Inactivation of p53 Enhances Sensitivity to Multiple Chemotherapeutic Agents

Douglas S. Hawkins, G. William Demers, and Denise A. Galloway

Cancer Biology Program, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

ABSTRACT

Many tumor types have p53 and/or RB mutations, and it is unclear what role the mutations of these tumor suppressor genes have on the efficacy of chemotherapeutic agents. The effect of p53 and RB inactivation on sensitivity to chemotherapeutic drugs was examined using a model system in which p53 or RB was inactivated in normal human foreskin fibroblasts (HFFs) by acute expression of human papillomavirus (HPV) 16E6 or 16E7. Cytotoxicity assays showed that HFFs expressing HPV 16E6 were 6- to 9-fold more sensitive to the DNA crosslinkers cisplatin and carboplatin and 7.8- to 11.5-fold more sensitive to the tubulin polymerizing agent paclitaxel than were LXSN-expressing cells. Analysis of mouse embryonal fibroblasts lacking p53 (p53−/−) compared with mouse embryonal fibroblasts homozygous (p53+/+) and heterozygous (p53+/−) for wild-type p53 confirmed the role of p53 in the enhanced sensitivity to cisplatin. Treatment with the alkylating agents melphalan and nitrogen mustard resulted in 3.8- to 7.3-fold greater sensitivity in HPV 16E6- or 16E7-expressing cells compared with LXSN-expressing cells. Enhanced sensitivity to cisplatin in cells lacking p53 function was explored by examination of its effects on the cell cycle progression after exposure. When treated with cisplatin, HFFs expressing 16E6 showed delayed progression through S phase relative to HFFs expressing LXSN. The delay in S phase progression was coincident with the induction of p53 protein levels in LXSN-containing HFFs, suggesting a role for p53 in DNA repair of cisplatin-induced damage. These results indicate that the inactivation of p53 in the absence of other genetic alterations leads to enhanced sensitivity to multiple chemotherapeutic agents rather than to increased resistance.

INTRODUCTION

Failure to control cellular proliferation is a characteristic feature of cancer (1, 2). Normal cells progress through the cell cycle under the regulation of an intricate system of cyclins, CDKs4, and CDK inhibitors (3–7). This cascade of regulatory proteins provides both the catalyst for growth and the mechanism to arrest growth at discrete “checkpoints” after cellular injury, particularly DNA damage (8–11). Checkpoints provide an opportunity for repair and a method to maintain genomic integrity. In contrast, malignant cells frequently acquire abnormalities in cell cycle control, allowing dysregulated proliferation and abrogation of normal checkpoints in spite of cellular damage (3, 12, 13).

p53 and RB are two key proteins involved in cell cycle control; they are frequently altered in cancer (14–16). After DNA damage, p53 levels increase and mediate multiple cellular responses: (a) G1 arrest via transcriptional induction of p21, a CDK inhibitor (17); (b) DNA damage repair via transcriptional induction of Gadd45 (18) and possibly by direct interaction with ERCC3 (19) and the TFIH-associated nucleotide excision repair pathway (20); and (c) induction of apoptosis in some cell types (21). Approximately one-half of all primary tumors have deleted or mutated p53 (22). In addition, individuals with Li-Fraumeni, a familial cancer susceptibility syndrome characterized by germline heterozygous alterations in p53, show an increased incidence of multiple malignancies, including breast cancer, sarcomas, brain tumors, and leukemias (23–25). RB also plays a role in the G1 checkpoint by binding the E2F family of transcription factors (26). Phosphorylation of RB by CDKs inactivates its growth-suppressing role by releasing E2F, which activates the expression of genes required for the G1-S phase transition. RB is deleted or mutated in multiple primary tumors, including osteosarcoma and bladder and lung cancer (27). Heterozygous germline alterations in RB are associated with familial RB in early childhood and in osteosarcoma.

