dependent elimination of tumour cells expressing NKG2D ligands has been well documented *in vitro* [39, 62, 68, 69] and *in vivo* in tumour transplant experiments [70, 71]. In humans, specific NKG2D gene polymorphisms were associated with susceptibility to cancer[72].

The most direct evidence supporting a role for NKG2D in tumour surveillance came from analysis of tumour incidence in gene-targeted mice that lack NKG2D, and also carrying transgenes that elevate the incidence of specific cancers [18]. In TRAMP mice, the incidence of a highly aggressive form of prostate adenocarcinoma was markedly increased when the mice were also deficient for NKG2D[18]. Similarly, in mice carrying the Eμ-Myc transgene that causes B lymphoma, onset of lymphoma was accelerated by 7 weeks if the mice were also deficient for NKG2D[18]. It has not yet been established whether NKG2D-dependent control of tumours in these models is mediated by NK cells or one or more type of NKG2D-expressing T cell, or by both NK cells and T cells.

The action of NKG2D in tumour surveillance in the TRAMP model was also suggested by the finding that many of the aggressive adenocarcinomas that arose in NKG2D-deficient mice expressed one or more of the NKG2D ligands, whereas similar tumours that arose in NKG2D-proficient mice generally lacked expression of NKG2D ligands. A likely explanation is that aggressive prostate adenocarcinomas tumours that arose in NKG2D-proficient mice were subjected to NKG2D-mediated immune surveillance, resulting in selection for variant tumour cells that had lost expression of the ligands (Figure 2). In contrast to this pattern in TRAMP mice, the B lymphomas that arose in Eμ-Myc mice commonly expressed NKG2D ligands whether or not the mice were NKG2D deficient. These data suggested that evasion of NKG2D-mediated surveillance occurs in some cases despite continued expression of NKG2D ligands, consistent with the fact that many primary tumours in normal animals or humans express NKG2D ligands.

A role for NKG2D in surveillance of skin cancer was suggested by the increased expression of transcripts for NKG2D ligands in carcinogen-treated skin samples [30]. Interestingly, transgenic mice that constitutively expressed high levels of NKG2D ligands, which results in dampened NKG2D function, exhibited an increased incidence of carcinogen-induced cutaneous malignancies [73]. These data were not definitive in implicating NKG2D in

cutaneous immune surveillance, however, because the NK cells in these transgenic mice exhibited defects in eliminating tumour cells that lacked NKG2D ligands, as well as those that expressed NKG2D ligands.

NKG2D deficiency did not result in the increased incidence or severity of some types of cancer, including a late arising, less malignant form of adenocarcinoma in TRAMP mice or fibrosarcomas induced by MCA[18]. The latter observation was surprising in light of the contrasting findings of an earlier study that used repeated injections of NKG2D-specific antibody to block the receptor[74]. It is possible that sustained engagement of NKG2D by antibodies impairs both NKG2D-dependent and -independent NK cell functions, as has been reported for sustained engagement of NKG2D by its ligands [73, 75–77]. In any case, the observation that the incidence or severity of tumours was unaffected by NKG2D deficiency in some models suggests that these types of tumours readily evade NKG2D-dependent immune surveillance, fail to express NKG2D ligands at a sufficiently early stage, or are otherwise sequestered or insensitive to immune destruction. Possible mechanisms of evasion include upregulation of inhibitory MHC class I molecules to counteract the higher levels of stimulatory ligands, and/or loss of distinct stimulatory or adhesion ligands by the tumor cells. In contrast, the DNAM1 receptor has been implicated in immune surveillance of MCA-induced sarcoma suggesting its action is not readily evaded in this system[34].

Considering the capacity for cancers to evolve in the host and the heterogeneity of oncogenic mechanisms that operate in different cancer models, it is not surprising that the NKG2D system, or any other system for that matter, is ineffective in impeding disease in certain cancer models. A detailed account of why NKG2D is ineffective in these specific models is not yet available, but it is notable that in some systems NKG2D ligands are expressed on the surface of tumor cells, but fail to promote tumor rejection, whereas in other cases NKG2D ligands are lost from the tumor cells. In the first case, it is likely that the response of NKG2D+ lymphocytes is suppressed or avoided, whereas in the latter case, tumor variants that lack expression of ligands may arise readily. Why some tumors may extinguish ligands more readily than other is unknown, but could be related to the fact that the milieu of activated oncogenes, mutated tumor suppressors and other mediators varies in tumors of different origins.

## Cancer-associated immune activation

Tumorigenesis is a complex process involving the dysregulation of many cellular pathways, in many cases resulting from mutations in oncogenes and tumour suppressor genes. Recognition of cancer cells by innate immune cells must depend on their ability to distinguish these dysregulated cells from normal cells. Many of the distinctive features of cancer cells, such as proliferation, invasiveness and repression of cell death pathways, are features that are exhibited by normal cells in other contexts. Other features are more specific for diseased cells, although untransformed stressed cells may also be similarly affected. For a host defense system against cancer to be effective, the system in the cancer cell that alerts the immune system most probably involves signalling pathways that process several types of information that collectively, but not individually, identifies the cell as a cancer cell.

Features that distinguish cancer cells from most normal cells are numerous. To mention only a few, mutations that activate oncogenes probably occur very early in the development of most tumours and provide persistent proliferative signals. Oncogene-induced signals activate tumour suppressors by at least two mechanisms, one involving p19ARF (encoded by the CDKN2A gene) and the other involving the DNA damage response**[G]**, which is activated after DNA damage is sensed by the ATM and ATR protein kinases (Box 2). Activated p19ARF and the DNA damage response can each independently induce p53

expression and other mediators that arrest the cell cycle and can induce cellular senescence (Box 3) or apoptosis when persistently activated [78, 79].

**Box 2**

**DNA damage response**

The DNA damage response protects the genome by facilitating repair of minor DNA damage in cells, and functions as a key barrier to tumorigenesis (Fig. 1). Rapid DNA replication in the context of oncogene activation is thought to result in the disruption of DNA replication forks ("replication stress") and accompanying DNA breaks, both of which can trigger the DNA damage response by activating key sensors in the DNA damage response pathway, the protein kinases ataxia-telangiectasia mutated (ATM) and ATM and RAD3 related (ATR) [143]. ATM and ATR initiate a cascade that ultimately induces cell cycle arrest and DNA repair functions. Depending on the cell type and other factors, prolonged or severe activation of the DNA damage response results in activation of apoptotic programmes or cellular senescence programmes. The DNA damage response therefore helps to preserve the integrity of the genome or eliminate severely damaged cells.

**Box 3**

**Cellular senescence**

Cellular senescence, wherein tumour cells survive for a time in an irreversibly senescent state, is associated with a blockade in cellular proliferation as well as a specific programme of gene activation that results in secretion of several pro-inflammatory cytokines and chemokines. Interestingly, many of these modulators paradoxically promote tumour cell growth, and studies suggest that the senescent state can, in some cases, promote tumorigenesis [144]. On the other hand, abundant evidence has accumulated that senescence is a barrier to tumorigenesis [78, 79, 145], by inhibiting tumour cell growth, inducing cell death and activating immune responses that help to eliminate cancer cells.

