The Cytidine Deaminase APOBEC3 Family Is Subject to Transcriptional Regulation by p53

Daniel Menendez, Thuy-Ai Nguyen, Joyce Snipe, and Michael A. Resnick

Abstract

The APOBEC3 (A3) family of proteins are DNA cytidine deaminases that act as sentinels in the innate immune response against retroviral infections and are responsive to IFN. Recently, a few A3 genes were identified as potent enzymatic sources of mutations in several human cancers. Using human cancer cells and lymphocytes, we show that under stress conditions and immune challenges, all A3 genes are direct transcriptional targets of the tumor suppressor p53. Although the expression of most A3 genes (including A3C and A3H) was stimulated by the activation of p53, treatment with the DNA-damaging agent doxorubicin or the p53 stabilizer Nutlin led to repression of the A3B gene. Furthermore, p53 could enhance IFN type-I induction of A3 genes.

Implications: Activated p53 can integrate chromosomal stresses and immune responses through its influence on expression of APOBEC3 genes, which are key components of the innate immune system that also influence genomic stability.

Introduction

The well-known tumor suppressor p53 is a transcriptional master regulator that regulates the expression of genes associated with a wide range of functions, including cell-cycle arrest, apoptosis, and senescence in response to genotoxic and nongenoxic stresses that challenge cellular genomic integrity (1). Following various stresses, the activated p53 protein is accumulated in the nucleus, where it binds to DNA as a tetramer at p53 response elements (p53RE; ref. 1). The commonly held view of p53 as a "guardian of the genome" has expanded greatly during the past few years to cover many biological processes (1–3), including the role of p53 in modulating the human immune system and antiviral defense (4–7). Previously, we found that activation of p53 by common antitumor agents in human primary and cancer cell lines directly alters the expression of several members of the innate immune Toll-like receptor (TLR) gene family, which is involved in host defense against invading pathogens (8, 9). This results in modulation of TLR downstream responses to cognate ligands (10, 11).

p53 chromatin immunoprecipitation (ChIP-seq) approaches in combination with transcriptome analysis have revealed many new p53-regulated target genes. From our p53 cistrome studies in human cancer (3) and human primary lymphocytes (unpublished data), we have identified new p53 targets involved in immune response signaling. Included are several members of the innate immune gene family APOBEC3 (A3; apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3).

The A3 genes, which consist of seven highly related DNA cytidine deaminases that are tandemly distributed on human chromosome 22, catalyze the deamination of cytidine to uracil (12). The A3 genes are a key component of the innate immune system in vertebrates that inhibit replication of a variety of retroviruses, endogenous retroelements, and DNA viruses (13, 14). Recently, the C-to-T hypermutagenesis genomic changes in multiple cancers have been attributed to A3A and A3B (15–17).

The A3 genes are generally viewed as constitutively expressed in immune-related cells as well as in immune-associated cancer cells. In the context of innate immune responses to infection, the expression of several A3 genes is modulated by IFNs that may be cell type and IFN-type dependent (18, 19). However, little is known about the transcription factors involved in the expression of the A3 genes in response to immune challenges and other environmental stresses.

Here, we describe a new role for p53: regulation of the A3 gene family in response to common anticancer drugs and direct activation of p53. Using both a panel of human cancer cell lines as well as primary immune cells obtained directly from human subjects, we discovered that most A3 genes are transcriptionally responsive to p53. Furthermore, cancer-associated p53 mutants can dramatically impact the pattern of expression of the A3 genes, including novel responses of the cancer hypermutator A3B gene. In addition, p53 can participate and influence the IFN-induced transcriptional responses of several A3 family members. Overall, we provide the first evidence that p53 is a key node linking DNA damage, immune-induced responses, and A3 gene expression in human cells.
Materials and Methods

Cell lines and treatments

Human cancer cell lines were cultured in RPMI1640, McCoy’s 5A, or DMEM supplemented with 10% of FBS and 100 U/mL penicillin/streptomycin as described elsewhere. More information about the cancer and primary human cells is provided in the Supplementary Material.

