Kaempferol Targets RSK2 and MSK1 to Suppress UV Radiation-Induced Skin Cancer

Ke Yao1,2, Hanyong Chen1, Kangdong Liu1,2, Alyssa Langfald1, Ge Yang1,2, Yi Zhang1,2, Dong Hoon Yu1, Myoung Ok Kim1, Mee-Hyun Lee1, Haitao Li1, Ki Beom Bae1, Hong-Gyum Kim1, Wei-Ya Ma1, Ann M. Bode1, Ziming Dong2, and Zigang Dong1

Abstract

Solar UV (SUV) irradiation is a major factor in skin carcinogenesis, the most common form of cancer in the United States. The MAPK cascades are activated by SUV irradiation. The 90 kDa ribosomal S6 kinase (RSK) and mitogen and stress-activated protein kinase (MSK) proteins constitute a family of protein kinases that mediate signal transduction downstream of the MAPK cascades. In this study, phosphorylation of RSK and MSK1 was upregulated in human squamous cell carcinoma (SCC) and SUV-treated mouse skin. Kaempferol, a natural flavonol, found in tea, broccoli, grapes, apples, and other plant sources, is known to have anticancer activity, but its mechanisms and direct target(s) in cancer chemoprevention are unclear. Kinase array results revealed that kaempferol inhibited RSK2 and MSK1. Pull-down assay results, ATP competition, and in vitro kinase assay data revealed that kaempferol interacts with RSK2 and MSK1 at the ATP-binding pocket and inhibits their respective kinase activities. Mechanistic investigations showed that kaempferol suppresses RSK2 and MSK1 kinase activities to attenuate SUV-induced phosphorylation of cAMP-responsive element binding protein (CREB) and histone H3 in mouse skin cells. Kaempferol was a potent inhibitor of SUV-induced mouse skin carcinogenesis. Further analysis showed that skin from the kaempferol-treated group exhibited a substantial reduction in SUV-induced phosphorylation of CREB, c-Fos, and histone H3. Overall, our results identify kaempferol as a safe and novel chemopreventive agent against SUV-induced skin carcinogenesis that acts by targeting RSK2 and MSK1.

Cancer Prev Res; 7(9) 958–67. ©2014 AACR.

Introduction

Over the past three decades, more people have developed skin cancer than all other cancers combined (1). One in five Americans will develop skin cancer in their lifetime (2). Skin cancers are typically diagnosed as basal cell carcinomas (BCC) or squamous cell carcinomas (SCC; ref. 1). Epidemiologic evidence suggests that solar ultraviolet (SUV) irradiation is the most important risk factor for any type of skin cancer (3, 4). SUV comprises approximately 95% UVA and 5% UVB. Both UVA and UVB can cause DNA damage, which is considered a primary etiologic factor contributing to the development of skin cancer. Activation of UV-induced cellular signaling pathways plays a vital role in UV-induced skin tumorigenesis. MAPKs are serine-threonine kinases that control fundamental cellular processes such as growth, proliferation, differentiation, migration, and apoptosis. The mammalian MAPK family consists of the ERKs, JNKs, and p38 (5). Among the MAPK family, the ERKs cascade has been a focus of cancer chemoprevention because of its importance in carcinogenesis (6, 7). Abnormalities in the ERKs pathway play a critical role in the development and progression of cancer and its deregulation has been reported in approximately one-third of all human cancers (8). The p38-related signal transduction pathway is also markedly affected by SUV exposure (9). The 90 kDa ribosomal S6 kinase (RSK) and mitogen and stress-activated protein kinase (MSK) proteins constitute a family of protein kinases that mediate signal transduction downstream of the MAPK cascades. RSK is activated by ERKs in response to growth factors, polypeptide hormones, neurotransmitters, chemokines, and other stimuli (10). MSK is also activated by ERKs in response to such stimuli, but in addition, MSK can be activated by p38 in response to various cellular stress stimuli and proinflammatory cytokines (11). RSK and...
MSK1 are located between ERKs or p38 and their target transcription factors. We previously reported that RSK2 plays a key role in neoplastic transformation of human skin cells and in skin cancer growth (12, 13). Mice lacking MSK1 show reduced skin tumor development in a two-stage chemical carcinogenesis model (14, 15). Therefore, targeting SUV-induced RSK2 and MSK1 might be an effective strategy for preventing skin tumorigenesis caused by SUV.