Mutations in tumour suppressor genes, or the pathways that activate tumour suppressors, enable continued cellular proliferation of nascent tumour cells, but dysregulate DNA replication and repair in a manner that ultimately results in instability of the genome and the accumulation of chromosomal abnormalities [80, 81].

Hence, at early stages of tumorigenesis, cells may have early warning signs such as activation of p19ARF, the DNA damage response and tumour suppressors, and activation of the gene programme that is associated with cellular senescence. At late stages the cells have other defects such as genomic instability. Furthermore, certain other stress pathways are commonly activated in cancer cells, including the heat shock response[82] and the unfolded protein response**[G]**[83]. The roles of some of these pathways as warning signals that trigger anticancer immune responses is discussed below.

## DNA damage response (see Box 2)

The role of the DNA damage response as a key barrier to tumorigenesis was highlighted by studies on human tissues showing that early pre-neoplastic lesions in the breasts, lungs, bladder and colon of patients show chronic activation of the DNA damage response, manifested by phosphorylation of ATM, CHK2 (checkpoint kinase 2 homologue) and histone γ-H2AX, another marker of DNA damage response activation [84, 85] (Figure 1). Other studies show that transgenic expression in mice of the proto-oncogene *MYC* leads to

DNA damage, consequent activation of ATM and p53, and apoptosis of affected cells *in vivo* [86, 87]. DNA damage induced by MYC may result from production of increased reactive oxygen species, independent of cell cycle entry, as well as from induction of rapid DNA replication [88]. ATM activation under these conditions provides a barrier to tumorigenesis, because deficiency of *Atm* increases MYC-induced tumorigenesis in mice [86, 87]. These data are consistent with the fact that mutations in *ATM* in humans, which cause the syndrome ataxia telangiectasia when present in the homozyogous state, increase the incidence of lymphoma, leukaemia and breast cancer [89].

A link between the DNA damage response and anti-tumour immune responses was initially established in studies of NKG2D ligands [90] (Table 2). Genotoxic stress that activates ATM or ATR can induce NKG2D ligand expression (RAE1, MULT1, H60a, MICA and ULBPs) on the surface of relatively normal cultured cells, including fibroblast cell cultures [8, 90]. Induction of NKG2D ligand expression was prevented by inhibiting, or knocking down, the expression of ATR or ATM, depending on the nature of the genotoxic stress [8, 90]. Most established tumour cell lines express NKG2D ligands constitutively, and knockdown studies established that constitutive ATM or ATR activation had an important role in maintaining constitutive ligand expression by those cells [8, 90]. These studies indicated that the display of NKG2D ligands on tumour cells is mediated in part by an activated DNA damage response. This upregulation of NKG2D ligand expression is accompanied by increased levels of the corresponding mRNA transcripts, but it has not been determined whether this is due to increased transcription or alterations in mRNA processing.

The DNA damage response also has a role in inducing the expression of ligands for a distinct NK cell receptor, as shown by a study showing that CD155, a ligand for DNAM1, was induced by DNA-damaging drugs on the surface of multiple myeloma cells in an ATM/ATR-dependent manner [57]. Finally, the DNA damage response directly regulates expression of the death receptor DR5, a ligand for TRAIL [91]. Engagement of DR5 by TRAIL induces apoptosis, and evidence indicates that TRAIL is expressed by NK cells and T cells and functions as an important effector molecule in tumour surveillance by these cells [28, 92].

## Cellular senescence (see Box 3)

Cellular senescence has been linked to immune mediated tumor elimination mechanisms by a study of liver tumours generated from transformed cells that initially lacked p53 expression. When p53 expression was subsequently switched on, the tumours underwent growth arrest, exhibited features of cellular senescence and were gradually eliminated by NK cells and other infiltrating cells [78]. These studies suggested that senescence associated with p53 activation in tumour cells is in some cases associated with the activation of immune responses that destroy cancer cells or their pre-malignant counterparts.

How the senescence programme is linked to anticancer immune responses has not been worked out in detail, but studies suggest that numerous ligands that stimulate immune responses are upregulated in senescent cells, including intercellular adhesion molecule 1 (ICAM1), a ligand for the adhesion receptor lymphocyte function-associated antigen 1 (LFA1) that has an important role in NK cell activation, NKG2D ligands such as MICA and ULBP2, and the DNAM1 ligand CD155 [78, 93] (Table 2). As already noted above, the expression of some of these ligands is induced by the DNA damage response. Hence, it remains unclear whether the induction of these immune-stimulating ligands is related to the senescence programme itself, which normally takes several days to establish, or the DNA damage response, which may be involved in establishing the senescence programme. Notably, the induction of NKG2D ligand expression in cultured cells by DNA-damaging agents did not require p53[90], whereas tumour senescence and NK-cell-dependent elimination of senescent tumour cells was induced by p53 reactivation *in vivo* [78]. Therefore,

it seems probable that both p53-dependent and p53-independent processes linked to tumorigenesis might regulate the sensitivity of tumour cells to NK cell-mediated elimination *in vivo*.

## The heat shock response

It has long been known that the heat shock response is activated in many forms of cancer [82]. Mice deficient in heat shock transcription factor 1 (HSF1), which functions as a key inducer of heat shock response, were less susceptible than wild-type mice to cancer in experimental models, suggesting that the heat shock response is hijacked by tumours to enhance their survival[94]. Linking antitumour immune responses to the heat shock response may therefore function to target a response that is otherwise beneficial to tumours, thus limiting the damage.

Studies in cultured human epithelial cells implicated the heat shock response in transcriptional activation of the human *MICA* and *MICB* genes[95] (Table 2). The promoter regions of both genes contain HSF1 binding elements, which were necessary for transcriptional activation [96]. Distinct elements in the promoters were required to support transcription in virus-infected cells or proliferating cells, suggesting that there was some independence in the control of MICA and MICB transcription under different conditions.

So far, transcriptional activation has not been observed in the case of other NKG2D ligand families, including human ULBP and RAET1 proteins and mouse RAET1, MULT1 and H60 proteins. In the case of MULT1, however, the heat shock response nevertheless has an important role in regulating cell surface expression of the protein at a posttranscriptional step (Table 2). In fibroblast cells and other cells, the cytoplasmic tail of MULT1 is subject to ubiquitylation that targets the protein for destruction in lysosomes [97]. Exposure of these cells to heat shock reversed the ubiquitin-dependent destruction of MULT1, resulting in a marked increase in levels of the protein at the cell surface. Interestingly, ultraviolet (UV) irradiation of fibroblasts had the same effect [97]. Although UV irradiation induces the DNA damage response, the stabilization of MULT1 protein by UV irradiation was independent of the DNA damage response. UV irradiation alters several other pathways in cells, and it is possible that its effects on MULT1 stabilization are exerted through a pathway that overlaps with the heat shock response.

MULT1 expression provides an example of combinatorial regulation by distinct cancer-associated stress pathways, the DNA damage response [90], and the heat shock response [97]. Coupling MULT1 expression to multiple stress pathways, in this case operating at different stages of biogenesis of the molecule, may represent a paradigm for mechanisms that restrict the display of immune-activating ligands to seriously diseased cells.

