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Review

The DNA damage response, immunity and cancer

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Abstract

The genome is constantly exposed to exogenous DNA damaging events in the form of radiation, viral infection and chemicals. Endogenous processes such as DNA replication and free radical formation also threaten the integrity of the genome. DNA damage is directly deleterious to cells and also promotes tumorigenesis. Eukaryotic organisms have evolved a signaling pathway, called the DNA damage response, to protect against genomic insults. Sensor proteins detect various forms of damage, and convey signals via a complex pathway regulated by protein phosphorylation, stabilization and transcriptional regulation. The DNA damage response causes cell cycle arrest and induction of DNA repair functions, such that cells with modest damage may survive. However, cells with more severe damage are induced to undergo apoptosis. Two compelling studies show that the DNA damage response is activated very early during tumorigenesis, providing evidence that the DNA damage response could function as a barrier in early tumorigenesis. We recently demonstrated that the DNA damage response alerts the immune system by inducing expression of cell surface ligands for the activating immune receptor NKG2D, which is expressed by natural killer cells (NK cells) and some T cells. In this review we discuss the DNA damage response and its link to the innate immune system and tumor surveillance. These findings might have important implications for the understanding of cancer therapies and for drug development.

Keywords: DNA damage; Cancer; Tumor surveillance

Contents

1. Surveillance against DNA damage

Maintenance of genome integrity after DNA damage is vital for eukaryotic cells. A failure can endanger the survival of the individual cell as well as of the organism. Following DNA damage, the PI3-kinase-relateded protein kinases ATM (Ataxia Telangiectasia, mutated) and ATR (ATM- and Rad3-Related) cooperate with other proteins to initiate the DNA damage response [4]. Double-strand breaks preferentially activate ATM, whereas stalled DNA replication induces ATR activity. In response to many genomic insults, however, both kinases are eventually activated, ultimately triggering the activation of their downstream substrates Chk1 and Chk2. These signal transducers phosphorylate effector proteins such as E2F1, p53 and Cdc25 family members, which inhibit cell cycle progression and activate DNA repair systems. If the DNA damage is too extensive the p53 family members induce apoptosis. Hence, the DNA damage response maintains genome integrity by activating DNA repair systems, thereby avoiding the replication of damaged DNA, or by inducing apoptosis if the damage is irreparable.

The importance of the DNA damage response in preventing tumorigenesis was shown in part by analysis of mutations prevalent in cancer cells and by studies of mutant mice that are deficient in its different components [5,6]. Some of the proteins implicated in detection or repair of DNA damage are well-known tumor suppressor genes, such as p53 or *BRCA1*, which are frequently mutated in many types of human cancers. Mice with mutations in these genes develop tumors at varying ages. These

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observations provide a strong link between the DNA damage response and tumorigenesis.

It has been known for some time that the DNA damage response is activated in many advanced tumors. Two recent reports by Bartkova et al. and Gorgoulis et al. show constitutive activation of the DNA damage response in precancerous and early cancerous lesions of human breast, bladder, lung and colon, but not in normal tissue [1,2]. The activation of the DNA damage response did not simply reflect the high proliferation rate of tumors cells, as DNA damage response markers are absent from normal proliferating epithelial cells and from inflammatory lesions. Nor was it correlated with widespread genomic instability as occurs in advanced tumors. Instead, *in vitro* and *in vivo* studies suggest that oncogene-driven proliferation induces aberrant cell cycles, which activate the DNA damage response. Analysis using microarrays for single nucleotide polymorphisms (SNPs) show preferential loss of heterozygosity at common fragile sites suggesting that the DNA damage response in precancerous and cancerous lesions may be attributed to problems with DNA replication. In addition SNP analysis of 35 bladder tumors at different stages shows that the DNA damage response is activated before p53 mutations arise. These studies imply that aberrant cell cycles activate DNA damage checkpoints resulting in p53-dependent cell cycle arrest or apoptosis. It was argued that this barrier to tumor progression creates selection pressure for precancerous cells to inactivate certain aspects of the DNA damage response, such as p53, and might account for the high frequency of p53 inactivation in human cancer. The discovery that the DNA damage response is activated early in tumorigenesis, as well as in some viral infections, raises the possibility that it could represent a distinctive property of diseased cells, which could allow the immune system to identify and attack such cells.