RNA isolation and qPCR

Total RNA was isolated with RNeasy Kits (Qiagen). Quantification and purity of the samples were determined using NanoDrop spectrophotometer (Thermo Fisher Scientific), and 1 μg total RNA was reverse transcribed using Transcriptor reverse transcriptase with random hexameric primers (Roche) following the manufacturer’s recommendations. qPCR was performed following established procedures, primers, and Universal Primary Library System probes as described previously (19) using 7000 ABI detection system (Applied Biosystems). All reactions were done in triplicate, and relative quantification values were calculated on the basis of the 2^−ΔΔCt method using expression from the housekeeping gene Tata-Binding Protein (TBP).

Statistical analysis

Analysis was performed using GraphPad Prism statistical software. Data are represented as mean ± SDs from at least three separate experiments. Two-tailed Student t test was applied for comparisons of two groups. P values <0.05 were considered significant.

Results

Chromosomal stress and p53 activation alters A3 gene family expression

Recently, in our p53 ChIP-seq study that included associated gene expression analysis in osteosarcoma U2OS cells following p53 activation by Nutlin-3 (Nutlin) or doxorubicin (DXR), we identified several new p53 target genes related to immune responses (3). Binding of p53 to the regulatory regions of the A3C and A3H members of the A3 gene family was associated with increased transcription based on TaqMan analysis. Given that homology is high among the A3 family sequences (20), we hypothesized that p53 responsiveness may have been conserved among other members of the A3 family.

Because of the redundancy in the regulatory and coding regions for A3 genes, there have been inconsistent conclusions about the transcriptional regulation of the A3 genes as well as the quantification of A3 expression by common approaches (microarray and qPCR; ref. 19). To validate our previous findings and explore the extent to which p53 regulates the expression of other A3 members, we measured expression of all A3 genes in U2OS cells following exposure to Nutlin and doxorubicin using the Universal Primary Library System technology [this technology, which is more robust than the general SYBR Green primers or Applied Biosystems TaqMan primers/probes assays for evaluating A3 mRNA changes (19), allows highly related sequences to be distinguished]. Activation of p53 by these agents led to time-dependent upregulation of several A3 genes (from ~2.5- to 13-fold), as shown in Fig. 1A.

The A3B gene differed in that both drug treatments repressed it. In addition to A3A and A3D, we confirmed upregulation of A3C and A3H as well as the p53 target gene CDKN1A (p21) used as a positive control. We also observed that doxorubicin, but not Nutlin, increased A3G mRNA. Except for repression of A3B, a similar pattern of expression of A3 genes was observed in A549 lung cancer cells (Supplementary Fig. S1A). The expression of A3A was not detected in A549 cells. Overall, we establish that the expression of A3 genes can be regulated in response to genotoxic stress and p53 activation.

Given that the induction of innate immune genes is relevant in disease and cancer treatments and to determine whether the impact of Nutlin and doxorubicin on A3 genes was general rather than cell type specific, we expanded the analysis for A3 responses to DNA-damaging agents and p53 activation in a panel of cell lines harboring wild-type (WT), null, and mutated p53 (Supplementary Table S2). Presented as heatmaps in Fig. 1B (Nutlin) and C (doxorubicin) are the mRNA fold changes for each A3 gene and cell line (see Supplementary Table S3 for raw data). Expression of A3A was mainly observed in cell lines of immune origin (LCL35, GM12878, THP1, Jurkat, and RAJI). The expression of the A3 genes was clearly induced by drug treatments only in WT p53 cells, where there was consistent induction of A3C and A3H as well as p21 among the cell lines. Cell type and agent-specific effects in mRNA changes were observed for A3A, A3D, A3F, and A3G. As observed in U2OS and A549, A3B was repressed in all WT p53 cell lines examined in response to Nutlin and doxorubicin. We also found that other chromosomal stresses, such as ionizing radiation, altered the A3 genes expression profile in various cancer cell lines in a p53-dependent manner (Supplementary Fig. S1B and S1C). Although there are dramatic differences in the expression spectra for some A3 genes between cell lines, we establish that many genes of the A3 family are responsive to genotoxic stress and p53 activation in human cancer cells.