## Other mechanisms that regulate NKG2D ligands

Evidence suggests additional modes of regulation of stimulatory ligands that activate NK cells. In F9 embryocarcinoma cells, the transcription of *Raet1* genes was induced by retinoic acid[98], which was the basis for the first identification of these genes. Retinoids exert growth suppressive effects on normal cells and tumour cells and have been considered promising agents for cancer therapy[99]. However, the generality of the role of retinoids in regulating NKG2D ligands remains unclear.

The transcription of *Raet1e* was inhibited by the transcription factor JUNB in cell lines and mouse tissues[100]. These findings are of particular interest in light of the evidence that JUN family transcription factors show complex regulation in conditions of stress and injury[101].

NKG2D is expressed by all γδ T cells that reside in the mouse epidermis [68]. These T cells participate in the destruction of cutaneous malignancies and wound healing[102, 103]. One NKG2D ligand, H60c, is expressed selectively in the epidermis [104, 105]. Engagement by H60c displayed on keratinocytes, by NKG2D on epidermal γδ T cells, is essential for triggering γδ T cell activation and lysis of the keratinocytes, suggesting that NKG2D functions as a key co-stimulatory receptor in the activation of epidermal T cells [105]. Interestingly, expression of *H60c* is upregulated in wounded skin, and cultured keratinocytes upregulate H60c but not other NKG2D ligands, suggesting that H60c may be the main NKG2D ligand involved in cutaneous immune surveillance[105] (Table 2). The mechanisms that upregulate H60c in diseased and stressed tissues are not yet known. Although human and mouse cutaneous T cells differ in many respects, it is interesting that one of the human NKG2D ligands, ULBP4, is also selectively expressed in human skin[106].

Regulation of NKG2D ligands by microRNAs was reported for MICA and MICB. In human cell lines, microRNAs that target the 3' untranslated regions of the *MICA* and *MICB* transcripts inhibited steady state MICA and MICB expression [107]. Stressing the cells amplified MICA and MICB expression to an extent that overcame the block imposed by the microRNAs. Whether these microRNAs are themselves regulated in normal tissues by stress remains to be established. However, it was of interest that certain tumours overexpressed the microRNAs, which may serve as a mechanism whereby tumours evade immunosurveillance [107].

An additional, important mechanism of regulation of NKG2D ligands is through shedding from the cell surface, which has been reported for MICA, MICB, ULBP2 and ULBP4 [108–111] but has not been documented in the case of the mouse NKG2D ligands. Shedding of NKG2D ligands is thought to be mediated by ADAM (a disintegrin and metalloproteinase) family metalloproteinases [112–114], and is assisted by ERP5 (endoplasmic reticulum protein 5; also known as PDIA6) [115]. The presence of cell free NKG2D ligands in the serum of patients with late stage cancer [109, 110] was correlated with reduced levels of NKG2D at the surface of NK cells and T cells and decreased function of these cells [108, 116, 117], and could therefore be a mechanism that enables tumours to escape immune surveillance [118]. . Shedding of NKG2D ligands is also seen in cases of autoimmune disease where there is no "evasion" process[66] **s**uggesting that shedding may occur spontaneously in the case of cells that express ligands. However, it is also possible that the extent of shedding is increased as tumors progress, perhaps reflecting selection for immune-escape variants.

## Influences of the tumour environment

In the environment of a tumour, numerous events are thought to have an impact on the expression and function of immunoreceptors, including NKG2D and its ligands. In principle, these influences may increase or decrease NKG2D-dependent immune surveillance. For example, as noted earlier, inflammation associated with tumorigenesis can, in some cases, increase tumour growth and suppress protective antitumour immune responses [23], and it is possible that inhibition of NKG2D function is one example of how such inhibition occurs. It is also conceivable that in some tumour environments, NKG2D signalling induces a pro-tumour programme. Although the roles of these regulatory events in promoting or inhibiting antitumour immune responses remain uncertain, they must be accounted for when considering the role of NKG2D in tumour immunity.

Cytokines present in the tumour environment are one important potential determinant of NKG2D ligand expression. RAE1 was downregulated following exposure to transforming growth factor-β (TGFβ)[114], which might therefore be one of the mechanisms of immunosuppression mediated by this cytokine. Surprisingly, IFNγ and IFNα downregulated H60a but not RAE1 or MULT1 expression by sarcoma cell lines [119]. Furthermore, the

expression of MICA and, in some cases, ULBP2 by melanoma and glioma cell lines was downregulated by IFNγ treatment [120, 121]. These findings were surprising because IFNs generally promote antitumour immune responses.

The expression and function of NKG2D itself is also influenced by the tumour environment. Pro-inflammatory cytokines that are involved in proliferation and survival of NK cells and T cells such as IL-2 and IL-15 stimulate NKG2D expression and potentiate its cytotoxic and IFNγ secretory function [122, 123]. Conversely, the immunosuppressive cytokine TGFβ secreted by several tumours and found in the serum of cancer patients[124] can directly induce NKG2D downregulation when secreted in the tumour environment or as a membrane-bound cytokine on regulatory immune cells, or when present in tumour-derived exosomes[125, 126]. Downregulation of NKG2D expression also occurs in response to another cytokine that favours tumour growth, macrophage migration inhibitory factor (MIF)[127].

In addition to cytokines, it is probable that NKG2D ligands expressed in the tumour environment can adversely affect NKG2D function. Sustained engagement of NKG2D by its ligands *in vivo* compromises the capacity of NK cells to attack tumours. Persistent engagement by RAE1 [73], H60a [128] and MICA [75], or by soluble forms of MICA and MICB [108], is known to induce NKG2D internalization and subsequent degradation. In some cases, such persistent NKG2D engagement impairs NK cell functions more broadly, even inhibiting responses to NK-cell-sensitive target cells that lack NKG2D ligands[73, 76]. These findings raise the possibility that when tumour growth outpaces protective responses, NKG2D and other NK cell functions are ultimately inhibited as a result of persistent stimulation.

## Concluding remarks

As a result of the signaling associated with tumorigenesis, many developing tumour cells display cell surface ligands that engage activating receptors expressed by NK cells and in some cases T cells. Some of the same signaling pathways that activate this extrinsic response are responsible for activating intrinsic tumor suppressor mechanisms such as p53-induced apoptosis and senescence. However, there are likely some differences in the specific mediators of the two types of responses, as suggested by the finding that induction of NKG2D ligands by the DNA damage response occurs in cells lacking p53 [90].

As exemplified by studies of the NKG2D ligand MULT1, anti-tumor responses dependent on NK receptors are in some cases regulated by cooperation of distinct stress pathways that act at different levels of biogenesis of the immune-activating ligands. Combinatorial regulation by distinct stress pathways presumably helps to prevent inappropriate responses to normal cells. On the downside, however, these features may provide multiple targets for mutational inactivation of immune recognition of tumor cells.