In addition to genetic alterations leading to p53 and RB abnormalities, viral oncoproteins, such as HPV E6 and E7, can cause inactivation of the growth-suppressive functions of p53 and RB, respectively (28). HPV 16E6 binds to p53 and targets it for degradation via a ubiquitin-dependent pathway (29). HPV E7 binds to RB, leading to the release of free E2F, independent of RB phosphorylation (26). Functional inactivation of p53 and RB provides a biological mechanism by which HPV infection contributes to the development of anogenital malignancies (30). Introduction of these viral oncoproteins into cells using retroviral vectors also provides a model for phenotypic p53 and RB deficiency.

It is unclear what direct effect mutations of p53 or RB have on the efficacy of cancer chemotherapeutic agents. Several lines of experimental investigation suggest that loss of p53 function leads to resistance to chemotherapy and radiation therapy. Primary tumors that have altered p53 are generally associated with a worse clinical outcome than are tumors that maintain wild-type p53 (25, 27). Tumor cell lines lacking p53 function are resistant to multiple chemotherapeutic agents (31) and radiation therapy (32), although this has not been found universally (33). MEFs derived from p53 knockout mice (homozygous p53−/−) are resistant to chemotherapy and radiation in vitro (21), as are fibrosarcoma cells transplanted into nude mice in an in vivo model (34). All of these investigations are complicated by the genetic instability of cells lacking native p53 function and the possibility of additional drug-resistance mechanisms acquired in the transformation process. Several of these studies have used p53−/− cells transformed by adenovirus E1a and H-ras, further complicating the analysis of drug resistance mechanism.

Because of the difficulty in separating the effect of p53 loss from the acquired genetic abnormalities allowed by the loss of p53, we used acute expression of HPV 16E6 and 16E7 in primary HFFs as an alternative model for p53 and RB inactivation. Unlike cells in other model systems, HFFs infected with HPV 16E6 or 16E7 display no gross chromosomal abnormalities and do not acquire a transformed phenotype until late in passage after cell crisis (35). This system provides a model with which to study the relationship between loss of p53 or RB and chemotherapy sensitivity.

Our results showed increased sensitivity to several chemotherapeutic agents in cells lacking p53 function. Further analysis of the
mechanism of increased sensitivity to cisplatin in p53-compromised cells revealed that these cells were inhibited in their progression through S phase. Furthermore, p53 levels in normal cells were increased at a time after exposure in which they had started to accumulate in G2-M. p53-deficient cells, lacking a p53 response to cisplatin, demonstrated delayed progression through S phase. This suggests that p53 may play a role in DNA repair, thereby allowing cells to progress more readily through S phase.

MATERIALS AND METHODS

Cell Culture and Media. Primary human fibroblasts were derived from neonatal foreskin and grown in DMEM supplemented with 10% FBS and penicillin/streptomycin (complete medium) at 37°C and 5% CO2. For cytotoxicity experiments, complete medium without phenol red was used. Cells were infected with amphotropic retroviruses containing vector LXSN, HPV16 genes for E6 or E7 (LXSN-16E6, LXSN-16E7, and LXSN-16E6E7) as described previously (36). Pooled populations of cells after G18 selection were used for 1 to 10 passages.

Primary MEFs were harvested from offspring of a p53 heterozygous (wild-type p53+/−) breeding pair (GenPharm). p53 status was determined by PCR as described previously (37).

Cytotoxicity Assay. Asynchronously growing cells were transferred into 96-well tissue culture plates (Costar Corp.) in 150 μl of medium. Each well contained 3.75 × 104 cells. After overnight incubation, the medium was aspirated off the adherent cells and fresh medium with variable drug concentrations was added. Drug-containing media were made fresh from stock solutions by serial 2-fold dilutions. Cisplatin and carboplatin stock solutions were in PBS in concentrations of 1 mg/ml and 5 mg/ml, respectively. Nitrogen mustard and melphalan were dissolved in 0.1 M hydrochloric acid at 167 mM and 13 mg/ml, respectively, and were freshly prepared for each experiment. Paclitaxel (Taxol) stock solution was in DMSO at 1 mg/ml. Cisplatin, carboplatin, nitrogen mustard, and melphalan were obtained from Sigma Chemical Co. and paclitaxel from Dr. Alan F. Wahl (Bristol-Meyers Squibb). Unless otherwise stated, drug exposure was continuous for 72 h. For 1-h pulsed exposure experiments, cells were treated at various drug concentrations for 1 h, then washed with PBS twice, followed by incubation in medium for 72 h.