As most of the A3 genes are expressed in immune cells, we examined their response to p53 induction in human primary lymphocytes. Relative levels of mRNA expression were determined in phytohemagglutinin-stimulated lymphocytes obtained from peripheral blood mononuclear cells freshly isolated from 11 healthy human volunteers. Despite variability among subjects, the overall A3 gene family expression profiles induced by Nutlin and doxorubicin were like those in WT p53 cancer cell lines, as described in Supplementary Fig. S2A and S2B. The expression of the internal controls p21 and Mdm2 were also induced in all donors after Nutlin or doxorubicin treatments. In agreement with previous reports (18, 19), A3A expression was undetectable in T lymphocytes. A3B mRNA was not detected in all donors, but in those that it was observed (8/11), both treatments induced repression of this gene. Pretreatment of lymphocytes from 4 volunteers with the p53 inhibitor pifithrin-α (21) prior to Nutlin or doxorubicin strongly altered expression, identifying a direct role for p53 in regulating Nutlin- and doxorubicin-induced A3 gene expression (Supplementary Fig. S2C and S2D).

p53 directly modulates A3 gene family expression in human cells

A direct comparison between the A3 expression profiles for HCT116 p53⁺ and its isogenic version lacking p53
(HCT116 p53−/−) revealed that most of the changes in A3 genes were dependent on p53 (Fig. 1B and C). Few significant changes in expression were observed for the A3 genes after Nutlin or doxorubicin in the null and mutant p53 cell lines. When changes were detected in the p53-mutant cell lines, they were typically opposite to the changes observed in WT p53 cell lines. An example is A3C, which was repressed in p53-mutant cells but significantly induced in WT p53 cells. However, the most dramatic example was found for the A3B response to doxorubicin in that it was upregulated in several mutant cell lines but repressed in WT p53 cells. The p53-dependent gene expression changes in A3 genes were addressed by expressing RNAi p53 from a cassette that was integrated in MCF7 (22) or in U2OS (3), or using a lentiviral vector harboring short hairpin RNAs that target TP53 mRNA (termed p53sh-3755 and p53sh-3756) in A549 and LCL35 cells (Fig. 2; Supplementary Fig. S3). As shown in Fig. 2A–C and Supplementary Fig. S3, the upregulation observed for A3 genes following doxorubicin or Nutlin exposure in control cells (parental or scrambled) was completely lost or reduced in all p53-depleted cell lines regardless of tissue origin, confirming that these are p53 targets. The A3B-induced repression was prevented by silencing of p53 expression.

To investigate further a direct connection between p53 and expression of A3 genes, WT p53 was overexpressed in the p53-null cancer cell lines SaOS2, HCT116 p53−/−, and H1299. Although cell type differences were observed, the functional restoration of WT p53 expression resulted in approximately 2.5- to 14-fold upregulation of several A3 genes along with p21 mRNA used as a positive control (Fig. 2D). The A3F gene was the only family member that was not modified in any of the cell lines transfected. There was a lack of basal A3A or A3H expression in SaOS2 cells that appeared to be due to the absence of p53 as transfection of WT p53 resulted in expression of both genes (Fig. 1B and C). Consistent with our previous observations, the restoration of functional p53 resulted in a significant inhibition of A3B gene expression in both transfected cell lines. Overall, these results confirm the direct participation of p53 in the regulation of A3 gene family members.
Activated p53 binds transcriptional regulatory regions of A3 genes

The p53-induced transcription of several A3 genes led us to investigate public p53 ChIP-seq datasets (summarized in Supplementary Table S4) for the impact of p53 across a broad range of cell types and exposures. Included are the responses of normal diploid fibroblasts, embryonic stem cells, lymphoblasts as well as breast, colon and bone cancer cells. As shown in Fig. 3A, p53 can bind to the transcriptional regulatory regions of most of the A3 genes, A3A, A3B, A3C, and A3H genes, in response to the following stress conditions that activate p53: doxorubicin, cisplatin, 5-FU, actinomycin-D, ionizing radiation, Nutlin, and RITA. To determine whether A3 genes are direct transcriptional targets of p53, we screened for potential p53REs in the promoter and regulatory regions of each A3 gene, from −5 to +1.7 kb flanking the transcription start site (TSS) as well as the regions where ChIP-seq p53-binding peaks were observed. Using established guidelines for transcriptional functionality of potential p53REs (23) and the p53Scan software (24), we identified several potential transactivation-responsive p53REs located in the transcriptional regulatory regions of all A3 genes (Fig. 3A).

