Expression of Dominant Negative c-jun Inhibits Ultraviolet B-Induced Squamous Cell Carcinoma Number and Size in an SKH-1 Hairless Mouse Model

Simon J. Cooper, Jacalyn MacGowan, James Ranger-Moore, Matthew R. Young, Nancy H. Colburn, and G. Tim Bowden

1Department of Radiation Oncology, Arizona Cancer Center and 2Department of Epidemiology and Biostatistics, College of Public Health, The University of Arizona, Tucson, AZ; and 3Basic Research Laboratory, National Cancer Institute, Frederick, MD

Abstract

UVB radiation is a complete carcinogen able to initiate, promote, and progress keratinocyte cells toward carcinogenesis. Exposure to UVB leads to the propagation of a number of signal transduction pathways resulting in increased DNA binding of transcription factors, including activator protein-1 (AP-1), and subsequent gene expression. To test the hypothesis that AP-1 activation plays a role in the promotion of UVB-induced skin tumors, a dominant negative c-jun (TAM67) mutant transgene was expressed in the epidermis of SKH-1 hairless mice and bred with mice expressing an AP-1 luciferase reporter gene. Single UVB exposure experiments showed a significant decrease in AP-1 activity, as measured by luciferase levels, in mice expressing TAM67 72 h postexposure. Transgenic and nontransgenic littersmates were placed into a chronic UVB exposure experiment, three exposures per week for 25 weeks. Expression of TAM67 reduced the number of tumors per mouse by 58% and tumor sizes were 79% smaller than the tumors present in the nontransgenic study group. These tumors were histologically identified as squamous cell carcinomas. TAM67 had no effect on UVB-induced hyperplasia because comparable epidermal thickening was observed in both study groups over a 5-day period post-UVB exposure. Immunohistochemical analysis showed a reduction in the number of cyclin D1-expressing cells in squamous cell carcinoma samples removed from the TAM67 study group. These data show that TAM67 can inhibit UVB-induced squamous cell carcinoma formation, suggesting that AP-1 is a good candidate target for the development of new chemoprevention strategies to prevent sunlight-induced skin cancers.

Introduction

Epidemiological studies have indicated that the application of sunscreens and sun blocks are not totally effective in preventing solar radiation-induced skin carcinogenesis; therefore, new-targeted approaches are needed for chemoprevention of skin cancer. Prevention as a means to lower incidence is a major research priority especially because each year new cases of skin cancer account for 50% of all new cancers reported. Although three regions of UV light (UVA, UVB, and UVC) are contained in solar radiation, only UVA and UVB reach the earth’s surface with UVC being filtered out by the ozone layer (1). Both UVB and to a lesser extent UVA are responsible for sunlight-induced cancers of the skin (2, 3). UVB has been demonstrated to be a complete carcinogen able to induce initiation, promotion, and progression of exposed epidermal cells although molecular mechanisms are still unclear as to how normal exposed cells are progressed toward a malignant tumor.

Exposure of mammalian cells to UVB radiation results in the activation of a number of transcription factor protein families including activator protein-1 (AP-1) and nuclear factor κB (NF-κB; 4, 5) leading to the activation of a number of genes, termed UV response genes. The AP-1 family of proteins has been shown to be involved in cell proliferation and survival and, therefore, plays an important role in tumor progression (6). There is continuing evidence that the AP-1 family of proteins is able to bring about these cell proliferation and survival effects by regulating the expression and function of a number of cell cycle regulators including Cyclin D1, p53, p21 (cip1/waf1), p19 (ARF), and p16 (6). Knockout strategies have also demonstrated that AP-1 protein family members such as c-Jun, JunB, and Fra-1 are essential for mouse embryonic development (7) and that the absence of c-Jun leads to elevated levels of both p53 and its target gene, p21, in fibroblasts (8).

Experiments in JB6 cells first provided evidence for the role of AP-1 in skin tumor promotion and progression (9). These experiments showed that cells sensitive to tumor promotion had an elevated AP-1 activity in response to tumor-promoting agents when compared to promotion-resistant cells. Later work identified constitutively active AP-1 in malignant versus benign tumor cells as measured by sequence-specific DNA binding and transactivation studies (10), suggesting that constitutive activation of AP-1 may lead to a sustained deregulation of gene expression within these malignant cells. A role for AP-1 in the
transformation of mammalian cells has further been brought to light by the use of dominant negative protein studies. The expression of a deletion mutant of the c-jun gene (TAM67) that is missing a portion of its transactivation domain is able to inhibit the transformation of normal rat embryo cells (11). The expression of this deletion mutant of the c-jun gene also inhibited transformation of rat embryo cells after exposure to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA; Ref. 11).