Cell viability was determined in sextuplicate wells for each drug concentration using the XTT/PMs viability dye assay (38, 39). Both XTT and PMS were obtained from Sigma. After 72 h of incubation, 50 μl XTT/PMs (1 mg/ml XTT and 7.5 μg/ml PMS) were added to each well and absorbance was measured at 450 nm. Cell viability was expressed as the percent absorbance of treated wells relative to the untreated control wells.

Cell Cycle Analysis. Cells were harvested from tissue culture plates in complete medium, then changed to DMEM with 0.5% FBS for 1 day. Cells were then split into fresh plates at 5 × 103 cells/100-mm plate in complete medium with 3 μg/ml aphidicolin for 24 h. Cells were then washed with PBS twice and incubated in DMEM with 10% FBS with or without cisplatin. Drug exposure was either 1 μg/ml continuously or 25 μg/ml for 1 h, followed by two PBS washes and postincubation in DMEM with 10% FBS.

Cell Cycle Synchronization. Cells were harvested at stated times after drug exposure. After washing with PBS once, cells were fixed with ice-cold 70% methanol, pelleted, resuspended in IFA (10 mM Hepes, pH 7.4-150 mM NaCl-4% FBS-0.1% sodium azide) with 10 μg/ml RNase and incubated for 30 min at 37°C. Cells were then incubated overnight in the dark at 4°C with propidium iodide (40). The stained cells were analyzed for DNA content on a fluorescence-activated cell sorter (FACScan, Becton Dickinson Instruments). Cell cycle fractions were quantified with Reproman (True Facts Software, Seattle, WA).

RESULTS

Sensitivity to Chemotherapeutic Drugs. To determine the effect of inactivation of p53 and RB on toxicity of chemotherapy to human fibroblasts, cells with vector LXSN were compared with cells expressing 16E6, 16E7, and 16E6E7. The XTT/PMs dye reduction assay allowed for determination of IC50 from dose response curves generated from multiple drug doses. Three classes of drugs were assayed: platinum compounds, alkylating agents, and a tubulin polymerizing agent. IC50 was defined as the drug dose resulting in 50% loss of cell viability relative to untreated cells; results are given in Table 1.

Using this method, cells expressing 16E6 or 16E6E7 were 6- to 9-fold more sensitive to platinum compounds cisplatin (Fig. 1A) and carboplatin (Fig. 1B) than were LXSN cells after 72-h continuous exposure and 3.5-fold more sensitive after 1-h pulsed exposure to cisplatin (Fig. 1F). Cells expressing 16E7 had an intermediate sensitivity to cisplatin and carboplatin. Treatment with the alkylators nitrogen mustard (Fig. 1C) and melphalan (Fig. 1D) resulted in 3.8- to 7.3-fold more sensitivity in the 16E6-, 16E7-, and 16E6E7-expressing cells than in the LXSN-expressing cells. Treatment with the tubulin polymerizing agent paclitaxel (Fig. 1E) demonstrated 7.8- to 11.5-fold increased sensitivity in cells expressing 16E6 compared with cells expressing LXSN or 16E7. Each of these compounds showed increased cytotoxicity in 16E6-expressing cells, whereas 16E7-expressing cells showed a varied relative sensitivity to each of the drug classes used in this study. In no case did the expression of 16E6 or 16E7, or the coexpression of 16E6 and 16E7, lead to enhanced resistance to the chemotherapeutic agents.

Because 16E6 expression in HFFs results in near elimination of detectable p53 protein, these cytotoxicity data suggested that the loss of p53 correlated with enhanced drug sensitivity. To support this hypothesis, MEFs in first passage were studied with the same colorimetric cytotoxicity assay. Fibroblasts with wild-type p53 (p53+/+) showed increased resistance to the chemotherapeutic agents.

p53 AND CHEMOTHERAPY SENSITIVITY

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (μg/ml)</th>
<th>SD</th>
<th>Mean</th>
<th>95% CI</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>3.7 ± 0.6</td>
<td>0.1</td>
<td>3.7</td>
<td>3.4</td>
<td>4</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>0.5 ± 0.1</td>
<td>0.05</td>
<td>0.5</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>2.1 ± 0.5</td>
<td>0.2</td>
<td>2.1</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Melphalan</td>
<td>0.4 ± 0.2</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1.3 ± 0.2</td>
<td>0.15</td>
<td>1.3</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Cisplatin, 1-h exposure</td>
<td>15 ± 4</td>
<td>0.5</td>
<td>15</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Carboplatin, 1-h exposure</td>
<td>3.3 ± 1.0</td>
<td>0.2</td>
<td>3.3</td>
<td>3.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

a ND, not done.