An interesting question is whether the ligand-induction mechanisms linked to tumor suppressor pathways evolved specifically for anti-tumor responses, or play a more general role in disease responses, such as in responses to infections. In considering this question, it is often argued that because cancer is predominantly a disease of older individuals who are presumed to be post-reproductive, natural selection cannot directly select for anti-cancer mechanisms. It should be kept in mind, however, that this argument applies equally to intrinsic tumor suppressor mechanisms and immune-based tumor suppressor mechanisms. In light of this, some of the assumptions behind this argument may be questioned. The various barriers to tumorigenesis presumably work together to ensure that cancer is usually delayed to a later stage of life.

Immune-based anti-tumor mechanisms have the potential to complement, as opposed simply supplement, intrinsic barriers in at least two respects. First, the triggers of the immune-based mechanisms may in some cases be operative in cases where intrinsic tumor suppressors are lost from tumor cells, such as in the example above where the induction of NKG2D ligands by the DNA damage response occurs in cells that have lost the p53 tumor suppressor [90]. Secondly, the innate immune system responses induced by these mechanisms may in some cases promote powerful adaptive immune responses that have the capacity for immune memory and sustained systemic protection.

Although NK cell receptor-dependent responses to tumours can be protective to the host, some tumours may avoid detection by down-regulating or shedding ligands, as suggested by studies of NKG2D ligands. The mechanisms whereby ligand expression is extinguished are incompletely understood and must be addressed in future studies. In some cases, it is probable that loss of ligand expression results from mutations in developing tumour cells that inactivate the same pathways that induce ligand expression in the first place, whereas in other cases the cells may acquire mutations in the ligand genes themselves. It is also likely that although expression of ligands for NK receptors is often protective against cancer, in some cases the tumor can evolve to exploit ligand expression to prevent a protective immune response. Desensitization of the NKG2D receptor as a result of shedding of NKG2D ligands is probably one example, but there are likely to be others.

A thorough understanding of the pathways involved in immune system activation by tumors may enable the design of therapeutic drugs. In cases where tumors acquire mutations that disable pathways that induce immune system ligands, a useful approach could be to design drugs that bypass the missing steps or re-induce ligands by another mechanism. It is possible, for example, that the efficacy of some chemotherapy drugs such as 5-FU and cisplatin may be related to increasing NKG2D ligand expression by tumour cells via activation of the DNA damage response [8, 129]. In addition, proteasome inhibitors and/or histone deacetylase inhibitors represent promising candidate for enhancing expression of NKG2D ligands[130, 131]. Given the likelihood that ligand expression is induced by the synergistic action of several signals associated with the cancerous state, such interventions may be quite selective in targeting tumour cells as opposed to normal cells. Where inhibitory cytokines have a role in suppressing ligand expression on tumour cells, blockade of cytokine action may serve as a useful therapeutic intervention. As an alternative approach, although NKG2D ligands are absent from some tumour cells *in vivo*, vaccines comprised of cells expressing tumour antigens and NKG2D ligands may nevertheless be effective at inducing effective adaptive immune responses against tumours in some cases [70, 132].

On the other hand, many if not most advanced cancers continue to express ligands for NKG2D and other activating receptors on NK cells with impunity (Figure 2). Tumour escape in these instances may be due to impaired functioning of NK cell receptors due to inhibitory cytokines in the microenvironment, in which case cytokine blockade may be beneficial. Shedding of ligands, such as NKG2D ligands, are thought to repress protective immune responses, suggesting that antibodies that systemically block or remove the shed proteins may be therapeutic[111]. In other cases, persistent stimulation of the NK cells via NKG2D and possibly other activating receptors may result in anergy. A deeper understanding of how such persistent stimulation inactivates NK cell receptors and NK cell activity may provide approaches to reverse the unresponsive state of NK cells. Finally, in cases where NKG2D ligands continue to be expressed by tumour cells, they represent an inviting target for therapeutic antibodies designed to eliminate ligand-expressing tumour cells.

## GLOSSARY

| | |
|---|---|
| **NKG2D** | (Natural killer group 2, member D). A lectin-type activating receptor encoded by *Klrk1* (killer cell lectin-like receptor subfamily K, member 1) located in the NK cell gene complex. NKG2D associates in the plasma membrane with signalling adaptor molecules, including DAP10 (in both humans and mice) and (in mice but not humans) DAP12. DAP10 activates phosphoinositide 3-kinase and its signalling mechanism resembles that of co-stimulatory receptors such as CD28. By contrast, DAP12 activates spleen protein tyrosine kinase (SYK) and its signalling resembles that of T and B cell receptors |
| **p53** | A tumour suppressor that is mutated in ~50% or more of all human cancers. The p53 protein is a transcription factor that is activated by DNA damage, anoxia, expression of certain oncogenes and several other stress stimuli. Target genes activated by p53 regulate cell-cycle arrest, apoptosis, cell senescence and DNA repair |
| **Kaposi's sarcoma** | A tumour of endothelial-cell origin that is found most frequently in immunosuppresed patients, particularly in HIV-infected individuals. Kaposi's sarcoma-associated herpesvirus has been implicated as a co-factor in the formation of Kaposi's sarcoma |
| **RAG proteins** | RAG1 and RAG2 are proteins that mediate V(D)J recombination in pre-B cells and thymocytes, which is necessary for the production of B cell receptors and T-cell receptors, and for the development of B cells and T cells |
| **Perforin** | A component of the cytolytic granules of cytotoxic T cells and natural killer cells that participates in the permeabilization of plasma membranes, allowing granzymes and other cytotoxic components to enter target cells |
| **SV40 virus large T antigen** | A multifunctional protein product of the simian virus 40 early region that is necessary to establish a permissive host cell environment for viral replication by interactions with host proteins. Large T antigen binds and functionally inactivates the tumour suppressor proteins RB and p53 |
| **γδ T cells** | A T cell that expresses a T-cell receptor consisting of a γ-chain and a δ-chain. These T cells are present in several epithelial locations as intraepithelial lymphocytes (IELs), and are also present in lymphoid organs. Although the functions of γδ T cells (or IELs) are still mostly unknown, it has been suggested that mucosal γδ T cells mediate innate-type mucosal immune responses, and epidermal γδ T cells in mice have been implicated in tumour surveillance and wound repair |
| **NKT cells** | A subpopulation of T cells that expresses both NK- and T-cell markers. In the C57BL/6 mouse strain, NKT cells express the NK1.1 (NKRP1C) molecule and the T-cell receptor (TCR). Some NKT cells recognize CD1d-associated lipid antigens and express a restricted repertoire of TCRs (iNKT). After TCR stimulation of naive mice, NKT cells rapidly produce interleukin-4 and interferon-γ |

| | |
|---|---|
| **Unfolded protein response** | A response that increases the ability of the endoplasmic reticulum to fold and translocate proteins, decreases the synthesis of proteins, and causes cell cycle arrest and apoptosis |
| **Ataxia-telengiectasia syndrome** | (Also known as Louis–Bar syndrome). A familial recessive disease that is characterized by progressive cerebellar ataxia, oculocutaneous telangiectases and susceptibility to pulmonary infections. It is caused by germline mutations in the *ATM* gene, which is a sensor that activates the DNA damage response |