Recent investigations have started to dissect the in vivo contributions of AP-1 activation to tumorigenesis. Studies utilizing malignant mouse epidermal cells stably transfected with TAM67 showed an inhibition in both the AP-1 transactivation response and in the cells’ ability to form s.c. tumors when injected into athymic nude mice (12). Young et al. (13) expressed the TAM67 mutant in the epidermis of transgenic mice, and after DMBA/TPA exposure, demonstrated for the first time that AP-1 transactivation was a required step for in vivo TPA-induced tumor promotion. Recent studies from this laboratory have identified that expression of the TAM67 mutant in the epidermis of ICR mice blocks okadaic acid (OA)-induced skin tumor promotion (14). In the present study, we demonstrate that the expression of TAM67 on an SKH-1 hairless mouse background decreases both skin tumor number and size after chronic exposure to UVB radiation. The inhibition of UVB-induced skin carcinogenesis was associated with an inhibition in the skin of UVB-induced AP-1 activation.

**Results**

Expression of the K14-TAM67 transgene in the skin of the transgenic mouse was determined by RNA analysis using a two-tube reverse transcription (RT)-PCR technique (Fig. 1). The primers used were specific for TAM67 and an exon of the human growth hormone gene located downstream of the TAM67 sequence. As a control for DNA contamination, reactions for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were included in these experiments. It was determined that TAM67 was actively transcribed in the epidermis of transgenic mice but not in their nontransgenic littermates.

Once it had been determined that the transgene was indeed being expressed in the epidermis of the transgenic mice, the effects of this expression were examined. K14-TAM67 mice on the SKH-1 hairless background were crossed with SKH-1 mice expressing AP-1 luciferase (Luc). Mice expressing both of these transgenes or only the AP-1 Luc transgene were used in an acute UVB exposure experiment. Pre- and 72 h post-UVB exposure samples (10 kJ/m²) were analyzed for Luc activity (Fig. 2). Expression of the TAM67 transgene was observed to have little effect on the basal AP-1 activity compared to non-TAM-expressing littermates. After UVB exposure, Luc activity in the wild-type mice was induced by approximately 38-fold above pre-UVB samples. Expression of TAM67 significantly reduced this UVB-induced AP-1 activation compared to wild-type mice ($P = 0.045$).

To extend these observations, TAM67-expressing transgenic mice and their negative littermates were placed into a chronic UVB exposure study to determine the effects of the transgene on UVB-induced skin carcinogenesis. The starting UVB dose (2.5 kJ/m²) was increased every week until the maximum dose of 9 kJ/m² was achieved at the 6th week. The appearance, the number, and the size of tumors were recorded throughout the 25-week study. The expression of TAM67 inhibited the appearance of UVB-induced tumors when compared to nontransgenic littermates. Tumors were first observed on nontransgenic littermates at week 10 compared to week 15 in the transgenic mice and all nontransgenic mice demonstrated at least one skin tumor at week 20 compared to week 24 for the transgene-expressing group (data not shown). The average number of tumors per mouse was also decreased in the TAM67 study group when compared to nontransgenic littermates. At the end of the study, TAM67 mice averaged 58% fewer tumors ($P = 0.007$), and the average volumes of these tumors were...
79% smaller ($P = 0.006$) than those of the nontransgenic littermates (Fig. 3). All tumors collected from these experiments were identified histologically as squamous cell carcinoma with no apparent differences in degree of differentiation. Utilization of the TUNEL assay and immunohistochemistry techniques showed no differences in the number of proliferating cells (measured by PCNA) or apoptotic cells when comparing the nontransgenic and TAM67 UVB-induced tumors (data not shown). Therefore, expression of the TAM67 transgene in the epidermis of the SKH-1 mice decreases chronically induced UVB skin cancer number and size.