Table 1. IC50 for HFFs expressing LXSN, 16E6, 16E7, or 16E6E7 treated with chemotherapeutic agents.
were compared with fibroblasts either homozygous null for p53 (p53−/−) or heterozygous for p53 (p53+/−; Fig. 2). p53+/+ and p53−/− fibroblasts had similar cisplatin sensitivity (IC50 = 2.9 μg/ml ± 0.7 and 3.0 ± 0.7, respectively), whereas p53−/− cells were 2.3-fold more sensitive after 72-h exposure (IC50 = 1.3 μg/ml ± 0.7). Similar results have been obtained using paclitaxel.5

Cell Cycle Alteration in Cisplatin-treated Cells. The increase in sensitivity to cisplatin in cells lacking p53 function was examined by analysis of cell cycle distribution after exposure to the drug. LXSN and 16E6 cells were initially studied in asynchronously growing culture with continuous exposure to 1 μg/ml cisplatin. This dose was intermediate between the IC50s for LXSN and 16E6. In asynchronously growing cells, exposure to cisplatin resulted in greater accumulation of 16E6-expressing cells in S phase relative to LXSN-expressing cells (data not shown). Little difference in the percent of cells remaining in G1 was seen comparing LXSN- and 16E6-expressing cells. The accumulation in S phase suggested that cells expressing 16E6 were less likely to proceed completely through S phase after exposure to cisplatin. Because chemotha-
6 h of release from aphidicolin and with continuous exposure to 1 
μg/ml cisplatin, LXSN cells exhibited significantly elevated p53 protein levels compared with untreated controls (Fig. 5A). Elevated p53 protein was observed at a time point at which the cells had progressed through S phase and were starting to accumulate in G2-M (Fig. 3C) and therefore is consistent with the hypothesis that p53 response is necessary for normal S-phase progression after cisplatin exposure. These levels continued to increase and remained elevated at 30 h after exposure. One-h exposure to 25 μg/ml cisplatin resulted in even greater levels of p53 (Fig. 5B), which were also persistent at 30 h after exposure. Although p53 protein was detectable in 16E6-

**Fig. 2.** Effect of p53 genotype in MEF cells treated with cisplatin continuously for 72 h. Representative data sets are shown. Points, mean and SD of six replicate wells using the XTT/PM5 assay; bars, SD. Data are typical of four separate experiments.

To further define the effect of cisplatin on progression through S phase, LXSN and 16E6 cells were synchronized at the G1-S phase transition with density arrest and serum starvation, followed by release from density into complete medium with aphidicolin, a reversible DNA polymerase-α inhibitor. This method accentuated the cell cycle effects of cisplatin treatment, allowing the study of a synchronized population of cells. Untreated cells progressed into S phase within 3 h after release from aphidicolin (Fig. 3, A and B) and had peak appearance in G2-M by 9 h after release (Fig. 3C), followed by progression through mitosis into G1 by 15 h (Fig. 3A). Untreated LXSN- and 16E6-expressing cells progressed through the cell cycle with the same kinetics. Both LXSN and 16E6 cells treated continuously with 1 μg/ml cisplatin progressed into S phase at the same rate as untreated cells (Fig. 3, A and B). LXSN cells treated with cisplatin progressed into G2-M at the same rate as untreated cells; however, entry into G2-M was delayed in 16E6 cells treated with cisplatin (Fig. 3C). Eventually, the 16E6-expressing cells accumulated in G2-M (Fig. 3C), and neither LXSN nor 16E6 cells progressed through mitosis into G1 after treatment (Fig. 3A).

