

The DNA Damage Response Arouses the Immune System

Stephan Gasser and David H. Raulet

Cancer Research Laboratory, Department of Molecular and Cell Biology, University of California, Berkeley, California

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- γ *in vitro*. Significantly, NKG2D expression is not restricted to NK cells, but is also expressed by subsets of γ/δ T cells, NKT cells, and most notably CD8⁺ T cells. All CD8⁺ T cells in humans, and all activated CD8⁺ T cells in mice, express NKG2D. Most studies, including our own, have shown that engagement of NKG2D on CD8⁺ 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

Requests for reprints: David H. Raulet, Department of Molecular and Cell Biology, University of California, Berkeley, 489 Life Science Addition, Berkeley, CA 94720-3200. Phone: 510-642-9521; Fax: 510-642-1443; E-mail: raulet@berkeley.edu.

©2006 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-05-4603

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-G₁, intra-S, and G₂-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,

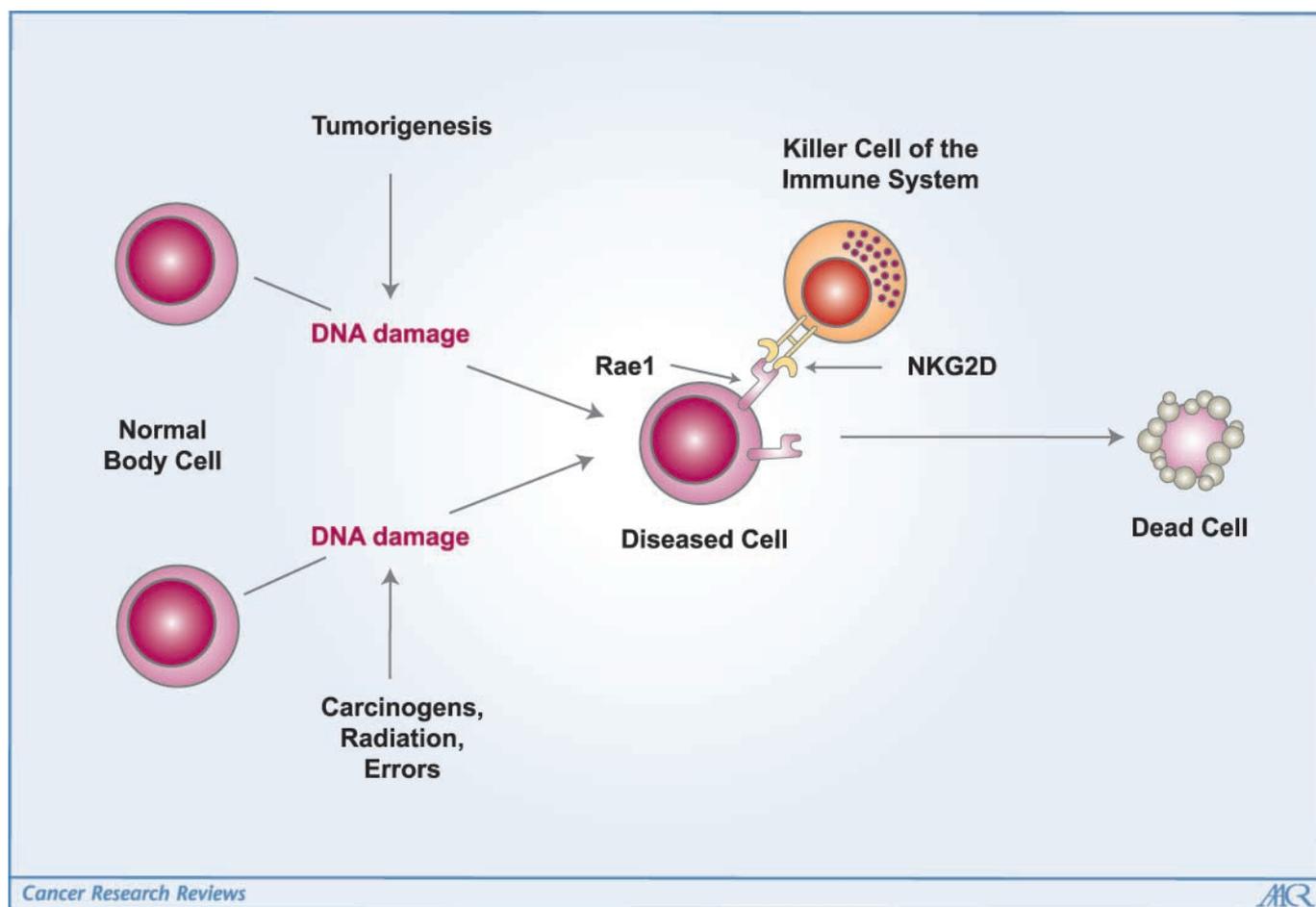


Figure 1. Linkage between tumorigenesis, the DNA damage response and the immune response. DNA-damaging agents or DNA lesions associated with tumorigenesis activate the DNA damage response, which results in up-regulation of Rae1 and other ligands of the NKG2D receptor. These ligands activate NK cells 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 G₂ 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,¹ 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- β and IFN- γ 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.

