Ultraviolet Irradiation Induces BRCA2 Protein Depletion through a p53-independent and Protein Synthesis-dependent Pathway

Shao-Chun Wang, Keishi Makino, Li-Kuo Su, Annie Y. Pao, Jeong Soo Kim, and Mien-Chie Hung

Abstract

It has been suggested that BRCA2, the protein product of the breast cancer susceptibility gene BRCA2, is involved in DNA damage repair. It is therefore likely that BRCA2 plays a role in a signaling pathway induced by DNA-damaging agents. To test this possibility, we examined the alteration of the BRCA2 protein level in human cell lines after UV irradiation. We found that UV irradiation down-regulated BRCA2 in a dose-dependent manner in all cell lines tested. The down-regulation of BRCA2 occurred soon (within 4 h) after UV treatment. Surprisingly, down-regulation of BRCA2 by UV does not require functional p53, which has been suggested to be required for the down-regulation of BRCA1 and BRCA2 mRNAs by DNA-damaging agents. Moreover, the proteosome- and calpain-mediated protein degradation pathways do not have an important role in the UV-induced BRCA2 depletion. However, blocking protein synthesis temporally inhibited the depletion of BRCA2 and BRCA1 in some cell lines. Ectopic expression of BRCA2 in cells increased resistance of cells to high-dose UV irradiation. These results demonstrate that BRCA2 is involved in a DNA-damaging signaling pathway induced by UV radiation and that expression of BRCA2 can protect cells from UV-mediated cell death.

Introduction

Patients carrying heterozygous germ-line mutations in the breast cancer susceptibility gene BRCA2 are predisposed to breast, ovarian, and other types of cancer (1–3). The wild-type allele of the BRCA2 gene is usually lost in tumors of patients carrying germ-line BRCA2 mutations, indicating a prominent role of BRCA2 in the development of cancer. Recently, we and others have demonstrated that the expressions of BRCA2 RNA and protein are regulated in a cell cycle-dependent manner, with a peak expression during S and M phases of the cell cycle (4–7). Studies using Brca2 knockout mice suggested a role of BRCA2 in DNA damage signaling and repair (reviewed in Refs. 1–3). Mouse embryos carrying homozygous mutations of Brca2 are developmentally retarded and usually die early in embryogenesis. Homozygous Brca2 mutant mice that survive to birth showed a wide range of defects, including the development of lethal thymic lymphomas. In addition, mouse embryonic stem cells and embryo fibroblast cells lacking functional Brca2 are deficient in DNA repair. These observations together suggest a guardian function for BRCA2 in maintaining genome integrity by participating in the repair of DNA damage. This suggestion is further supported by the physical association between BRCA2 and RAD51, a human homologue of the Escherichia coli DNA double-strand-break repair protein RecA.

UV radiation is a potent DNA-damaging agent that activates a wide spectrum of signaling pathways in cells. One of such responses is the increased level of wild-type p53, probably because of its increased protein stability by site-specific phosphorylation induced by UV (8). Mouse embryos lacking functional Brca2 have been shown to be hypersensitive to UV irradiation (9). This observation suggests that Brca2 is essential for the mouse embryo cells to survive DNA damage caused by UV. However, it remains to be determined whether BRCA2 also involves in stress responses to UV irradiation in human adult cells.

BRCA2 has been shown to physically associate with the tumor suppressor p53 and therefore is linked to the p53-dependent transcription function (reviewed in Ref. 9 and references therein). Consistently, homozygous Brca2 mutant mouse embryos develop better when they also lack a functional p53 gene, suggesting a functional association between these two genes. It has been shown that loss of the p53 checkpoint function combined with BRCA2 deficiency may trigger tumor formation and cancer progression (10). The functional and physical link between BRCA2 and p53 raises an intriguing question as to whether the response of BRCA2, if any, to DNA damage caused by genotoxic agents requires functional p53.

In this report, we show that UV irradiation results in depletion of BRCA2 protein, and that this process does not require functional p53 but requires protein synthesis. We also show that forced expression of BRCA2 increased cell resistance to UV irradiation.