## References


1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70. [PubMed: 10647931]
2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–67. [PubMed: 2188735]
3. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med 2000;248:171–83. [PubMed: 10971784]
4. Pardoll D. Does the immune system see tumors as foreign or self? Annu Rev Immunol 2003;21:807–39. [PubMed: 12615893]
5. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24–37. [PubMed: 16397525]
6. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol 2008;9:503–10. [PubMed: 18425107]
7. Long E. Regulation of immune responses through inhibitory receptors. Annual Review of Immunology 1999;17:875–904.
8. Gasser S, Raulet DH. Activation and self-tolerance of natural killer cells. Immunol Rev 2006;214:130–42. [PubMed: 17100881]
9. Raulet DH. Interplay of natural killer cells and their receptors with the adaptive immune response. Nat Immunol 2004;5:996–1002. [PubMed: 15454923]
10. Shankaran V, et al. IFN gamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001;410:1107–1111. [PubMed: 11323675]
11. Street SE, et al. Innate immune surveillance of spontaneous B cell lymphomas by natural killer cells and gammadelta T cells. J Exp Med 2004;199:879–84. [PubMed: 15007091]
12. Smyth MJ, et al. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. J Exp Med 2000;192:755–60. [PubMed: 10974040]
13. Zerafa N, et al. Cutting edge: TRAIL deficiency accelerates hematological malignancies. J Immunol 2005;175:5586–90. [PubMed: 16237043]
14. Street SE, Trapani JA, MacGregor D, Smyth MJ. Suppression of lymphoma and epithelial malignancies effected by interferon gamma. J Exp Med 2002;196:129–34. [PubMed: 12093877]
15. Savage PA, et al. Recognition of a ubiquitous self antigen by prostate cancer-infiltrating CD8+ T lymphocytes. Science 2008;319:215–20. [PubMed: 18187659]
16. Fasso M, et al. SPAS-1 (stimulator of prostatic adenocarcinoma-specific T cells)/SH3GLB2: A prostate tumor antigen identified by CTLA-4 blockade. Proc Natl Acad Sci U S A 2008;105:3509–14. [PubMed: 18303116]
17. Liu Z, et al. Protective immunosurveillance and therapeutic antitumor activity of gammadelta T cells demonstrated in a mouse model of prostate cancer. J Immunol 2008;180:6044–53. [PubMed: 18424725]
18. Guerra N, et al. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity 2008;28:571–80. [PubMed: 18394936]
19. Nepal RM, et al. AID and RAG1 do not contribute to lymphomagenesis in Emu c-myc transgenic mice. Oncogene 2008;27:4752–6. [PubMed: 18408759]

20. Unni AM, Bondar T, Medzhitov R. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. Proc Natl Acad Sci U S A 2008;105:1686–91. [PubMed: 18223157]
21. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science 2008;319:1352–5. [PubMed: 18323444]
22. Kaplan DH, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proceedings of the National Academy of Sciences of the United States of America 1998;95:7556–61. [PubMed: 9636188]
23. Swann JB, et al. Type I NKT cells suppress tumors in mice caused by p53 loss. Blood. 2009
24. Qin Z, Blankenstein T. A cancer immunosurveillance controversy. Nat Immunol 2004;5:3–4. author reply 4–5. [PubMed: 14699396]
25. van den Broek ME, et al. Decreased tumor surveillance in perforin-deficient mice. J Exp Med 1996;184:1781–90. [PubMed: 8920866]
26. Street SE, Cretney E, Smyth MJ. Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. Blood 2001;97:192–7. [PubMed: 11133760]
27. Qin Z, Kim HJ, Hemme J, Blankenstein T. Inhibition of methylcholanthrene-induced carcinogenesis by an interferon gamma receptor-dependent foreign body reaction. J Exp Med 2002;195:1479–90. [PubMed: 12045246]
28. Finnberg N, Klein-Szanto AJ, El-Deiry WS. TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. J Clin Invest 2008;118:111–23. [PubMed: 18079962]
29. Cretney E, et al. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. J Immunol 2002;168:1356–61. [PubMed: 11801676]
30. Girardi M, et al. Regulation of cutaneous malignancy by gammadelta T cells. Science 2001;294:605–9. [PubMed: 11567106]
31. Gao Y, et al. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. J Exp Med 2003;198:433–42. [PubMed: 12900519]
32. Crowe NY, Smyth MJ, Godfrey DI. A critical role for natural killer T cells in immunosurveillance of methylcholanthrene-induced sarcomas. J Exp Med 2002;196:119–27. [PubMed: 12093876]
33. Smyth MJ, Crowe NY, Godfrey DI. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. Int Immunol 2001;13:459–63. [PubMed: 11282985]
34. Iguchi-Manaka A, et al. Accelerated tumor growth in mice deficient in DNAM-1 receptor. J Exp Med 2008;205:2959–64. [PubMed: 19029379]
35. Girardi M, et al. The distinct contributions of murine T cell receptor (TCR)gammadelta+ and TCRalphabeta+ T cells to different stages of chemically induced skin cancer. J Exp Med 2003;198:747–55. [PubMed: 12953094]
36. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991–8. [PubMed: 12407406]
37. Pessino A, et al. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. Journal of Experimental Medicine 1998;188:953–60. [PubMed: 9730896]
38. Sivori S, et al. NKp46 is the major triggering receptor involved in the natural cytotoxicity of fresh or cultured human NK cells. Correlation between surface density of NKp46 and natural cytotoxicity against autologous, allogeneic or xenogeneic target cells. European Journal of Immunology 1999;29:1656–66. [PubMed: 10359120]
39. Pende D, et al. Role of NKG2D in tumor cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin. European Journal of Immunology 2001;31:1076–86. [PubMed: 11298332]
40. Vitale M, et al. Identification of NKp80, a novel triggering molecule expressed by human NK cells. Eur J Immunol 2001;31:233–42. [PubMed: 11265639]
41. Pende D, et al. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. Journal of Experimental Medicine 1999;190:1505–16. [PubMed: 10562324]