Other end points were examined to determine the action of TAM67 in tumor growth inhibition. Previous reports have identified that hyperplasia is induced at early time points after exposure of the skin to UVB in SKH-1 hairless mice (15). Mice were exposed to a single dose of UVB (10 kJ/m$^2$) and dorsal skin samples removed at 0–120 h postexposure to determine if TAM67 was able to affect UVB-induced hyperplasia. The expression of the transgene (Fig. 4) had no effect on the onset or degree of hyperplasia in response to UVB exposure of the epidermis when compared to nontransgenic littermates.

A number of recent studies have suggested a role for cyclin D$_1$ in murine skin tumor development (16–18). To determine whether cyclin D$_1$ expression may have played a role in the differences seen in tumor development between transgenic and nontransgenic littermates in the long-term UVB exposure experiments, tumors from these samples were stained for cyclin D$_1$. Expression of TAM67 in the epidermis of SKH-1 mice showed no effect on basal levels of cyclin D$_1$ in untreated skin when compared to nontransgenic littermates (Fig. 5). Expression of TAM67 in the epidermis of SKH-1 mice showed a significant reduction in UVB-induced levels of cyclin D$_1$ in those squamous cell carcinomas arising from the TAM67-expressing epidermis ($P = 0.029$), suggesting that cyclin D$_1$ plays an active role in tumor growth and may be regulated by AP-1.

**Discussion**

AP-1 has been implicated in tumor promotion using chemical and viral agents, but not physical agents; therefore, we sought to determine the role of AP-1 in UVB-induced skin carcinogenesis. The present study demonstrates the ability of TAM67 expressed in the epidermis of SKH-1 mice to inhibit UVB-induced AP-1 activation and to delay and reduce UVB-induced squamous cell carcinoma incidence. This is important, because UVB is the major causative agent of human skin squamous cell carcinoma. This current study established for the first time that skin carcinoma induction and growth is AP-1 dependent. Thus, AP-1-dependent gene regulation contributes not only to early but also to late events in UVB-induced skin carcinogenesis.

**FIGURE 3.** TAM67 expression reduces UVB-induced skin tumor size and number. TAM67$^+$ and TAM67$^-$ mice were exposed to a 25-week course of UVB starting at a dose of 2.5 kJ/m$^2$ with a dosage increase of 1.5 kJ/m$^2$ every week until the maximum dosage of 9 kJ/m$^2$ was achieved at the 6th week. Tumor appearance, size, and number per mouse were measured weekly throughout the experiment and recorded. Significant differences between TAM67$^+$ and TAM67$^-$ groups were apparent in tumor number (A) at week 25, $P = 0.007$, and when comparing the slopes of the curves, $P < 0.001$, and in tumor volume (B) at week 25, $P = 0.006$ and differences between the slopes, $P = 0.012$.
The ability of TAM67 to inhibit AP-1 transactivation is due to its ability to sequester Jun and Fos family members into low-activity transcription factor complexes (11). The transactivation of AP-1-dependent genes has been reported to be necessary for tumor promotion by TPA (13) and OA (14) in vivo, and for tumor promoter-induced transformation in mouse epidermal JB6 cells (19). In addition, expression of TAM67 in mouse keratinocytes is able to block TPA-induced invasion (20), and in malignant epidermal cell lines inhibits the formation of tumors when these cells are injected into athymic nude mice (12).

Early responses in the skin of SKH-1 mice after an acute UVB exposure include increased number of cells expressing both p21 and p53 and thickening of the epidermis (15). Interestingly, the expression of TAM67 had no effect on UVB-induced epithelial hyperplasia agreeing with similar findings observed in chemically induced carcinogenesis models using both OA and TPA (13, 14), as well as in Human Papillomavirus Type-16 (HPV) E7-induced hyperplasia (21). Chronic UVB exposure of SKH-1 mice results in modulation of the expression of cell cycle markers, with p53 and cyclin D1 overexpression correlating with the development of skin tumors (17). Therefore, this expression of cyclin D1 in squamous cell carcinomas contributes to the tumor phenotype even in the presence of elevated p53 levels. Elevated cyclin D1 expression is seen in squamous carcinoma and papillomas but not in normal or hyperproliferative skin (22) while decreased expression leads to reduced skin carcinogenesis (16). Squamous cell carcinomas removed from chronically exposed UVB SKH-1 mice in our investigation showed increased in cyclin D1 protein levels when compared to normal dorsal skin sections at the end of the 25-week experiment. Expression of TAM67 significantly reduced cyclin D1 protein expression in UVB-induced squamous cell carcinoma samples but did not modulate basal levels of cyclin D1 in the untreated transgenic mouse skin.