Aphidicolin-synchronized cells were also treated with 25 μg/ml cisplatin for 1 h. Under these conditions, both LXSN- and 16E6-expressing cells demonstrated profound cell cycle arrest, although LXSN cells progressed partially into S phase, and 16E6 cells remained arrested at the G1-S phase transition (Fig. 4). The cell cycle analysis of cisplatin-treated cells suggested that the increased sensitivity of cells lacking p53 function was correlated to the hampered ability of 16E6 cells to progress through S phase.

**p53 and p21 Protein Levels.** Cells containing p53 were less affected in their cell cycle progression through S phase than cells without p53 function, suggesting that p53 response to cisplatin exposure may be important in mediating cell cycle progression. To determine the p53 response, levels of p53 protein were analyzed by Western blot in lysates from aphidicolin-synchronized cells. Within
expressing cells, even after exposure to cisplatin, levels were consistently below those found in untreated LXSN cells.

p21 (WAF1), an inhibitor of multiple CDKs, is transcriptionally regulated by p53 (17) and allows an assessment of the p53 transactivation response. Cell lysates were also analyzed for p21 protein levels by Western blot. LXSN cells treated with continuous exposure to 1 mg/ml cisplatin after aphidicolin release exhibited significantly increased p21 protein levels at 30 h but not at 6 h after treatment (Fig. 5C). One-h exposure to 25 mg/ml cisplatin resulted in even greater levels of p21 at 30 h but not at 6 h after treatment (Fig. 5D). Although p21 was detectable in 16E6-expressing cells, it was consistently below the basal level seen in untreated LXSN cells. This suggests that p53 can respond to cisplatin-induced damage during S phase and transactivate p53-responsive genes, one of which is p21.

DISCUSSION

A number of previous studies, particularly those using cancer cell lines, have shown that the loss of p53 function resulted in increased resistance to chemotherapeutic agents (21, 31, 32, 34). In contrast, the present study found increased cytotoxicity to a wide variety of drugs in primary, nontransformed cells lacking p53. Several explanations for these conflicting results are possible. First, HPV 16E6 may have additional effects beyond degradation of p53. However, enhanced chemosensitivity to paclitaxel and cisplatin in MEFs that are p53−/− suggests that these results are not due to a p53-independent activity of HPV 16E6. The results with the MEFs also rule out the possibility that the differences are species specific in human versus mouse cells. Second, in the process of becoming an established cell line, tumor cells may acquire additional genetic abnormalities that confer drug resistance. Comparisons between different tumor cell lines may be clouded by these uncharacterized abnormalities. Similarly, genetic instability is characteristic of the p53-deficient state (41, 42); MEF p53−/− cells have been shown to develop aneuploidy by the seventh passage in culture (43). Therefore, cells lacking p53 studied after multiple passages may have acquired additional abnormalities conferring drug resistance. Early passage human cells expressing 16E6 may more closely approximate a system in which p53 deficiency is the only alteration. Third, some cell types may display a predominant apoptotic response to chemotherapy (44). In those cell types, apoptosis in p53+/+ cells may be the principal explanation for their relative sensitivity to chemotherapeutic agents. Recent studies using a breast cancer cell line that does not have a predominant apoptotic response showed enhanced cisplatin sensitivity in cells expressing HPV 16E6 or having a dominant negative p53 mutant (45). Similarly, HFFs also do not have a predominant apoptotic response, and a sub-G1 population consistent with apoptotic cells was not seen after cisplatin exposure. Tissue-specific responses to cellular injury will likely influence the role of p53 in drug sensitivity. Fourth, the role of p53 in drug sensitivity may be dependent on the class of chemotherapeutic agents studied.

Having observed enhanced cisplatin sensitivity in cells lacking p53 function, we explored the mechanism of drug sensitivity by evaluating cell cycle progression after cisplatin treatment. For these studies, we chose aphidicolin synchronization to accentuate cell cycle effects because experiments in asynchronously growing cells suggested a relative accumulation of 16E6-expressing cells in S phase. Because we sought a mechanism to explain the differential toxicity of cisplatin, an equimolar dose intermediate between the IC50 of LXSN- and 16E6-expressing cells was chosen for cell cycle experiments. The clinical efficacy of a chemotherapeutic agent is dependent upon the therapeutic window (i.e., the range between toxic doses for normal tissue and malignant cells), so that a given dose produces a higher cell kill rate in cancerous cells than in normal tissue. By analogy, studying the cell cycle effects within...
the range of differential toxicity between LXSN- and 16E6-expressing cells allowed us to address the role that p53 loss might have in shifting the therapeutic window.