42. von Lilienfeld-Toal M, et al. Activated gammadelta T cells express the natural cytotoxicity receptor natural killer p 44 and show cytotoxic activity against myeloma cells. Clin Exp Immunol 2006;144:528–33. [PubMed: 16734623]
43. Halfteck GG, et al. Enhanced in vivo growth of lymphoma tumors in the absence of the NK-activating receptor NKp46/NCR1. J Immunol 2009;182:2221–30. [PubMed: 19201876]
44. Mandelboim O, et al. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. Nature 2001;409:1055–60. [PubMed: 11234016]
45. Arnon TI, Markel G, Mandelboim O. Tumor and viral recognition by natural killer cells receptors. Semin Cancer Biol 2006;16:348–58. [PubMed: 16893656]
46. Bloushtain N, et al. Membrane-associated heparan sulfate proteoglycans are involved in the recognition of cellular targets by NKp30 and NKp46. J Immunol 2004;173:2392–401. [PubMed: 15294952]
47. Pogge von Strandmann E, et al. Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. Immunity 2007;27:965–74. [PubMed: 18055229]
48. Welte S, Kuttruff S, Waldhauer I, Steinle A. Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. Nat Immunol. 2006
49. Boles KS, Stepp SE, Bennett M, Kumar V, Mathew PA. 2B4 (CD244) and CS1: novel members of the CD2 subset of the immunoglobulin superfamily molecules expressed on natural killer cells and other leukocytes. Immunol Rev 2001;181:234–49. [PubMed: 11513145]
50. Schatzle JD, et al. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. Proc Natl Acad Sci U S A 1999;96:3870–5. [PubMed: 10097130]
51. Lee KM, et al. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. J Exp Med 2004;199:1245–54. [PubMed: 15123744]
52. Chlewicki LK, Velikovsky CA, Balakrishnan V, Mariuzza RA, Kumar V. Molecular Basis of the Dual Functions of 2B4 (CD244). J Immunol 2008;180:8159–67. [PubMed: 18523281]
53. Vaidya SV, et al. Targeted disruption of the 2B4 gene in mice reveals an in vivo role of 2B4 (CD244) in the rejection of B16 melanoma cells. J Immunol 2005;174:800–7. [PubMed: 15634901]
54. Shibuya A, et al. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. Immunity 1996;4:573–81. [PubMed: 8673704]
55. Tahara-Hanaoka S, et al. Tumor rejection by the poliovirus receptor family ligands of the DNAM-1 (CD226) receptor. Blood 2006;107:1491–6. [PubMed: 16249389]
56. Bottino C, et al. Identification of PVR (CD155) and Nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. J Exp Med 2003;198:557–67. [PubMed: 12913096]
57. Soriani A, et al. ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. Blood 2009;113:3503–11. [PubMed: 19098271]
58. El-Sherbiny YM, et al. The Requirement for DNAM-1, NKG2D, and NKp46 in the Natural Killer Cell-Mediated Killing of Myeloma Cells. Cancer Res 2007;67:8444–8449. [PubMed: 17875681]
59. Carlsten M, et al. DNAX accessory molecule-1 mediated recognition of freshly isolated ovarian carcinoma by resting natural killer cells. Cancer Res 2007;67:1317–25. [PubMed: 17283169]
60. Gilfillan S, et al. DNAM-1 promotes activation of cytotoxic lymphocytes by nonprofessional antigen-presenting cells and tumors. J Exp Med 2008;205:2965–73. [PubMed: 19029380]
61. Wu J, et al. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 1999;285:730–2. [PubMed: 10426994]
62. Bauer S, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999;285:727–9. [PubMed: 10426993]
63. Saez-Borderias A, et al. Expression and function of NKG2D in CD4+ T cells specific for human cytomegalovirus. Eur J Immunol 2006;36:3198–206. [PubMed: 17109473]
64. Azimi N, et al. Immunostimulation by induced expression of NKG2D and its MIC ligands in HTLV-1-associated neurologic disease. Immunogenetics 2006;58:252–8. [PubMed: 16568261]

65. Groh V, Smythe K, Dai Z, Spies T. Fas ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. Nat Immunol 2006;7:755–62. [PubMed: 16732291]
66. 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 U S A 2003;100:9452–7. [PubMed: 12878725]
67. Taneja V, et al. Requirement for CD28 may not be absolute for collagen-induced arthritis: study with HLA-DQ8 transgenic mice. J Immunol 2005;174:1118–25. [PubMed: 15634938]
68. Jamieson AM, et al. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity 2002;17:19–29. [PubMed: 12150888]
69. 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;107:159–66. [PubMed: 16150947]
70. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001;413:165–71. [PubMed: 11557981]
71. 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 U S A 2001;98:11521–6. [PubMed: 11562472]
72. Hayashi T, et al. Identification of the NKG2D Haplotypes Associated with Natural Cytotoxic Activity of Peripheral Blood Lymphocytes and Cancer Immunosurveillance. Cancer Res 2006;66:563–70. [PubMed: 16397273]
73. Oppenheim DE, et al. Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. Nat Immunol 2005;6:928–37. [PubMed: 16116470]
74. Smyth MJ, et al. NKG2D function protects the host from tumor initiation. J Exp Med 2005;202:583–8. [PubMed: 16129707]
75. Wiemann K, et al. Systemic NKG2D Down-Regulation Impairs NK and CD8 T Cell Responses In Vivo. J Immunol 2005;175:720–9. [PubMed: 16002667]
76. Coudert JD, Scarpellino L, Gros F, Vivier E, Held W. Sustained NKG2D engagement induces cross-tolerance of multiple distinct NK cell activation pathways. Blood 2008;111:3571–8. [PubMed: 18198346]
77. Ogasawara K, et al. Impairment of NK Cell Function by NKG2D Modulation in NOD Mice. Immunity 2003;18:41–51. [PubMed: 12530974]
78. Xue W, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007;445:656–60. [PubMed: 17251933]
79. Ventura A, et al. Restoration of p53 function leads to tumour regression in vivo. Nature 2007;445:661–5. [PubMed: 17251932]
80. Zhu C, et al. Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. Cell 2002;109:811–21. [PubMed: 12110179]
81. Dominguez-Sola D, et al. Non-transcriptional control of DNA replication by c-Myc. Nature 2007;448:445–51. [PubMed: 17597761]
82. Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 2000;92:1564–72. [PubMed: 11018092]
83. Ma Y, Hendershot LM. The role of the unfolded protein response in tumour development: friend or foe? Nat Rev Cancer 2004;4:966–77. [PubMed: 15573118]
84. Bartkova J, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005;434:864–70. [PubMed: 15829956]
85. Gorgoulis VG, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907–13. [PubMed: 15829965]
86. Pusapati RV, et al. ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. Proc Natl Acad Sci U S A 2006;103:1446–51. [PubMed: 16432227]
87. Reimann M, et al. The Myc-evoked DNA damage response accounts for treatment resistance in primary lymphomas in vivo. Blood 2007;110:2996–3004. [PubMed: 17562874]

88. Vafa O, et al. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. Mol Cell 2002;9:1031–44. [PubMed: 12049739]

89. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 2003;3:155–68. [PubMed: 12612651]

90. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005;436:1186–90. [PubMed: 15995699]

91. Wu GS, et al. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 1997;17:141–3. [PubMed: 9326928]

92. Smyth MJ, et al. Nature's TRAIL--on a path to cancer immunotherapy. Immunity 2003;18:1–6. [PubMed: 12530970]

93. Krizhanovsky V, et al. Senescence of activated stellate cells limits liver fibrosis. Cell 2008;134:657–67. [PubMed: 18724938]

94. Dai C, Whitesell L, Rogers AB, Lindquist S. Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. Cell 2007;130:1005–18. [PubMed: 17889646]

95. Groh V, et al. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proceedings of the National Academy of Sciences of the United States of America 1996;93:12445–50. [PubMed: 8901601]

96. Venkataraman GM, Suciu D, Groh V, Boss JM, Spies T. Promoter region architecture and transcriptional regulation of the genes for the MHC class I-related chain A and B ligands of NKG2D. J Immunol 2007;178:961–9. [PubMed: 17202358]

