

# The DNA Damage Response Arouses the Immune System

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## Abstract

**Although there is considerable knowledge of how DNA damage triggers cell cycle arrest, DNA repair, and apoptosis, little was known about its potential role in immune responses. Recently, we showed that genotoxic stress and stalled DNA replication forks induce the expression of ligands for the NKG2D receptor found in natural killer cells and certain T cells, cell types that are able to attack tumor cells. Chronic activation of this response in tumor cells may contribute to immune recognition, but it also imposes a selection mechanism for immune escape and malignant progression. This unique arm of the DNA damage response may have implications for understanding therapeutic responses, many of which induce the DNA damage response, and for designing more effective regimens to treat cancer.** (Cancer Res 2006; 66(8): 3959-62)

## Tumor Cell Recognition by Natural Killer Cells

Recent genetic studies have led to a renaissance of the concept that the innate and adaptive immune systems play roles in tumor surveillance. Natural killer (NK) cells recognize many tumor cells but not normal cells, and they are thought to aid in the elimination of nascent tumors. NK cell recognition of tumor cells is regulated by inhibitory and stimulatory receptors expressed by the NK cells. After considerable progress in uncovering the mechanisms of NK cell inhibition by MHC molecules, which are often down-regulated on tumor cells, much recent effort has been directed at understanding how NK cells are stimulated by transformed cells. One theme of recognition is exemplified by a group of stimulatory NK receptors, including NKp46, NKp44, NKp30, and NKG2D. These receptors are believed to recognize normal self molecules, the expression of which are up-regulated in diseased, transformed, and infected cells (1). This theme of "induced self-recognition" remains hypothetical in the cases of NKp46, NKp44, and NKp30, largely because the ligands for these receptors remain unidentified. In the case of NKG2D, however, the identification of ligands has provided direct evidence for the proposition that ligands are induced in diseased cells and could lead to potent immune responses (2-4). Much evidence demonstrates that mouse and human NKG2D ligands are often expressed poorly on normal cells but their expression levels are up-regulated on tumor cells or virus-infected cells. A given tumor cell line or primary tumor typically expresses one or more NKG2D ligands, although not usually all of them, suggesting a complexity to their regulation, or the action of selection by the immune system leading to sporadic loss of some ligands. The NKG2D ligands include two broad families of proteins that are distant relatives of MHC class I molecules: the MIC and RAET1 families. MICA and MICB are encoded in the human MHC.

No MIC homologues have been found in mice. The RAET1 family, in contrast, is shared by mice and humans. Three subfamilies of mouse *Raet1* genes have been identified by our group and others: *Rae1*, *H60*, and *Mult1*. They are structurally related and localized to chromosome 10, but are relatively distinct in amino acid sequence (3-6). *Rae1* consists of several highly related isoforms encoded by different genes. Only one *H60* gene and one *Mult1* gene have been reported. The human *RAET1* gene family, also called *ULBPs*, consists of several linked genes encoding proteins with a relatively low degree of homology to each other, comparable to the diversity observed among mouse *Raet1* family members (7, 8).

Ectopic expression of NKG2D ligands in cells that do not express endogenous NKG2D ligands renders the cells sensitive to NK cell-mediated lysis *in vitro*, and dramatically reduces the tumorigenicity of tumor cell lines *in vivo* (3, 4, 9, 10). Antibody blocking studies suggest that NKG2D is one of the major "natural cytotoxicity receptors" necessary for lysis of tumor cell lines *in vitro*, although not the only one (11). Furthermore, engagement of NKG2D on mouse NK cells by ligands presented on transfected cells or by cross-linking the NKG2D receptor with antibody induces the production of inflammatory cytokines such as IFN- $\gamma$  *in vitro*. Significantly, NKG2D expression is not restricted to NK cells, but is also expressed by subsets of  $\gamma/\delta$  T cells, NKT cells, and most notably CD8<sup>+</sup> T cells. All CD8<sup>+</sup> T cells in humans, and all activated CD8<sup>+</sup> T cells in mice, express NKG2D. Most studies, including our own, have shown that engagement of NKG2D on CD8<sup>+</sup> T cells enhances T cell responsiveness and could induce higher levels of T cell immunity *in vivo* (10-12). In our studies, expression of NKG2D ligands by transfected tumor cells rendered them immunogenic, resulting, in some instances, in long-lasting T cell immunity (10).