Acknowledgments

Received 12/22/2005; revised 1/17/2006; accepted 1/20/2006.

We thank Tim Nice for comments on the manuscript and other colleagues for helpful discussions.

¹ Unpublished data.

References

- Moretta A, Bottino C, Vitale M, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu Rev Immunol* 2001; 19:197–223.
- Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 1999;285:727–9.
- Cerwenka A, Bakker ABH, McClanahan T, et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 2000;12:721–7.
- 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;1:119–26.
- 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.
- 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.
- Cosman D, Müllberg J, Sutherland CL, et al. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 2001;14:123–33.
- Radosavljevic M, Cuillerier B, Wilson MJ, et al. A cluster of ten novel MHC class I related genes on human chromosome 6q24.2-q25.3. *Genomics* 2002;79:114–23.
- 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.
- Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* 2001;413:165–71.
- Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* 2002;17:19–29.
- Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T. Costimulation of CD8 $\alpha\beta$ T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* 2001;2:255–60.
- Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A* 1996;93:12445–50.
- 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.

15. DiTullio RA, Jr., Mochan TA, Venere M, et al. 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat Cell Biol* 2002;4:998-1002.
16. Gorgoulis VG, Vassiliou LV, Karakaidos P, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* 2005;434:907-13.
17. Bartkova J, Horejsi Z, Koed K, et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* 2005;434:864-70.
18. Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, et al. ATM activation in normal human tissues and testicular cancer. *Cell Cycle* 2005;4:838-45.
19. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 2001;15:2177-96.
20. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 2004;73:39-85.
21. Halazonetis TD. Constitutively active DNA damage checkpoint pathways as the driving force for the high frequency of p53 mutations in human cancer. *DNA Repair (Amst)* 2004;3:1057-62.
22. Smyth MJ, Swann J, Cretney E, Zerafa N, Yokoyama WM, Hayakawa Y. NKG2D function protects the host from tumor initiation. *J Exp Med* 2005;202:583-8.
23. Oppenheim DE, Roberts SJ, Clarke SL, 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.
24. Salih HR, Rammensee HG, Steinle A. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 2002;169:4098-102.
25. 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.
26. Salih HR, Antropius H, Gieseke F, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. *Blood* 2003;102:1389-96.
27. Raffaghello L, Prigione I, Airoidi I, et al. Down-regulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia* 2004;6:558-68.
28. Holdenrieder S, Stieber P, Peterfi A, Nagel D, Steinle A, Salih HR. Soluble MICA in malignant diseases. *Int J Cancer* 2006;118:684-7.
29. Jinushi M, Takehara T, Tatsumi T, et al. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. *J Hepatol* 2005;43:1013-20.
30. Coudert JD, Zimmer J, Tomasello E, et al. Altered NKG2D function in NK cells induced by chronic exposure to NKG2D ligand-expressing tumor cells. *Blood* 2005;106:1711-7.
31. Lee JC, Lee KM, Kim DW, Heo DS. Elevated TGF- β 1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. *J Immunol* 2004;172:7335-40.
32. Zhang C, Tian ZG, Zhang J, Feng JB, Zhang JH, Xu XQ. The negative regulatory effect of IFN- γ on cognitive function of human natural killer cells. *Zhonghua Zhong Liu Za Zhi* 2004;26:324-7.
33. Viktorsson K, De Petris L, Lewensohn R. The role of p53 in treatment responses of lung cancer. *Biochem Biophys Res Commun* 2005;331:868-80.
34. Soussi T, Lozano G. p53 mutation heterogeneity in cancer. *Biochem Biophys Res Commun* 2005;331:834-42.
35. Ehrke MJ. Immunomodulation in cancer therapeutics. *Int Immunopharmacol* 2003;3:1105-19.
36. Zagazdzon R, Golab J. Immunomodulation by anticancer chemotherapy: more is not always better (review). *Int J Oncol* 2001;18:417-24.
37. DiPaola RS, Durivage HJ, Kamen BA. High time for low-dose prospective clinical trials. *Cancer* 2003;98:1559-61.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

The DNA Damage Response Arouses the Immune System

Stephan Gasser and David H. Raulet

Cancer Res 2006;66:3959-3962.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/66/8/3959>

Cited articles This article cites 37 articles, 10 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/66/8/3959.full#ref-list-1>

Citing articles This article has been cited by 21 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/66/8/3959.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/66/8/3959>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.