Overall, these results support a broad role for p53 in the A3 gene family regulation and suggest that the p53-binding regions containing the putative p53REs may regulate the expression of A3 genes. The top predicted functional p53REs are shown in Supplementary Table S1. A complete list of p53REs associated with A3 genes is presented in Supplementary Table S5. We note that for some of the putative p53REs, the sequences were 95% to 100% conserved between several A3 genes, as found for p53REs of the A3D, A3F, and A3G genes, which is consistent with the evolutionary duplication of the A3 genes (20). Although potential p53REs in A3B, A3D, A3F, and A3G were identified around their TSS, none exhibited binding in reported p53 ChIP-seq studies. However, for the case of A3B, an intronic region containing a p53RE was bound by p53. The p53RE associated with A3F was previously reported as a p53-bound region in a ChIP-PET assay (25). As a lack of binding in previous reports might be due to relatedness of the A3 genes, we investigated p53 binding to specific sites using a ChIP-PCR analysis that targets each of the putative p53REs (due to high sequence conservation, no reliable primers were identified to analyze some of the top predicted functional p53REs for A3D, A3F, and A3G). With this approach, we confirmed that...
activated p53 can directly bind to the p53RE sequences identified in silico and/or previously found to bind in vivo for several A3 genes after Nutlin or doxorubicin treatment in U2OS and A459 cells, as described in Fig. 3B and C and Supplementary Fig. S4. Binding of p53 to the promoter region of its target gene p21 was used as positive control, while a random region of the GAPDH promoter was used as negative control (Supplementary Fig. S4). Collectively, these results demonstrate that most p53RE sites considered as associated with p53-induced expression of the A3 family members can be directly bound by p53 and strongly indicate that in response to chromosomal stresses, p53 is a major transcription factor regulating the expression of A3 genes.

Tumor-associated p53 mutants can promote A3B expression

As described above, all the A3 genes were upregulated in WT p53 cancer cell lines in response to p53 activation except A3B, which was repressed. However, in cell lines with mutant p53 alleles, A3B was generally upregulated. As shown in Fig. 4A, transient expression in SaOS2 p53-null cells of the cancer-associated, hotspot p53 mutants R175H, G245S, R248Q, R273H, and R280K resulted in upregulation of the A3B gene with little effect on expression of A3C, A3H, or the internal control p21. The results were similar when the same p53 mutants were transiently overexpressed in HCT116 p53−/− cells, confirming that mutant p53 induces the expression of A3B in human cancer cells (Fig. 4B). Interestingly, change-of-spectrum mutants A138Y, H178Y, and
Altogether, these results imply that tumor-associated p53 mutants can influence the expression of several A3 genes, especially increasing the A3B mRNA levels.

**p53 modulates IFN-induced expression of A3 genes in cancer cells**

Consistent with its antiviral roles, several A3 genes are upregulated by various IFNs (18, 19). We therefore assessed whether p53 can influence the expression of A3 genes in response to IFN type-I (IFN-I) in various cancer cell lines that differed in TP53 status.

As shown in the heatmap of Fig. 5A, all A3 genes were modestly upregulated after 24 hours of IFN-I treatment in most cell lines regardless of p53 functional status, except for the substantial increase in A3B. To address whether p53 directly affects IFN-I-mediated gene expression of A3 genes, U2OS cells with p53shRNAi or scramble shRNAi were treated with IFN-I. The IFN-I treatment stimulated the expression of all A3 genes (Fig. 5B), except A3B and A3C (Supplementary Fig. S5A) in a time-dependent manner in U2OS scramble cells. Consistent with previous findings (27), there was a modest approximately 3-fold increase in TP53 mRNA levels after 24 hours of IFN-I treatment (Fig. 5) in the scramble cells. Although the absence of p53 due to p53shRNAi does not seem to have any impact on the early upregulation of the A3 genes by IFN-I challenge (1 to 6 hours), the presence of p53 was clearly required to maintain the upregulation of the A3A, -D, -F, -H, and -G genes at 24 hours (Fig. 5B). Correspondingly, the protein levels of total p53 and phosphorylated Ser15 p53, a marker for activation, were increased (Fig. 5C) at 24 hours.