The finding that NKG2D ligand expression is commonly up-regulated in transformed or infected cells suggests that hard-wired mechanisms in the cell sense infection or other correlates of disease and up-regulate the ligands in response to these signals. A proposed mechanism of ligand up-regulation is cell "stress," usually vaguely defined. It has been reported that heat shock induces the expression of human MIC molecules, and that *MIC* genes contain heat shock gene regulatory elements (13). In our studies, numerous forms of cell stress including heat shock, hypoxia, hyperoxia, pH extremes, and serum starvation did not induce RAET1 family ligands in cell cultures, suggesting that *RAET1* genes are governed by different principles (14).

## NKG2D Ligand Expression by Genotoxic Stress

Studies based on cell lines and tumor samples suggest that constitutive activation of the genotoxic stress-response pathway, also called the DNA damage response, is common in human cancer (15-18). The pathway is initiated when ATM and ATR cooperate with other molecules to sense different DNA lesions (19, 20). ATM is primarily responsible for detecting double-strand breaks, whereas ATR is predominantly responsible for detecting stalled DNA replication ("replication stress"). Replication stress is a consequence of many forms of DNA damage, and may occur when

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cells proliferate inappropriately. Directly or indirectly, most genomic insults ultimately activate both kinases, at least to some extent, initiating a signal transduction cascades that involve Chk1 and Chk2 Serine/threonine kinases and Cdc25 phosphatases. The signals exert multiple outcomes, including inhibition of cell cycle progression by inactivating cyclin-dependent kinases, up-regulation of the proapoptotic proteins p53, p63, and p73, and induction of DNA repair functions. The p53 family members induce apoptosis under some conditions, but under less severe conditions, they induce arrest of the cell cycle. In summary, activation of the DNA damage response leads to two main outcomes. When damage is "manageable," the pathway induces cell cycle arrest (at the S-G<sub>1</sub>, intra-S, and G<sub>2</sub>-M phases) to avoid replicating damaged DNA, and activates DNA repair functions to restore genomic integrity. If the DNA damage is too extensive, however, apoptosis is induced.