Materials and Methods

Materials. 293 cells were maintained in high-glucose DMEM-10% fetal bovine serum containing 25 mM HEPES. Other cell lines were maintained in DMEM/F-12 medium with 10% fetal bovine serum. The monoclonal antibody N61 against BRCA2 has been described (7). Antibodies against p53 (Oncogene Science, Cambridge, MA), BRCA1 (Neomarker, Union City, CA), pRB (Santa Cruz Biotechnology, Santa Cruz, CA), and PARP3 (PharMingen, San Diego, CA) were purchased. Genistein, MG115, MG132, and ALLN were purchased from Calbiochem (La Jolla, CA). z-VAD-fmk was purchased from Sigma Chemical Co. (St. Louis, MO).

BRCA2 Expression Plasmids. Fragments of the wild-type human BRCA2 cDNA were isolated by RT-PCR and completely sequenced. The full-length BRCA2 cDNA was then assembled from these fragments and was inserted into a modified pcDNA3 plasmid. The m1 BRCA2 mutant, which mimics a pathogenic germ-line mutation and has a two-nucleotide deletion at codon 17, was generated using mutagenesis PCR.

Immunoblot Analysis. Cells were harvested and lysed in ice-cold NETN buffer [150 mM NaCl, 1 mM EDTA, 20 mM Tris (pH 8.0), and 0.5% NP40]. Lysates containing an equal amount of protein (60–120 μg) were separated by SDS-PAGE and blotted to polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA).

Received 4/10/00; accepted 2/13/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This study is supported in part by the M.D. Anderson Breast Cancer Research Program (to M.-C. H. and L.-K. S.) and NIH Grants R01 CA58880, CA60856, and CA77858. K. M. is a postdoctoral fellow supported by Department of Defense Breast Program (to M-C. H. and L-K. S.) and NIH Grants RO1 CA58880, CA60856, and CA60856.

1. The abbreviations used are: PARP, poly(ADP-ribose) polymerase; z-VAD-fmk, z-Val-Ala-Asp-fluoromethyl ketone; TNF, tumor necrosis factor; EGFR, epidermal growth factor receptor; NF-κB, nuclear factor-κB.
UV induced BRCA2 depletion

Proteins were then identified using antibodies per the manufacturer’s instructions.

UV Survival Assays. 293 cells were plated in six-well plates or 60-mm dishes and transfected with the appropriate plasmid DNA as described in the figure legends using the cationic liposome LPD1 (a gift kindly provided by Drs. Leaf Huang and Song Li, University of Pittsburgh, Pittsburgh, PA). Sixteen hours later, the transfected cells were split. For the luciferase assay, the cultures in six-well plates were split into two wells and treated with UV radiation (80–120 J/m²) 36 h after plating. The cultures were maintained, and the medium was changed every 3 days. Ten days later, the cells were lysed, and the luciferase activity was determined using a luciferase assay system (Promega Corp., Madison, WI) following the manufacturer’s instructions. Results from four independent experiments were averaged. For the clonogenic assay, 1 × 10⁵ cells were transfected in 60-mm plates and then plated in 100-mm tissue culture dishes 48 h after transfection. After incubation for 36 h, the cells were treated with UV irradiation (80–120 J/m²), and the cultures were maintained until the surviving cells formed colonies.

Results

UV Irradiation Altered BRCA2 Protein Expression. To investigate the effect of UV irradiation on BRCA2 protein expression, we examined a panel of human cell lines including an immortalized normal breast epithelial cell line (MCF-10A), an osteosarcoma cell line (U-2 OS), a pancreatic cancer cell line (CAPAN-1), two breast cancer cell lines (MDA-MB-435 and MDA-MB-231), and an ovarian cancer cell line (SKOV3-ip1; Table 1). UV irradiation down-regulated BRCA2 protein levels in a dose-dependent manner in each cell line tested (Fig. 1A). Although low-dose UV irradiation down-regulated BRCA2 to various extents among these cell lines, UV doses >12 J/m² consistently reduced the BRCA2 protein in all cell lines tested. The CAPAN-1 cell line expresses a truncated BRCA2 because of a single nucleotide deletion (6174dT) in exon 11 of the BRCA2 gene (7). The truncated BRCA2 protein, which is not able to associate with p53 (11), was still down-regulated by UV irradiation. More importantly, UV-induced down-regulation of BRCA2 protein did not require functional p53. BRCA2 protein depletion by UV can be observed in cell lines expressing wild-type p53 (MCF-10A and U-2 OS), mutant p53 (MDA-MB-231, MDA-MB-435, and CAPAN-1), or no p53 (SKOV3-ip1). As expected, the p53 protein level was induced by UV irradiation in a dose- and time-dependent manner in the two cell lines expressing wild-type p53 (MCF-10A and U-2 OS) and was not altered in cell lines expressing mutant p55 (Fig. 1A). It is known that BRCA2 expression is reduced in cells grown arrested at the G0-G1 phases of the cell cycle (5, 7). To rule out the possibility that BRCA2 down-regulation by UV may be a secondary effect caused by cell cycle arrest at G0-G1 phases after UV irradiation, we examined the effects of UV treatment on cell cycle progression. As shown in Fig. 1B using MCF-10A and U-2-OS cells as examples, the BRCA2 protein down-regulation by UV irradiation did not correlate with cell cycle alteration based on fluorescence-activated flow cytometry (FACS). Thus, the down-regulation of BRCA2 by UV treatment cannot be attributed to the effects of UV irradiation on cell cycle progression.