Similarly, p53 occupancy at the p53REs for A3A and A3C was greatly increased only at 18 hours of IFN-I treatment (Fig. 5D), suggesting that activated p53 is needed for IFN-I regulation of A3 genes at late times. The impact of p53 on IFN-I–induced expression and p53 occupancy of several A3 genes was also observed in A549 cells (Supplementary Fig. S5B and S5C). Unlike for U2OS cells, the expression of the A3B gene in the A549 cells was initially upregulated after IFN-I at early time points (3 and 6 hours) when p53 was not bound in the A3B transcriptional regulatory region. Notably, A3B mRNA levels were returned to basal levels after 24 hours, while p53 occupancy increased overall, consistent with a p53-repressive activity on this gene.

The role of p53 in modulating IFN-I–induced expression of A3 genes was further confirmed in SaOS2 p53 null cells transiently transfected with WT p53. Restoration of WT p53 enhanced the IFN-I–induced gene expression of most of the A3 genes except for A3C (Supplementary Fig. S5D). There is a difference with overexpressed p53 compared with nontransfected cells, in that IFN-I treatment in parental cells failed to induce the A3A and A3H genes over the undetectable basal levels, while in p53 transfected cells, the mRNA levels of both genes along with other A3 genes were synergistically increased, except for A3B. Consistent with our other observations, A3B was repressed after p53 expression. Although IFN-I had no effect on its own, the IFN-I abolished the p53 induced repression of A3B, returning its expression to basal levels. Collectively, our results demonstrate a role for p53 in IFN-mediated expression of A3 gene family members.

**Discussion**

The A3 cytosine deaminases are key innate immune effector proteins providing defense against a range of viruses and retroelements through their ability to mutagenize viral DNA, restrict viral replication, and silence retrotransposition (28, 29). As the A3 proteins can deaminate cellular DNA and contribute to genomic instability, a fine-tuned control of expression of the A3 genes and enzymatic activities is needed to avoid collateral damage to the host genome. However, little was known about the transcriptional control of the A3 genes at basal or stress conditions beyond...
regulation by type-I IFN stimulation, estrogen, and recently by replication stress (18, 19, 30, 31).

Here, we show that p53 can be a major transcriptional regulator of all A3 genes in response to chromosomal stress. Consistent with these observations, depletion of p53, inhibition by pifithrin-α, and expression of loss-of-function tumor-associated p53 mutants alters A3 expression. Also, all A3 genes had p53 binding near TSSs, many of which were bound by activated p53. We observed that although p53 binding to these novel p53REs was relatively lower when compared with the binding of p53 to the p21 target gene, the binding of p53 in the A3 regulatory regions was consistent in two cell lines observed, not only following activation with the classical p53 activator drugs doxorubicin and Nutlin but also after IFN-I treatment. Furthermore, we found that numerous CHIP-seq datasets across cell lines, exposures, and tissue types provide further evidence for direct regulation of the A3 family by p53. Although we attempted to relate mRNA to protein changes with commercially available antibodies, results for A3 proteins were inconsistent, presumably due to the polyclonal nature of the antibodies used and/or multiple nonspecific signals. However, a strong correlation has been reported for A3F and A3G mRNA and protein expression using noncommercial antibodies (19).

We also found that p53 can influence expression of the A3 gene family in response to IFN related immune challenges, suggesting p53 may act as a central mediator of global innate immune responses. IFNs are the major cytokines produced by the innate immune system in response to viral infections, and several viral infections trigger p53 (32). Included in the IFN-stimulated group of genes (33) are TP53 (27) and A3 genes. (18, 19, 27). p53 was found to only influence late IFN-mediated transcriptional responses of the A3 genes. We suggest that p53 can enhance the antiviral arm of the innate immune response via the upregulation of A3 genes as well in consort with the antiviral response itself.

On the basis of our observations, we propose that activated p53 can serve as an integrator of DNA damage and immune responses. This is consistent with the recent report in which drug-induced replication stress can promote A3 activation (31). The role of p53 was not assessed in this work; however, replication stress typically involves p53 activation (31). We liken the action of p53 to a two-edged sword that has the potential to both increase genome stability through actions on viruses and retrotransposons or to decrease genome stability through mutagenesis of chromosomal DNA by the A3 deaminase activity. Through increased expression of A3 genes, p53...
might guard against retrotransposition of large repeats that are active in the genome (34–37). Wyllie and colleagues (37) demonstrated that WT p53 through its interaction with components of the piRNA (piwi-interacting RNA) pathway suppressed transposon mobility in normal cells, while mutant p53 in cancer cells could not, resulting in the activation of LINE mobility in cancer cells. This is consistent with depletion of A3C leading to greater LINE retrotransposition activity in a cancer cell line (38).