97. Nice TJ, Coscoy L, Raulet DH. Posttranslational regulation of the NKG2D ligand Mult1 in response to cell stress. J Exp Med 2009;206:287–298. [PubMed: 19171762]

98. Nomura M, Takihara Y, Shimada K. Isolation and characterization of retinoic acid-inducible cDNA clones in F9 cells: one of the early inducible clones encodes a novel protein sharing several highly homologous regions with a Drosophila polyhomeotic protein. Differentiation 1994;57:39–50. [PubMed: 8070621]

99. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. Oncogene 2003;22:7305–15. [PubMed: 14576840]

100. Nausch N, et al. Cutting Edge: The AP-1 Subunit JunB Determines NK Cell-Mediated Target Cell Killing by Regulation of the NKG2D-Ligand RAE-1{epsilon}. J Immunol 2006;176:7–11. [PubMed: 16365389]

101. Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. Oncogene 2001;20:2413–23. [PubMed: 11402337]

102. Hayday A, Tigelaar R. Immunoregulation in the tissues by gammadelta T cells. Nat Rev Immunol 2003;3:233–42. [PubMed: 12658271]

103. Jameson J, et al. A role for skin gammadelta T cells in wound repair. Science 2002;296:747–9. [PubMed: 11976459]

104. Takada A, et al. Two novel NKG2D ligands of the mouse H60 family with differential expression patterns and binding affinities to NKG2D. J Immunol 2008;180:1678–85. [PubMed: 18209064]

105. Whang MI, Guerra N, Raulet DH. Costimulation of dendritic epidermal gammadelta T cells by a new NKG2D ligand expressed specifically in the skin. J Immunol 2009;182:4557–64. [PubMed: 19342629]

106. Chalupny NJ, Sutherland CL, Lawrence WA, Rein-Weston A, Cosman D. ULBP4 is a novel ligand for human NKG2D. Biochem Biophys Res Commun 2003;305:129–35. [PubMed: 12732206]

107. Stern-Ginossar N, et al. Human microRNAs regulate stress-induced immune responses mediated by the receptor NKG2D. Nat Immunol 2008;9:1065–73. [PubMed: 18677316]

108. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T- cell activation. Nature 2002;419:734–8. [PubMed: 12384702]

109. Salih HR, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003;102:1389–96. [PubMed: 12714493]

110. Waldhauer I, Steinle A. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. Cancer Res 2006;66:2520–6. [PubMed: 16510567]
111. Jinushi M, et al. MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. Proc Natl Acad Sci U S A. 2008
112. Waldhauer I, et al. Tumor-associated MICA is shed by ADAM proteases. Cancer Res 2008;68:6368–76. [PubMed: 18676862]
113. Boutet P, et al. Cutting edge: The metalloproteinase ADAM17/TNF-alpha-converting enzyme regulates proteolytic shedding of the MHC class I-related chain B protein. J Immunol 2009;182:49–53. [PubMed: 19109134]
114. Eisele G, et al. TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. Brain 2006;129:2416–25. [PubMed: 16891318]
115. Kaiser BK, et al. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. Nature 2007;447:482–6. [PubMed: 17495932]
116. Doubrovina ES, et al. Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. J Immunol 2003;171:6891–9. [PubMed: 14662896]
117. Wu JD, et al. Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. J Clin Invest 2004;114:560–8. [PubMed: 15314693]
118. Wu JD, Atteridge CL, Wang X, Seya T, Plymate SR. Obstructing shedding of the immunostimulatory MHC class I chain-related gene B prevents tumor formation. Clin Cancer Res 2009;15:632–40. [PubMed: 19147769]
119. Bui JD, Carayannopoulos LN, Lanier LL, Yokoyama WM, Schreiber RD. IFN-Dependent Down-Regulation of the NKG2D Ligand H60 on Tumors. J Immunol 2006;176:905–13. [PubMed: 16393975]
120. Schwinn N, et al. Interferon-gamma down-regulates NKG2D ligand expression and impairs the NKG2D-mediated cytolysis of MHC class I-deficient melanoma by natural killer cells. Int J Cancer 2009;124:1594–604. [PubMed: 19089914]
121. Yadav D, Ngolab J, Lim RS, Krishnamurthy S, Bui JD. Cutting edge: Down-regulation of MHC class I-related chain A on tumor cells by IFN-gamma-induced microRNA. J Immunol 2009;182:39–43. [PubMed: 19109132]
122. Roberts AI, et al. NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. J Immunol 2001;167:5527–30. [PubMed: 11698420]
123. Horng T, Bezbradica JS, Medzhitov R. NKG2D signaling is coupled to the interleukin 15 receptor signaling pathway. Nat Immunol 2007;8:1345–1352. [PubMed: 17952078]
124. 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;172:7335–40. [PubMed: 15187109]
125. Ghiringhelli F, et al. CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med 2005;202:1075–85. [PubMed: 16230475]
126. Li H, Han Y, Guo Q, Zhang M, Cao X. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta1. J Immunol 2009;182:240–9. [PubMed: 19109155]
127. Krockenberger M, et al. Macrophage migration inhibitory factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. J Immunol 2008;180:7338–48. [PubMed: 18490733]
128. Coudert JD, et al. Altered NKG2D function in NK cells induced by chronic exposure to NKG2D ligand-expressing tumor cells. Blood 2005;106:1711–7. [PubMed: 15886320]
129. Cerboni C, Ardolino M, Santoni A, Zingoni A. Detuning CD8+ T lymphocytes by down-regulation of the activating receptor NKG2D: role of NKG2D ligands released by activated T cells. Blood 2009;113:2955–64. [PubMed: 19124832]
130. Diermayr S, et al. NKG2D ligand expression in AML increases in response to HDAC inhibitor valproic acid and contributes to allorecognition by NK-cell lines with single KIR-HLA class I specificities. Blood 2008;111:1428–36. [PubMed: 17993609]

131. Poggi A, et al. Effective in vivo induction of NKG2D ligands in acute myeloid leukaemias by all-trans-retinoic acid or sodium valproate. Leukemia 2009;23:641–8. [PubMed: 19151770]

132. Zhou H, et al. DNA-based vaccines activate innate and adaptive antitumor immunity by engaging the NKG2D receptor. Proc Natl Acad Sci U S A 2005;102:10846–51. [PubMed: 16040807]

133. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004;10:789–99. [PubMed: 15286780]

134. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003;3:781–90. [PubMed: 14523385]

135. 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. Nature Immunology 2000;1:119–126. [PubMed: 11248803]

136. Cerwenka A, et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. Immunity 2000;12:721–727. [PubMed: 10894171]

137. 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;169:4079–83. [PubMed: 12370332]

138. 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;33:381–91. [PubMed: 12645935]

139. Groh V, et al. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proceedings of the National Academy of Sciences of the United States of America 1999;96:6879–84. [PubMed: 10359807]

140. Friese MA, et al. MICA/NKG2D-mediated immunogene therapy of experimental gliomas. Cancer Res 2003;63:8996–9006. [PubMed: 14695218]

141. Unni AM, Bondar T, Medzhitov R. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. Proc Natl Acad Sci U S A. 2008

142. Lanier LL. Evolutionary struggles between NK cells and viruses. Nat Rev Immunol 2008;8:259–68. [PubMed: 18340344]

143. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev 2001;15:2177–96. [PubMed: 11544175]

144. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A 2001;98:12072–7. [PubMed: 11593017]

145. Braig M, et al. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature 2005;436:660–5. [PubMed: 16079837]

**Figure 1.**

Stepwise cancer progression and intrinsic and extrinsic barriers to cancer.