Kim et al. (17) reported that the magnitude of cyclin D1 overexpression correlates well with skin tumor progression, and the onset of cyclin D1 accumulation coincides with the sudden increase in the number of tumors per animal. Considering the persistent expression of cyclin D1 during UVB-induced murine skin carcinogenesis, Kim et al. suggested that cyclin D1 might contribute to the neoplastic process by providing a growth advantage during the early stages of tumor promotion. Finally, these authors concluded that the high levels of cyclin D1 in individual tumors suggest an important role during malignant progression. Because UVB-mediated responses observed in murine skin parallel those reported in human skin cancer development, it is likely that alterations in cyclin D1 expression are important in human skin carcinogenesis caused by chronic sun exposure.

Experiments in fibroblasts have identified that c-Jun mediates G1 cell cycle progression by a mechanism that involves direct transcriptional control of the cyclin D1 gene (23). Our observations suggest a direct mechanism for AP-1 in regulating cyclin D1 expression because lower levels of cyclin D1 protein expression are observed in the squamous cell carcinomas of the TAM67-expressing mice. Further investigation of this mechanism by Hennigan and Stambrook (24) established that a GFP-TAM67 fusion was able to arrest human fibrosarcoma cells in culture predominantly in the G1 phase of the cell cycle, not by decreasing the expression of cyclin D1 but by inhibiting the activation of cyclin D1 and cyclin E kinase complexes. Unlike TAM67, however, the GFP-TAM67 failed to interact with other leucine zipper proteins. Recent findings in NIN3T3 mouse fibroblasts demonstrate that expression of a dominant negative c-Jun is able to inhibit the activation of the cyclin D1 promoter and that AP-1 activity is essential for the activation of the cyclin D1 promoter by protein kinase C-pha; and -e (25). Together these results suggest that transcriptional activation of cyclin D1 may not be the only role AP-1 plays in regulating the cell cycle. In Fig. 5, we show a decrease in the expression of cyclin D1 in UVB-induced squamous cell carcinoma samples from TAM67 SKH-1 mice when compared to nontransgenic littermates, suggesting that transcriptional activation of cyclin D1 by AP-1 is the active mechanism in our studies.

![FIGURE 4. Expression of TAM67 has no effect on UVB-induced hyperplasia. Dorsal skin samples from TAM67 and TAM67 /C0 mice were isolated 0–120 h after exposure to 10 kJ/m² UVB. Samples were fixed in buffered formalin, embedded in paraffin, and stained with H&E. Distances from the top of the basement membrane to the bottom of the stratum corneum were measured, three measurements per field, 10 fields per sample.](mcr.aacrjournals.org)
The cyclin D1 promoter also contains NF-κB sites (26), suggesting that cyclin D1 may also be a target of NF-κB as well as AP-1-dependent regulation. The proteins of the NF-κB transcription factor family are known to regulate the transcription of many genes involved in cell cycle, cell proliferation, and apoptosis (27). It has also been reported that p65, the most transcriptionally active member of the NF-κB family, is able to interact with Fos/Jun members of the AP-1 family (28). More recent investigations have identified transactivation of NF-κB and AP-1 after exposure of TPA or TNF-α to JB6 cells (29, 30). Inhibition of these transactivation events in the presence of the antioxidant pyrrolidine dithiocarbamate or by expressing a nondegradable transactivation of NF-κB and AP-1 after exposure of TPA or TNF-α to JB6 cells (29, 30). Inhibition of these transactivation events in the presence of the antioxidant pyrrolidine dithiocarbamate or by expressing a nondegradable mutant of hBp suggests that transformation via TPA or TNF-α is dependent on both of these transcription factor families.

More recent investigations have suggested that TAM67 physically interacts with p65 in the nucleus of human keratinocytes and that the expression of TAM67 is able to inhibit the expression of NF-κB and AP-1 target genes (31). Together, these investigations suggest that inhibition of UVB-induced tumor growth and burden in the SKH-1 mouse model via the expression of TAM67 may also be due in part to an inhibition of NF-κB transactivation.

In conclusion, these observations from a SKH-1 hairless mouse system show that signaling pathways that are blocked by the expression of TAM67 play an important role in UVB-induced skin carcinogenesis. Previous studies examining tumor promotion by chemical agents or viral oncogenes have shown similar results as seen herein. Together, these data suggest that TAM67 blocks specific signaling pathways that are involved in both chemically and physically induced skin carcinogenesis. Further identification of the TAM67 target pathways is needed so that the search for chemopreventive agents may be directed toward compounds that inhibit these tumor-promoting events.