Table 1 The p53 status of cell lines used in this work

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>p53 status</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-10A</td>
<td>Breast epithelium</td>
<td>Wild-type</td>
</tr>
<tr>
<td>U-2 OS</td>
<td>Osteosarcoma</td>
<td>Wild-type</td>
</tr>
<tr>
<td>MDA-MB-435</td>
<td>Breast cancer</td>
<td>Mutant</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast cancer</td>
<td>Mutant</td>
</tr>
<tr>
<td>SKOV3-ip1</td>
<td>Ovarian cancer</td>
<td>Null</td>
</tr>
<tr>
<td>CAPAN-1</td>
<td>Pancreatic cancer</td>
<td>Mutant</td>
</tr>
<tr>
<td>A431</td>
<td>Epidermoid cancer</td>
<td>Mutant</td>
</tr>
</tbody>
</table>

UV-mediated down-regulation of BRCA2 protein. A, human cell lines were irradiated with different doses of UV (Lane 1, no treatment; Lane 2, 6 J/m²; Lane 3, 12 J/m²; Lane 4, 24 J/m²; Lane 5, 32 J/m²; Lane 6, 42 J/m²). Cells lysates were prepared 12 h after the UV treatment, and proteins were detected by immunoblotting. α-Actin was used as a control for equal loading of protein. The BRCA2 protein in CAPAN-1 cells is a COOH-terminal truncated mutant (7). B, UV effects of the cell cycle. Cell cycle progression of MCF-10A and U-2-OS in A was determined by FACS analysis 12 h after UV treatment. The percentage of cells in each cell cycle phase is shown. C, rapid BRCA2 down-regulation induced by UV. MDA-MB-231 and U-2-OS cells were treated with 24 J/m² of UV and incubated for different times (h) as indicated before the cells were lysed. NT, no treatment.

Requirement of Protein Synthesis of UV-mediated Depletion of BRCA2 and BRCA1. To further address the mechanism of the UV-induced BRCA2 down-regulation, we determine the kinetics of BRCA2 depletion in response to UV irradiation (Fig. 1C). UV-mediated down-regulation of BRCA2 protein occurred within 4 h after UV irradiation in U-2 OS and MDA-MB-231 cells.

The rapid depletion prompted us to test whether protein degradation contributed to the UV-mediated BRCA2 down-regulation by blocking protein synthesis with the protein translation inhibitor cycloheximide. To our surprise, cycloheximide treatment of U-2 OS cells actually prevented BRCA2 depletion within 9.5 h of incubation after UV irradiation, suggesting the involvement of protein synthesis in the down-regulation process (Fig. 2A). However, in U-2 OS cells, the inhibitory effect of cycloheximide was relieved after prolonged incubation (15 h; Fig. 2C). On the other hand, BRCA2 depletion by UV irradiation in MDA-MB-231 cells remained blocked by cycloheximide, even after prolonged incubation (17 h; Fig. 2, B and D). In this experiment, the inhibition of protein neosynthesis was confirmed by the lack of p53 induction in UV in cycloheximide-treated U-2 OS cells (8). In both cell lines tested, UV treatment also caused down-regulation of BRCA1 protein accompanied with a slower migration of the BRCA1 protein, which likely resulted from protein phosphorylation induced by UV, as reported previously (12, 13). Interestingly, although cycloheximide treatment could not block UV-mediated BRCA1 down-regulation in U-2 OS cells, cycloheximide completely blocked BRCA1 protein depletion by UV irradiation in MDA-MB-231 cells (Fig. 2).