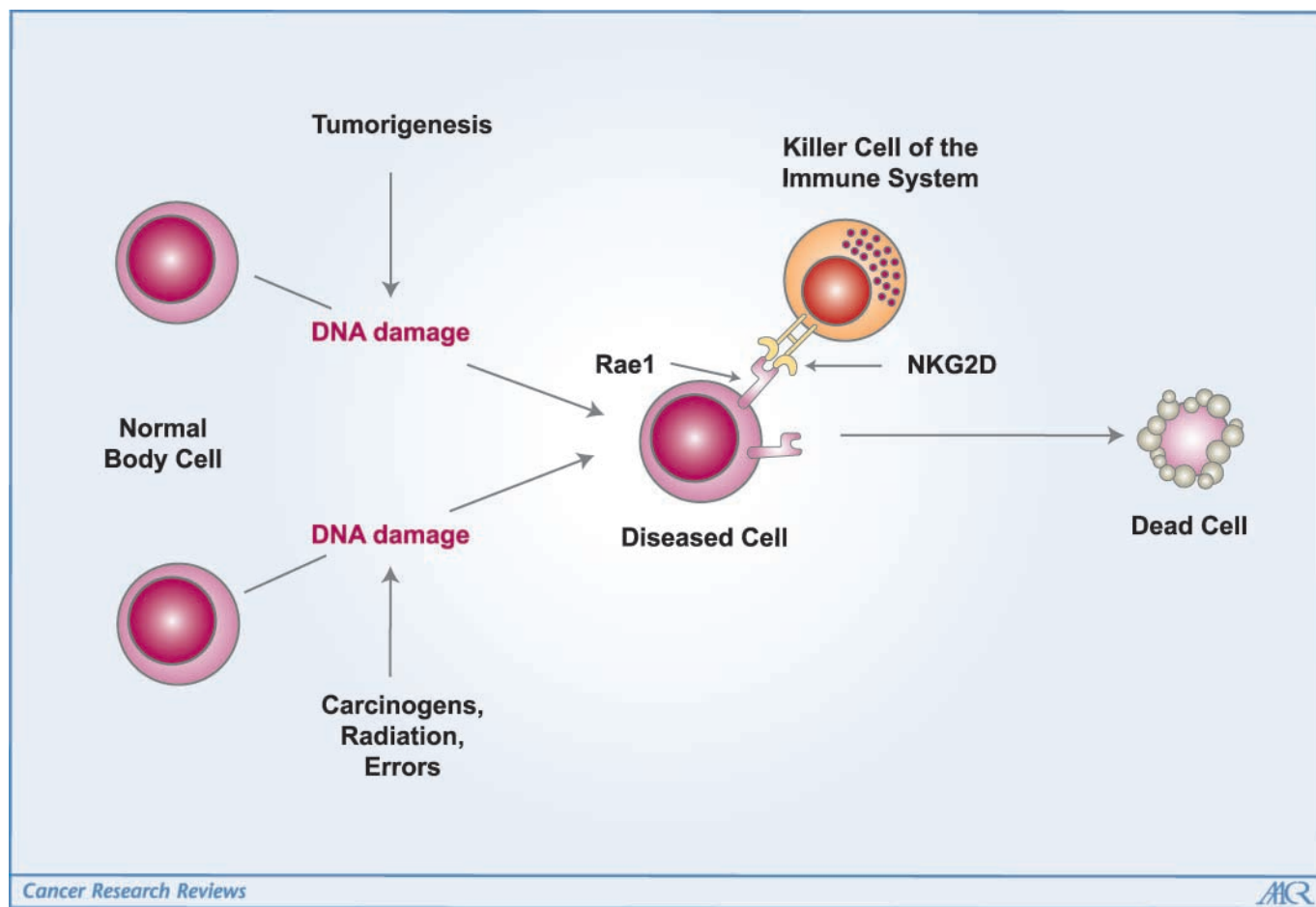
Recent reports have suggested that the DNA damage response is activated in more than half of the resected human lung and breast tumors, and striking recent findings show that human precancerous lesions reproducibly up-regulate the DNA damage response (15–18). Genotoxic stress may therefore represent a more distinctive feature of diseased cells than other correlates of tumorigenesis or infection. The resulting activation of the DNA damage response could serve as a means for a cell to sense "danger," and be linked to signaling that ultimately stimulates the immune system. Direct

evidence for such a role of the DNA damage response was provided by our finding that DNA-damaging agents or DNA replication inhibitors induce the expression of NKG2D ligands, and that blocking the function of ATM, ATR, or Chk1 inhibited ligand induction (14).

In summary, we recently provided evidence that expression of NKG2D ligands is induced in untransformed mouse cells by agents that damage the DNA or impart DNA replication stress, but not by other common forms of stress (14).

### DNA Damage Response: a Link to NKG2D Ligand Expression in Tumor Cells?

The NKG2D ligand inducers all activate the DNA damage response, a pathway that plays a central role in maintaining genomic integrity, suppressing tumors, and regulating the cell cycle. In this pathway, the sensor kinases ATM and/or ATR, which activate the checkpoint kinases, Chk2 and Chk1, and many other molecules, including p53, detect genomic lesions. A connection to cancer is suggested by previous reports that the DNA damage response is activated in precancerous lesions of the bladder, breast, lung, and colon. Proliferating normal tissues, in contrast, do not activate the DNA damage response (15–18). The early activation of the pathway occurs before the occurrence of genomic instability, but is coincident with allelic imbalances at common fragile sites,



**Figure 1.** Linkage between tumorigenesis, the DNA damage response and the immune response. DNA-damaging agents or DNA lesions associated with tumorigenesis activate the DNA damage response, which results in up-regulation of Rae1 and other ligands of the NKG2D receptor. These ligands activate NK cells and other lymphocytes to attack the diseased cells.

which are prone to DNA double-strand break formation when DNA replication is compromised. These findings suggest that activation of the DNA damage response occurring as a consequence of inappropriate proliferation results in cell cycle arrest and may account for the failure of some lesions to progress to cancer. It was proposed that tumor progression under these circumstances requires the appearance of mutations that dysregulate the DNA damage response pathway, such as p53 mutations, which allow the cell cycle block and allow tumor outgrowth (21). In contrast to p53, other components of the DNA damage response, such as ATR and Chk1, are rarely mutated in tumors, as these proteins are also essential for cell proliferation and maintenance of G<sub>2</sub> checkpoints.