Several studies have shown a correlation between aberrant expression of A3B mRNA in multiple tumors and a specific mutation signature (15, 17, 39). We have established that WT p53 represses A3B expression while p53 hotspot tumor-associated mutants can lead to upregulation of A3B. These p53 hotspot mutants have lost the wild-type ability to interact with p53RE sequences, opening the possibility that upregulation of A3B induced by p53 mutants could be through gain-of-function activities that enhance transactivation activities of other transcription factors, such as NF-kB (40). Consistent with this hypothesis, Maruyama and colleagues recently found that the classical NF-kB pathway is responsible for activation of A3B mRNA in cancer cells (41).

A recent study found that A3B mRNA expression and enzymatic activity were upregulated following transfection of a high-risk HPV genome. This effect was abrogated by inactivation of viral E6, a protein that causes degradation of p53 (42). Notably, in breast tumor samples and derived cancer cell lines A3B upregulation correlates with inactivation of TP53, strongly suggesting that p53 loss and increased A3B could be a tumorigenic event allowing cells to bypass DNA damage checkpoints triggered by A3B (15).

Recently, the A3A protein was found to be a mutator in human cancers (16, 17), as there is a stronger A3A mutagenesis signature than that associated with A3B (16). Furthermore, A3A itself can generate DNA breaks and activate the DNA damage response (DDR; ref. 43, 44) for which p53 is a major downstream regulator and effector (45). In our study, both WT and p53 mutant could upregulate A3A in response to DNA damage. Moreover, as chronic inflammation is commonly related to cancer and as A3A expression correlates with inflammatory environments (such as IFN-I), A3A-induced damage may contribute to somatic mutation selection in cancers (46). Both A3A and A3B are induced in bladder and breast cancer cells after bleomycin treatment (47). Consistent with our results, the induction of A3B is more robust in cell lines harboring mutant p53 than those with WT p53 (47).

Among the seven A3 genes, both A3C and A3H were consistently upregulated in a p53-dependent manner. However, beyond their immune sentinel functions (19), there is little information about potential mutagenicity toward host genomes. Elevated expression of A3C along with lowered expression of A3B in breast cancer patients correlate with improved clinical outcome (48), consistent with our results where p53 activation promotes expression of A3C and represses expression of A3B. A role for A3H in A3 cancer-associated mutagenesis has been indicated by an A3H-I haplotype and associated A3 mutation pattern in breast tumors lacking A3B expression (49).

Thus, it appears that p53 regulation of the A3 genes can have both antitumor and protumor consequences. Our results suggest an A3/p53 immune axis where activation of WT p53 in normal cells in response to internally and externally induced lesions (including replication collapse) could also influence A3 expression (particularly A3A and A3B) and subsequent appearance of mutations that have been identified in cancers.

We propose that after cancer establishment, the p53+ or p53− status along with cancer therapy may influence the potential for subsequent A3A and A3B mutagenesis as part of tumor evolution and metastasis. Most cancer therapies involve agents such as ionizing radiation, doxorubicin, and etoposide that induce WT p53. As described, mutant p53 proteins can strongly influence expression of A3A and A3B as well as other A3 genes. Even the lack of p53 can lead to upregulation of A3B. Given that more than 50% of cancers (some >90%) have altered p53, we suggest that specific p53 mutation status should be considered in treatments. Along this line, it would be interesting to address p53 status and mutagenesis patterns in secondary tumors as well as cancers that arise in association with Li–Fraumeni disease (50), which is due to a germline defect in TP53.

Overall, our results provide molecular insights into p53 direct control of immunosurveillance under stress conditions, integrating p53 tumor suppressor activities with innate immune stimuli. Further studies are needed to establish the functional impact of A3 transcriptional regulation by p53. For example, under conditions of viral infection how does the p53–A3 axis impact the host immune function and the likelihood of mutation in the host and the pathogen. The integration of immune response and DDR may have implications for understanding therapeutic approaches. Understanding the relationship could provide novel ways to incorporate cell-extrinsic and cell-intrinsic defenses against tumorigenesis by pharmacologic activation of p53, thereby achieving more effective therapies against cancer and other diseases.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: D. Menendez, M.A. Resnick
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.-A. Nguyen
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