Materials and Methods

Breeding of Transgenic Mouse Lines

K14-TAM67 (13) and AP-1 Luc (32) mice were obtained from Dr. Colburn on a DBA/2 genetic background. These transgenes were bred onto the SKH-1 genetic background by crosses with SKH-1 mice obtained from the Charles Rivers Laboratories (Kingston, NY). Mice were used in experiments after generation N5. Heterozygous offspring carrying either the K14-TAM67 transgene, the AP-1 Luc transgene or both were identified by PCR analysis of tail DNA using the following primers: hK14 primers (GenBank accession no. U11076) bp 1693–1717 and 2205–2181, with H-Ras primers 7816 cacccccactaagctttgtgagcgag and 7817 gctagcatagggctcaccgactgtgatga, or Luc primers ggaattcttcgaaatgtcc and cctaggttaacccagtagatcc and cctaggttaacccagtagatcc (13).

RNA Isolation

Tissues for RNA expression were harvested from animals after CO2 asphyxiation. For RT-PCR, tissue sections were harvested from the back of the mouse and snap frozen in liquid nitrogen. The epidermal layer was separated from the whole skin sample and pulverized in liquid nitrogen with a mortar and pestle. The powdered epidermis was immediately placed in extraction buffer, homogenized, and the RNA extracted using the Totally RNA kit (Ambion, Austin, TX). The purified RNA was then DNase treated before undergoing RT-PCR analyses.

RT-PCR

Qiagen’s protocol for Omniscript reverse transcriptase was used for performing two-tube RT-PCR. A total of 1 μg RNA per sample was primed using 200 ng of antisense primers for mouse GAPDH (5’-ggcccctctgattattgg-3’) and for the human growth hormone downstream of the TAM67 transgene (5’-tggataagggaattgggg-3’). The resulting cDNA products underwent 40 cycles of PCR (94°C, 1 min, 55°C, 1 min, 72°C, 1 min) after an initial 72°C hot start before a 3-min denaturing step at 94°C. Sense primers used in these reactions were for GAPDH (5’-aagttctgcaatgcac-3’) and for TAM67 (5’-aaccatcttcagggaa_cagg-3’). Products were analyzed on a 1.5% agarose gel.

Assays of AP-1 Luc Activity

AP-1 Luc activity was measured in AP-1 Luc+/K14-TAM67+ mice and TAM67+ control mice and TAM67+ control mice and TAM67+ control mice. Skin tumors isolated from TAM67+ (n = 3) and TAM67+ (n = 4) mice were formalin fixed, paraffin embedded, and then analyzed using immunohistochemistry for the expression of cyclin D1. Ten random fields per slide were counted with cells containing a brown precipitate recorded as positive. Positively stained cells per tumor were averaged across all replicate fields to obtain a single reliable value. Statistically significant differences between UVB-exposed TAM67+ (control) mice and TAM67+ (control) mice and TAM67+ (control) mice.
mice and in their AP-1 Luc+K14-TAM67+ siblings. Mice were anesthetized with Aventin at a dosage of 0.017 ml/g of body weight before three 1.5-mm skin punches were taken from the right ear. These samples were placed in liquid nitrogen before being stored at −80°C. The mice while anesthetized were treated with a UVB dose of 10 kJ/m². UVB exposure times were calculated using a UVX radiometer (Ultra-violet Products, San Gabrial, CA). Mice were euthanized 72 h post-UVB exposure, and 3 × 1.5 mm punches were taken from the left ears and treated and stored as before. A bichinonic acid (BCA) protein assay (Pierce, Rockford, IL) was performed on the car punches and the Luc activity of the samples determined using 20 μg of protein.

Skin Carcinogenesis

Eight-week-old K14-TAM67+ mice and their negative littermates were exposed to UVB three times a week. The dose of radiation received was increased slowly over the first 6 weeks. Exposure was started at a dose of 2.5 kJ/m² with a dosage increase of 1.5 kJ/m² every week until the maximum dosage of 9 kJ/m² was achieved at the 6th week. The presence of UVB-induced tumors and the size of these tumors were measured weekly throughout the experiment and recorded. Treatments of 9 kJ/m² continued until the end of the study at 25 weeks when mice were euthanized, tumor samples collected, and prepared for analysis.