The finding that the DNA damage response induces NKG2D ligand expression led us to propose that genomic abnormalities in established tumor cell lines trigger the DNA damage response, resulting in constitutive expression of NKG2D ligands (Fig. 1). In this hypothesis, the DNA damage response alerts the immune system to the fact that precancerous or potentially cancerous cells are present, and aids in triggering NK cell and possibly T cell responses to counter this threat. In support of this idea, we found that inhibiting ATM or Chk1 in the murine ovarian epithelial tumor cell line T2 (14) or in other tumor cell lines,<sup>1</sup> resulted in a substantial decrease of Rae1 levels at the cell surface. These data suggest that constitutive ligand expression in tumor cell lines is dependent on components of the DNA damage response pathway (14). These findings suggest a possible role of the immune system via the DNA damage response and NKG2D in the elimination of precancerous cells and cancer cells. In accordance with this hypothesis, recent studies imply an important role for NKG2D in controlling the incidence and progression of cutaneous carcinogenesis (22, 23). However, further studies will be necessary to firmly establish a link between the DNA damage response, NKG2D ligand expression in tumor cells and immune surveillance of cancer.

The potential linkage of the DNA damage response, NKG2D ligands and tumor surveillance has numerous implications. An important question is whether the events that enable developing tumors to escape from normal cell cycle controls impact the induction of immune responses. Interestingly, events such as p53 mutations that enable precancerous cells to progress beyond the cell cycle block imposed by the DNA damage response will not

necessarily abrogate NKG2D ligand expression, because we found that p53-deficient cell lines could be induced to express ligands by the DNA damage response (14). In fact, considering that mutation of p53 results in increased genomic instability, it is interesting to speculate that the resulting accumulation of genomic lesions could in some instances ultimately lead to increased activation of the DNA damage response and enhanced expression of NKG2D ligands.

How do precancerous or cancerous cells that express NKG2D ligands evade NKG2D-mediated tumor surveillance? It has been shown that a soluble form of MICA is released from some types of human tumors (24–29). The elevated levels of MICA in the serum are associated with down-regulated NKG2D expression and impaired activation of NK cells. NKG2D function in NK cells is also altered by chronic exposure to NKG2D ligand-expressing tumor cells (30). Furthermore, it has been reported that the inflammatory cytokines transforming growth factor- $\beta$  and IFN- $\gamma$  decrease NKG2D expression levels on NK cells (31, 32). Finally, it is likely that some tumors are selected for loss of NKG2D ligands. Thus, several mechanisms may enable progressing tumors to evade NKG2D-mediated tumor surveillance.

Radiation and chemotherapeutic drugs activate the DNA damage response and induce NKG2D ligands. It is generally accepted that the cell autonomous apoptotic response dependent on p53 family members is an important component of efficacious chemotherapy (33, 34). It is plausible, however, that part of their efficacy stems from enhanced NK or T cell-mediated rejection of the cells due to activation of the DNA damage response *in vivo*. In several experimental tumor models, low doses of chemotherapeutics were shown to greatly enhance host antitumor immunity (35, 36). Moreover, a number of studies suggest that low dose treatment with chemotherapeutics is sometimes equal or even superior to high-dose chemotherapy, which is often immunosuppressive (37). Combining low-dose chemotherapy with simultaneous NK cell activation protocols or infusion of NK cells might further potentiate the immunomodulatory effects of some chemotherapeutics. A better understanding of the pathway leading to NKG2D ligand expression may allow the design of chemotherapeutics that specifically enhance the immunogenicity of tumor cells yet reduce toxic side effects.

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