To circumvent the unexpected effect of cycloheximide, a pulse-chase experiment was performed. MDA-MB-231 cells were metabolically pulse labeled with [35S]methionine for newly synthesized protein before being irradiated with UV. The BRCA2 protein level was monitored at different chasing time points after UV irradiation by immunoprecipitation with an anti-BRCA2 antibody (Fig. 2F). There was a clear reduction in the BRCA2 protein level by 2 h after UV irradiation in MDA-MB-231 cells. To circumvent the unexpected effect of cycloheximide, a pulse-chase experiment was performed. MDA-MB-231 cells were metabolically pulse labeled with [35S]methionine for newly synthesized protein before being irradiated with UV. The BRCA2 protein level was monitored at different chasing time points after UV irradiation by immunoprecipitation with an anti-BRCA2 antibody (Fig. 2F). There was a clear reduction in the BRCA2 protein level by 2 h after UV irradiation in MDA-MB-231 cells.
irradiation. The result strongly suggests that UV irradiation causes BRCA2 protein degradation.

**BRCA2 Protein Depletion by UV through a Novel Pathway.** Although the regulation of BRCA2 RNA level by UV has been reported, the regulation of BRCA2 protein by UV irradiation has not been addressed (12, 14). The ubiquitin-proteasome pathway is an important mechanism to modulate protein stability. To determine whether the proteosome pathway is involved in UV-mediated BRCA2 depletion, three proteasome inhibitors, MG132, MG115, and ALLN (15), were used to treat U-2 OS cells upon UV irradiation. Although treatment with these inhibitors resulted in a significantly greater level of p53, which is controlled by ubiquitin-mediated protein depletion (16), these three inhibitors did not inhibit BRCA2 depletion induced by UV (Fig. 3A).

We also investigated the potential involvement of other proteases in the UV-induced BRCA2 depletion. p53 is also known to be a target of calpain-mediated proteolysis (17). Treatment with the calpain inhibitor ALLN (18) stabilized p53 but did not inhibit BRCA2 depletion induced by UV. The involvement of CPP32/caspase-3-like protease in UV-induced BRCA2 down-regulation has also been excluded because treatment with z-VAD-fmk, a broad-spectrum caspase inhibitor (19), failed to affect the stability of BRCA2 after UV irradiation (Fig. 3B).

It has been shown that UV signaling can be mediated through the activity of tyrosine kinase receptors on the cell membrane (20, 21). The possibility of the involvement of EGFR in UV-mediated BRCA2 down-regulation was investigated using the human epidermoid carcinoma cell line A431. A431 cells overexpress EGFR (22) and harbor mutated endogenous p53 (23). Treatment of cells with the tyrosine kinase receptor inhibitor genistein inhibited EGFR activity, as indicated by the decrease of tyrosine phosphorylation of EGFR, but had no effect on the UV-mediated BRCA2 depletion (Fig. 3C). In addition, treatment with TNF-α, a cytokine that acts in a synergistic manner with UV in stress signaling (24), did not result in BRCA2 depletion (Fig. 3D). These results suggest that UV-mediated BRCA2 protein depletion occur through a novel mechanism.

**BRCA2 and UV Protection.** Because UV irradiation caused BRCA2 down-regulation, we tested whether forced expression of BRCA2 could protect cells from UV-mediated cell killing using a clonogenic assay and a luciferase reporter assay. 293 cells, whose endogenous BRCA2 decreased upon UV irradiation similar to other cell lines examined (Fig. 4B), were transfected with a plasmid expressing either wild-type or m1 mutant BRCA2 cDNA and then irradiated with UV. UV-treated cells were cultured for a few weeks, and surviving colonies were counted. There were about twice as many surviving colonies from cells transfected with the wild-type BRCA2-expressing plasmid than from cells transfected with the m1 BRCA2 mutant expressing plasmid (Fig. 4C).