2. The DNA damage response induces expression of ligands for the NKG2D receptor

Direct evidence for such a role of the DNA damage response was provided by our finding that DNA damaging agents or DNA replication inhibitors, but not other common forms of stress, induce expression of cell surface protein ligands for the NKG2D receptor in an ATM or ATR dependent fashion [3]. NKG2D is an activating receptor expressed on NK cells, subsets of γ/δ T cells, NKT cells, and cytotoxic $CD8^+$ T cells [7–9]. In humans, all $CD8^+$ T cells express NKG2D. In contrast, NKG2D expression in mice is limited to activated $CD8^+$ T cells. NKG2D expressed on NK cells is one of the major receptors required for NK cell mediated lysis of tumor cell lines *in vitro*, although other receptors also participate. Furthermore, engagement of NKG2D on activated NK cells is sufficient to activate production of inflammatory cytokines, such as IFN-γ, at least in mice. On $CD8^+$ T cells, NKG2D acts as a co-stimulatory signal resulting in enhanced T cell responsiveness and higher levels of T cell immunity *in vivo*.

The NKG2D ligands are distant relatives of MHC class I molecules and can be grouped into *MIC* and *RAET1* gene families [7]. The *MIC* gene family members, *MICA* and *MICB*, are localized in the human major histocompatibility complex, whereas no *MIC* homologs have been found in mice to date. Humans and mice, in contrast, share the *RAET1* gene family. The human *RAET1* gene family, also referred to as ULBPs, consists of 10 genes, which share comparatively little homology to each other. In mice, *Raet1* genes can be further subdivided into *Rae1*, *H60* and *Mult1* gene subfamilies, which are relatively distinct in amino acid sequence to each other. *Rae1* consists of several highly related isoforms encoded by different genes. In contrast, only one *H60* and one *Mult1* gene have been reported.

A number of studies have implicated NKG2D and its ligands in tumor surveillance by the immune system. NKG2D ligands are not detectable at the cell surface of normal cells, whereas many tumor cells or virus-infected cells express significant levels. In mice, ectopic expression of NKG2D ligands in rare cell lines that lack endogenous NKG2D ligands renders the cells sensitive to NK cell mediated attack *in vitro* and *in vivo* [9–11]. Several reports suggest that ectopic expression of NKG2D ligands by tumor cells increases their immunogenicity *in vivo*, in some cases imparting long-lasting T cell-mediated immunity [10,12]. Furthermore, combining NKG2D ligands and tumor antigens in a vaccine in some cases provokes immune responses that can reportedly eliminate established tumors [13]. In addition, recent studies imply an important role for NKG2D in controlling the incidence and progression of cutaneous carcinogenesis [14,15]. In humans, a role for NKG2D in tumor defense has been suggested by the finding that soluble MICA and ULBP2 are present in the serum of many cancer patients [16,17]. The elevated levels of MICA or ULPB2 in the serum are associated with down regulated NKG2D expression and impaired activation of NK cells. The presence of soluble NKG2D ligands may therefore represent an immune escape mechanism that develops in tumors as they evolve in the host.

Based on recent evidence that the DNA damage response is activated in precancerous and cancerous lesions as well as in established tumor cell lines, we tested whether the constitutive expression of NKG2D ligands in such cells depends on the underlying genotoxic stress [18]. We observed that inhibiting ATM or Chk1 in the murine ovarian epithelial tumor cell line T2 or in other tumor cell lines (unpublished results) leads to a substantial decrease of Rae1 levels at the cell surface [3]. These findings support the idea that constitutive ligand expression in tumor cell lines depends on the DNA damage response. In summary, our study suggests that the induction of NKG2D ligand expression by DNA damage and the resulting activation of the immune system may represent an additional barrier in tumorigenesis by recruiting killer cells of the immune systems to lyse diseased cells (Fig. 1). However, more experimental evidence is required to firmly support a role of the DNA damage response and NKG2D in tumor surveillance.

Potential p53- or NKG2D-mediated tumor surveillance in response to DNA damage would have significant implications. An important question is if the two mechanisms are linked or provide independent protection against the development of malignant cells. In favor of the latter idea, we found that NKG2D ligands could be induced in cells that lacked p53 (Fig. 1) [3].