There may be many pathways in which p53 participates that can result in greater sensitivity to chemotherapeutic drugs when p53 is lost. We have begun to determine the role of p53 in response to cisplatin by examining progression through the cell cycle. One likely possibility is that the loss of p53 results in impaired DNA repair of platinum-DNA adducts, as evidenced by delayed progression through S phase. Cell lines deficient in the nucleotide excision repair pathway, such as xeroderma pigmentosa and Fanconi's anemia, have enhanced sensitivity to cisplatin (45). p53 has been linked to this repair pathway via interaction with ERCC3 (19). p53 may also play an indirect role in the repair of radiation-induced damage by transcriptional regulation of Gadd45, which interacts with proliferating cell nuclear antigen to enhance DNA repair (18).

Because cisplatin-DNA adducts are removed by the nucleotide excision repair pathway, the recently described interaction between p53 and the TFIIH-associated nucleotide excision repair provides a possible mechanism correlating p53 function and cisplatin sensitivity (20). Specific evidence linking p53 to cisplatin-DNA adduct repair was recently demonstrated using a platinated chlorophenicol acetyltransferase reporter plasmid construct (46). In this system, chlorophenicol acetyltransferase activity was markedly reduced in cells infected with HPV 16E6. p53 has been shown to play a key role as a checkpoint in response to DNA damage, controlling progression from G1 to S phase, but does not seem to mediate the G2-M arrest. Our data examining drug exposure concomitant with release from aphidicolin block suggest that p53 can be activated in S phase and may have a role facilitating progression through S phase.

Much less is known regarding the relationship between RB status and sensitivity to chemotherapy. The embryological lethality of homozygous RB deficiency has hampered the development of in vitro and animal models (47). Clinical studies in leukemia (48) and bladder cancer (49) have demonstrated an association between abnormal RB protein expression and primary chemotherapy resistance, as well as between abnormal RB protein expression and worse tumor-free survival rates. To our knowledge, there are no in vitro tumor cell line studies that have addressed the role of RB protein on chemotherapy sensitivity. In the present study, the similar sensitivity to alkylators in both HPV 16E6- and 16E7-expressing cells suggests that both p53 and RB may be involved in pathways of DNA repair of alkylator damage or in the cellular response to this damage. Both 16E6 and 16E7 have been shown to be able to bypass a DNA damage-induced G1 arrest using distinctly different mechanisms (50).

In summary, using a model of functional p53 and RB deficiency resulting from the expression of HPV 16E6 and 16E7 in primary human fibroblasts, we demonstrate that cells lacking p53 are more sensitive to a variety of chemotherapeutic agents. For cisplatin, enhanced sensitivity is associated with a delay in progression through S phase. p53 protein levels are induced in a time course coincident with the delay in progression through S phase. Cells lacking either p53 or RB function are more sensitive to alkylators. These alterations in chemotherapeutic sensitivity suggest that p53 is involved in multiple pathways, some of which overlap with RB pathways and some of which are independent. Future studies will explore the mechanism of enhanced sensitivity with particular attention to DNA repair. Further definition of the role of p53 and RB in drug sensitivity may lead to the rational selection of chemotherapy based on molecular features of an individual tumor.

ACKNOWLEDGMENTS

The authors would like to thank Dr. R. Blanton for establishment of primary MEFs from the p53+/- mice and Dr. Alan F. Wahl for his advice in setting up the cytotoxicity assay.

REFERENCES

p53 AND CHEMOTHERAPY SENSITIVITY


Inactivation of p53 Enhances Sensitivity to Multiple Chemotherapeutic Agents

Douglas S. Hawkins, G. William Demers and Denise A. Galloway


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/4/892

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/56/4/892.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.