A luciferase expression assay was also used to test the UV protection function of BRCA2. 293 cells were transfected with a luciferase reporter plasmid together with a plasmid expressing either the wild-type or the m1 mutant BRCA2 cDNA. Transfected cells were treated with UV and cultured for ~10 days. Luciferase activity expressed in these cells was measured and used as an indicator for cell survival. Consistent with the result of the clonogenic experiment, there were more surviving cells in cells transfected with wild-type BRCA2-expressing plasmid than in those transfected with m1 mutant BRCA2-expressing plasmid (Fig. 4D). These results showed that increased expression of wild-type BRCA2 protected cells from UV-induced cell killing.

---

[Fig. 2. BRCA2 protein depletion caused by UV irradiation requires protein synthesis. U-2 OS (A and C) and MDA-MB-231 (B and D) cells, treated or not treated with 50 μm of cycloheximide (CHX), were irradiated with UV (24 J/m2) and incubated for different periods of time as indicated (A and B, 9.5 h; C and D, 15 h). Proteins were detected by immunoblotting. The levels of BRCA2 were quantified using the NIH Image program and shown as columns. The BRCA2 level in cells without treating with UV was set as 1. E, UV irradiation causes BRCA2 protein degradation. MDA-MB-231 cells were pulse labeled with [35S]methionine (1 mCi/ml) for 2 h, irradiated with 42 J/m2 of UV, then incubated in the absence of [35S]methionine for different periods of time before being lysed. BRCA2 protein was immunoprecipitated and detected by autoradiography. The intensities of the BRCA2 protein signals were measured using Phosphorimage as shown by the columns. The BRCA2 level of the sample irradiated with UV and incubated for 4 h before lysis was set as 1. Lane 1, the pulse-labeled cells were lysed immediately after labeling; Lane 2, labeled cells were incubated for 4 h after labeling without UV irradiation; Lane 3, labeled cells were incubated for 2 h, irradiated, and further incubated for another 2 h; Lane 4, labeled cells were irradiated with UV right after labeling and incubated for 4 h before lysis; Lane 5, same as Lane 2 but used mouse plus rabbit IgG for immunoprecipitation. NS, non-specific.](image)

[Fig. 3. UV-induced BRCA2 protein depletion via a proteosome-independent, calpain-independent, and TNF-α-independent pathway. A, U-2 OS cells were first treated with proteosome inhibitor MG132 (5 μm), MG115 (25 μm), or calpain inhibitor ALLN (10 μm), or with DMSO as the solvent control for 30 min. Cells were then mock irradiated or irradiated with 24 J/m2 of UV. Cell lysates were prepared after incubation for 4 h in the presence of the inhibitors, and the indicated proteins were detected by immunoblotting. The retinoblastoma protein (RB) was used as an equal loading control. The accumulation of p53, which is subject to proteosome- and calpain-mediated depletion, in mock UV-irradiated cells demonstrated the effectiveness of inhibitor treatments. B, U-2 OS cells were treated with the calpain and caspase 3 inhibitor z-VAD (40 μm), and the level of the BRCA2 protein was determined by immunoblotting as in A. The level of PARP was lower after UV irradiation because of cleavage by caspase-3. PARP cleavage by caspase-3 was inhibited in the presence of z-VAD-fmk. C, A431 cells, which overexpress EGFR, were treated (+ gen) or not treated (− gen) with genistein (20 ng/ml) for 14 h in medium containing 5% serum. The cells were then irradiated with UV (24 J/m2) and incubated for 6 h before lysis. Cell lysates were then prepared, and proteins were detected by immunoblotting. EGFR-p was activated, therefore tyrosine phosphorylated, EGFR. D, the breast cancer cell line MDA-MB-231 was treated with TNF-α (10 ng/ml) for 5, 9, or 24 h, and the level of BRCA2 protein was determined by immunoblotting. Concomitant depletion of IκB by TNF-α as reported previously (27) was shown. NT, no treatment.](image)
Discussion

We showed in this report that BRCA2 protein level was reduced within 4 h after cells were irradiated with UV. The rapid depletion of BRCA2 suggests that the down-regulation of BRCA2 was not the result of UV-induced cell cycle arrest. This suggestion was also supported by our observation that the down-regulation of BRCA2 protein did not correlate with the cell cycle alteration after UV irradiation. UV irradiation has been shown to down-regulate the BRCA2 mRNA level (12, 14). The rapid reduction of BRCA2 protein level after UV irradiation indicates that the reduction of BRCA2 protein is not simply the consequence of the reduction of BRCA2 mRNA level.