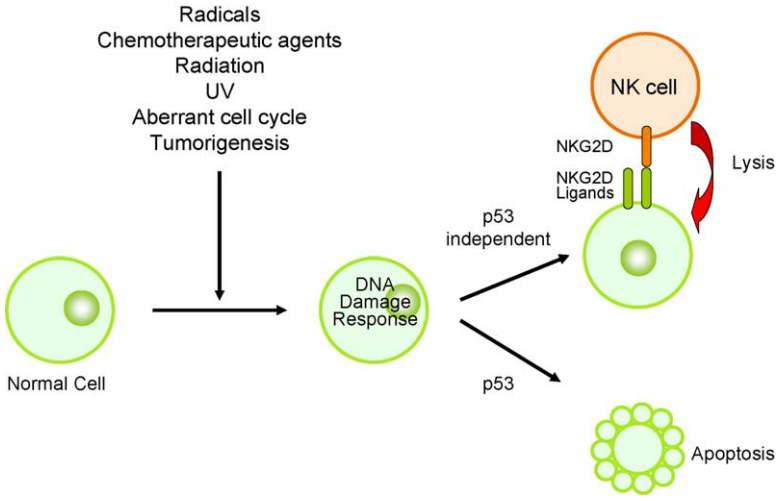

Fig. 1. Linkage between the DNA damage response, cancer and the immune response. Genomic insults or DNA damaging agents related to tumorigenesis activate the DNA damage response. This may lead to upregulation of ligands of the activating NKG2D receptor independently of p53. Expression of NKG2D ligands on diseased cells leads to attack and lysis by NK cells and other lymphocytes.

Whereas p53 is not required for expression of NKG2D ligands in tumor cell lines or in cells with DNA damage, it is possible that other p53 family members, along with p53, function in a partially redundant fashion to induce NKG2D ligand expression. Intriguingly, loss of p53 is implicated in the loss of genomic stability and it is plausible that the resulting lesions could enhance activation of the DNA damage response and increase expression of NKG2D ligands.

The potential linkage of the DNA damage response, NKG2D ligands and tumor surveillance may have important implications for the design and implementation of cancer therapies. A large body of evidence has shown that chemotherapeutic agents or radiation, which we showed induce expression of NKG2D ligands in a p53-independent fashion, induce apoptosis in a cell autonomous manner, which is dependent on p53 function in many tumor types [19]. However, disruption of the intrinsic apoptotic pathway is very common in cancer cells and p53 is the most frequently mutated gene in human tumors. Furthermore, disruption of p53 downstream effectors, such as PTEN, Bax, Bak and Apaf-1 or upstream regulators, like Mdm-2 and p19ARF, occur in human tumors [20]. Because induction of NKG2D ligands is at least partly p53-independent, it is possible that chemotherapy and/or radiotherapy enhances expression of NKG2D ligands on tumor cells, accounting for some of the efficacy of these drugs. Indeed, it has been shown that low doses of certain chemotherapeutic agents enhance host antitumor immunity in a number of experimental tumor models [21]. Moreover, low dose chemotherapy is sometimes equal or even superior to high-dose chemotherapy, which often is immunosuppressive [22]. Protocols combining chemotherapy or radiotherapy with concurrent regimens to enhance NK cell and T cell immunity may hold great promise for treatment of cancer. A more detailed understanding of the signaling events downstream of the DNA damage response that regulate NKG2D ligands and other aspects of immunity may also aid in the design of novel chemotherapeutic drugs with greater efficacy and less toxicity.

3. Conclusion

We have recently shown that ligands for the activating immune receptor NKG2D are induced by the DNA damage response, which is activated early in tumorigenesis as well as during certain virus infections. Genotoxic stress may therefore represent an important means by which the immune system distinguishes diseased cells from normal cells. The resulting activation of the DNA damage response would allow a cell to recognize the “danger” posed by diseased cells and trigger signals that induce apoptosis and alert the immune system. In future studies, it will be important to test in detail the stage in tumorigenesis or infection where NKG2D ligands are expressed and how the expression correlates with the DNA damage response. Also of interest is whether other components of the immune response, in addition to NKG2D ligands, are regulated by the DNA damage response. Finally, a better understanding of the role of different immune cells and cell interactions in tumor surveillance controlled by the DNA damage response may be helpful in designing more effective regimens to treat cancer.

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