Based on histopathological, clinical and molecular data generated from analysis of colon carcinomas, it was proposed that cancer generally develops in a stepwise fashion as depicted in a "Vogelgram" diagram which is the basis of the figure [2, 133]. Although the order of certain specific events is likely to vary in different instances of cancer, certain early events, including oncogene activation, result in DNA replication stress and DNA damage and therefore activation of the DNA damage response (DDR). Oncogene activation leads to induction of p19ARF by a distinct mechanism. With some independence, the DDR and activated p19ARF activate key tumour suppressors such as p53. Depending on numerous factors, activated p53 results in cell cycle arrest, cellular senescence or apoptosis, all of which represent intrinsic barriers to tumorigenesis. The DDR also induces expression of ligands for the NKG2D receptor, and probably other immune receptors, which can activate extrinsic, anti-tumor immune responses. Similarly, cellular senescence induced by p53 triggers immune mechanisms that eliminate the senescent cells, though the specific receptors involved in that case have not yet been defined. Because these barriers typically arise downstream of oncogene activation, selection for homozygous p53 mutations is usually delayed relative to oncogene activation and often correlates with a transition to malignancy. Subsequently, the tumor undergoes additional evolution that optimizes its fitness and capacity to metastasize.

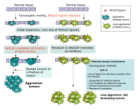

**Figure 2.**

Model of NKG2D-mediated tumour surveillance of prostate adenocarcinoma.

In C57BL/6 TRAMP mice, NKG2D-dependent immune responses limit the development of an early arising, highly malignant form of prostate adenocarcinoma, but not a late developing, less malignant form. As depicted on the left, the highly malignant tumours in NKG2D-deficient TRAMP mice generally express NKG2D ligands, whereas the rarer tumours of this type in NKG2D-proficient mice generally lack NKG2D ligands, suggesting that the immune response shapes these tumours by selecting for variant tumour cells that fail to express NKG2D ligands. In contrast, as depicted on the right, the less aggressive, late developing tumours express NKG2D ligands (albeit heterogeneously) whether or not NKG2D is expressed, suggesting that these tumours evade NKG2D-dependent elimination by a distinct mechanism.

## Table 1

Specific immune deficiencies associated with greater tumour incidence or severity in mice

| Strain | Tumour enhancement | Type of tumour | Defective immune component | Refs |
|---|---|---|---|---|
| **Spontaneous tumours** | | | | |
| 129/Sv | None | colon & lung | RAG2 | 10 |
| 129/Sv | None | colon & mammary | RAG2, STAT1 | 10 |
| C57BL/6, BALB/c | None | B cell lymphoma | $\beta_2$-microglubulin, perforin | 11, 14 |
| C57BL/6 | None | lymphoma | TRAIL | 13 |
| C57BL/6 | None | lymphoma | perforin | 12 |
| **Transgenic and knockout cancer models** | | | | |
| 129/Sv | *p53*−/− | Lymphoid/other | STAT1 | 22 |
| 129/Sv | *p53*−/− | Lymphoid/other | IFNγ receptor | 22 |
| C57BL/6 | *p53*+/− | Lymphoid/other | TRAIL | 13 |
| C57BL/6 | *p53*+/− | Lymphoid | Perforin* | 12 |
| C57BL/6 | *p53*+/− | Lymphoid/other | TCR Jα28,CD1d | 23 |
| C57BL/6 | TRAMP | prostate | NKG2D | 18 |
| C57BL/6 | TRAMP | prostate | TCRδ | 17 |
| C57BL/6 | Eμ-Myc | B-cell lymphoma | NKG2D | 18 |
| C57BL/6 | Eμ-Myc | B-cell lymphoma | TRAIL receptor | 28 |
| C57BL/6 | Eμ-Myc | B-cell lymphoma | RAG1 | 19 |
| **Carcinogen-induced tumours** | | | | |
| 129/Sv | MCA | fibrosarcoma | RAG2# | 10 |
| 129/Sv | MCA | fibrosarcoma | IFNγ receptor | 10, 22 |
| 129/Sv | MCA | fibrosarcoma | IFNγ receptor | 27 |
| 129/Sv | MCA | fibrosarcoma | STAT1 | 10, 22 |
| 129/Sv | MCA | fibrosarcoma | RAG2, STAT1 | 10 |
| C57BL/6 | MCA | fibrosarcoma | IFNγ | 26, 31 |
| C57BL/6 | MCA | fibrosarcoma | Perforin& | 25, 26 |
| C57BL/6 | MCA | fibrosarcoma | TRAIL | 29 |
| C57BL/6 | DEN | hepatocarcinoma | TRAIL receptor | 28 |
| C57BL/6 | MCA | fibrosarcoma | TCR Jα28 | 26 |
| FVB | MCA | fibrosarcoma | TCRβ | 30 |
| FVB | MCA | fibrosarcoma | TCRδ | 30 |
| FVB | DMBA/TPA | cutaneous | TCRδ | 30, 35 |

| Strain | Tumour enhancement | Type of tumour | Defective immune component | Refs |
|---|---|---|---|---|
| C57BL/6 | MCA | fibrosarcoma | TCRδ | 31 |

*Contrary results in a mixed 129/Sv x C57BL/6 genetic background[27].

#Contrary results in RAG1-deficient C57BL/6 mice[24].

&Contrary results[24].

MCA, methylcolanthrene; DEN, diethylnitrosamine; DMBA, dimethylbenz[a]anthracene; TPA, 12-O-tetradecanoyl phorbol acetate; RAG, recombinase activating gene; TRAIL, TNF-related apoptosis-inducing ligand; TCR, T cell receptor; IFNγ, interferon-γ; STAT, Signal transducer and activator of transcription.

## Table 2

Induction of NKG2D ligands by stress pathways associated with tumorigenesis and wounding

| Regulatory level | Underlying pathway | Ligands regulated | Specific regulation | |
|---|---|---|---|---|
| mRNA (transcription/mRNA stabilization) | | | | |
| | Heat shock response | MICA, MICB (human) | Heat shock transcription factors | 96 |
| | DNA damage response (DDR) | ULBP, MICA (human); Rae1, MULT1, H60a (mouse) | ATR, ATM, Chk1-dependent; p53 not required | 90 |
| | Cellular Senescence | MICA, MICB, ULBP2 (human) | ATR, ATM dependent in some cases | 57, 93 |
| | Wounding | H60c (mouse) | Not determined | 105 |
| Protein stabilization | | | | |
| | Heat shock response; UV-irradiation | MULT1 (mouse) | Independent of DDR | 97 |