It has been reported that the BRCA1 and BRCA2 mRNAs were reduced in cells treated with Adriamycin and UV in a p53-dependent manner (14). It also has been reported that BRCA1 expression was reduced in response to Adriamycin and mitomycin C in the presence of wild-type p53 (25). However, our results clearly show that both BRCA2 and BRCA1 protein levels were decreased by UV irradiation, regardless of the p53 status of cells investigated. Consistently, the COOH-terminal region of BRCA2, which binds to p53 and modulates its transcriptional function (11), was not required for the UV-mediated depletion of BRCA2. The COOH-terminus-truncated BRCA2 protein in CAPAN-1 cells was degraded by UV similar to wild-type BRCA2 in other cell lines.

The depletion of BRCA2 protein by UV irradiation appeared to require protein synthesis, because the protein synthesis inhibitor cycloheximide blocked this process. It is possible that UV irradiation induces the expression of a short-lived factor(s) that is critical for the degradation of BRCA2. Identification of such factor(s) would be a significant step in understanding BRCA2 regulation mechanism under genotoxic stresses. Extended incubation overcame the cycloheximide inhibition and resulted in further BRCA2 protein depletion in the absence of protein synthesis (Fig. 2C). This result indicates that protein degradation contributes to the UV-mediated BRCA2 depletion, which is also supported by the result of the pulse-chase experiment (Fig. 2E). It is worth mentioning that cycloheximide blocked BRCA1 protein depletion by UV irradiation in MDA-MB-231 but not in U-2 OS cells, indicating that UV may modulate BRCA2 and BRCA1 protein levels through different mechanisms in different cell types.

Our results strongly suggest that down-regulation of BRCA2 in UV-irradiated cells is mediated by a new mechanism. It does not rely on the function of the proteosome and calpain systems, which are the most common biological mechanisms for protein depletion. Inhibitors for proteosome (MG132, MG115, and ALLN) and caspase (z-VAD-fmk) did not prevent UV-induced BRCA2 down-regulation. Calpain inhibitors ALLN (15) and z-VAD-fmk (19) also did not inhibit this process. Because calpain is necessary for the degradation of IκB for the induction of NF-κB in UV-irradiated cells (26), our result suggests that NF-κB does not participate in the UV-induced BRCA2 down-regulation. Activation of the signaling pathway of TNF-α receptor, which has been shown to contribute to UV-induced apoptosis (24), did not result in significant BRCA2 down-regulation, suggesting that the TNF-α pathway is not involved in this process. Similarly, the UV-mediated down-regulation of BRCA2 does not depend on signaling through EGFR, which has been shown as a target of UV signaling (21). Additional studies are necessary to further understand in detail the mechanism(s) for UV-mediated BRCA2 and BRCA1 down-regulation.

A role of BRCA2 in the repair of UV-induced DNA damage has been hypothesized. Our results that ectopic expression of wild-type BRCA2 protected cells from UV-induced cell killing support this hypothesis. It is therefore surprising that the BRCA2 level decreased in cells irradiated with UV. However, the BRCA2 level was reduced not completely diminished. The amount of remaining BRCA2 may still provide protection to cells. This is consistent with the fact that homozygous Brca2 mutant cells, which do not have any functional Brca2, are more sensitive to UV than the wild-type or heterozygous Brca2 mutant cells (9). One possible consequence of the reduced BRCA2 level in UV-irradiated cells could be to render cells that sustain extensive DNA damage to cell death rather than to be transformed because of their carrying mutated DNA. Further investigations will be necessary to better understand the role of BRCA2 in UV-induced stress responses.

References


Ultraviolet Irradiation Induces BRCA2 Protein Depletion through a p53-independent and Protein Synthesis-dependent Pathway

Shao-Chun Wang, Keishi Makino, Li-Kuo Su, et al.

Cancer Res 2001;61:2838-2842.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/7/2838

Cited articles
This article cites 27 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/7/2838.full#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/7/2838.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, contact the AACR Publications Department at permissions@aacr.org.