UVB Induction of Hyperproliferation and AP-1 Activation

Four K14-TAM67+ mice or four negative littermates per group were treated with 10 kJ/m² UVB under Aventin anesthesia. Dorsal skin samples were collected over a time period of 0–120 h posttreatment. Five-micrometer sections were stained with H&E and UVB-induced hyperplasia was determined from these H&E-stained slides. The distance from the top of the basement membrane to the bottom of the stratum corneum was measured (three measurements per field, 10 fields per sample). These distances were measured using the Pro image analysis program (Media Cybernetics, Silver Springs, MD).

Measurement of Cyclin D1 by Immunohistochemistry

Monoclonal mouse anti-cyclin D1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Paraffin sections of tumors from the 25-week UVB exposure experiment were baked at 60°C for 1 h before being deparaffinized and placed in PBS. Slides were placed in Dako Target Retrieval Solution for 10 min, 98°C, and after cooling, immersed in 3% H₂O₂ for 5 min. Slides were processed with the “Mouse on mouse” detection kit (Vector Laboratories, Inc., Burlingame, CA) as directed by the company. The sections were incubated with anti-cyclin D1 for 1 h (1:20 dilution) at room temperature. Samples were then incubated with a biotinylated anti-mouse antibody for 10 min at room temperature followed by incubation with VECTASTAIN ABC reagent for 5 min. Color development was achieved by incubation with liquid DAB (DAKO Corporation, Carpenteria, CA) for 10 min at room temperature. Slides were counterstained with hematoxylin and dehydrated before addition of coverslips. A positive reaction was observed as a brown precipitate and the percentage of cyclin D1-expressing cells calculated from 10 random fields of view per slide.

Statistical Analysis

All values are expressed as mean ± SE. Statistical analysis was performed using the statistical analysis program Stata 7.0 (Statacorp 2001). For inhibition of UVB-induced AP-1-driven Luc activity in TAM67+ mice studies, three experiments were pooled for the statistical analysis. An analysis of covariance that explicitly modeled an experimental effect yielded essentially identical results (data not shown), confirming the stability of pooling the data. Comparisons were made between TAM67+ mice and their negative littermates at 25 weeks for tumor multiplicity and volume. For multiplicity, an unpaired t test was conducted on the raw data. For tumor volume at week 25, the distribution of the data was strongly right skewed, so an unpaired t test was conducted on logarithmically transformed data. Additionally, post hoc analysis of the slopes over time for tumor multiplicity and volume were conducted using linear regression, conditioned on the time points at which graphical analysis showed trajectories departing from baseline levels. A square root transformation provided the best normalization and linear slope for tumor volume over the time period. For statistical analysis of cyclin D1 levels in UVB-induced squamous cell carcinomas, the percentage of cyclin D1 positively stained cells per tumor were averaged across all replicate fields to obtain a single reliable value. Comparisons between TAM67+ mice and their negative littermates were conducted at week 25 using an unpaired t test. Statistical significance of difference was assessed using an unpaired t test. In all studies, results were identified as statistically significant if P < 0.05.

Acknowledgments

We thank Nancy K. Hart and the other members of the Dr. David S. Albert’s laboratory for help with the immunohistochemical and hyperplasia studies.

References

8. Schreiber, M., Kolbus, A., Piu, F., Szabowski, A., Mohle-Steinlein, U., Tian, ...
TAM67 Inhibits UVB-Induced Skin Carcinogenesis


Expression of Dominant Negative c-jun Inhibits Ultraviolet B-Induced Squamous Cell Carcinoma Number and Size in an SKH-1 Hairless Mouse Model

1National Institutes of Health Grants CA27502 and the Cancer Research Foundation of America Fellowship Grant.

Simon J. Cooper, Jacalyn MacGowan, James Ranger-Moore, et al.


Updated version
Access the most recent version of this article at:
http://mcr.aacrjournals.org/content/1/11/848

Cited articles
This article cites 29 articles, 16 of which you can access for free at:
http://mcr.aacrjournals.org/content/1/11/848.full.html#ref-list-1

Citing articles
This article has been cited by 27 HighWire-hosted articles. Access the articles at:
http://mcr.aacrjournals.org/content/1/11/848.full.html#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.