Flavonoids are ubiquitously found in fruits and vegetables as well as popular beverages, and reportedly exhibit antioxidant, antitumor, and anti-inflammatory effects (16–18). In particular, their antitumor activity has attracted much attention as a possible dietary prevention strategy against carcinogenesis. Kaempferol, a natural flavonol, isolated from tea, broccoli, grapes, apples, and other plant sources, is believed to have anticancer activity, but its molecular mechanisms and direct target(s) in cancer chemoprevention are still unclear. Herein, we report that kaempferol suppresses SUV-induced activation of signal transduction by directly inhibiting RSK2 and MSK1 in mouse skin cells. Moreover, kaempferol strongly suppresses tumor incidence in a SUV-induced skin carcinogenesis mouse model. Thus, kaempferol acts as an inhibitor of RSK2 and MSK1 and is expected to have beneficial effects in the prevention of SUV-induced skin carcinogenesis.

Materials and Methods

Chemicals

Eagle’s minimum essential medium and basal medium Eagle (BME) were purchased from Invitrogen. FBS was purchased from Gemini Bio-products. The antibodies against phosphorylated MEK, ERKs, JNKs, p38, RSK, MSK1, cAMP-responsive element binding protein (CREB), histone H3, and total MEK, ERKs, JNKs, p38, CREB, and histone H3 were purchased from Cell Signaling Biotechnology. The Ki-67 antibody was purchased from Abcam. Kaempferol and the antibody against β-actin were purchased from Sigma-Aldrich. The Protein Assay Kit was from Bio-Rad, and the CellTiter 96 AQueous One Solution Cell Proliferation Assay Kit and the luciferase assay substrate Bio-Rad, and the CellTiter 96 AQueous One Solution (20 L; Promega) was added and cells incubated for 1 hour in a 37° C, 5% CO2 incubator. Absorbance was read at 492 and 690 nm.

Pull-down assays

Kaempferol (2.5 mg) was coupled to CNBr-activated Sepharose 4B (GE Healthcare Biosciences) matrix-beads (0.5 g) in 0.5 mol/L NaCl and 40% DMSO (pH 8.3) overnight at 4°C, according to the manufacturer’s instructions. Active RSK2 and MSK1 or JB6 P+ cell lysates (500 µg) were mixed with kaempferol-conjugated Sepharose 4B beads or with Sepharose 4B beads alone as control (30 µL, 50% suspension). Binding was examined by Western blot analysis.

Molecular modeling

The computer modeling of kaempferol with RSK2 and MSK1 was performed using the Schrödinger Suite 2013 software programs (19). RSK2 and MSK1 crystal structures were prepared under the standard procedures of the Protein Preparation Wizard (Schrödinger Suite 2013). Hydrogen atoms were added consistent with a pH of 7 and all water molecules were removed. The ATP-binding site-based receptor grid was generated for docking. Kaempferol was
prepared for docking by default parameters using the LigPrep program (Schrodinger). Then, the docking of kaempferol and proteins was accomplished with default parameters under the extra precision (XP) mode using the program Glide. Herein, we could get the best-docked representative structures.

Keratinocyte isolation

Dorsal skin from SKH-1 mice (6–8 weeks old) was harvested and digested with 2.5% trypsin without EDTA for 1.5 hour at 32°C. The epidermis was scraped off into 10% FBS-SMEM (Gibco) and stirred at 100 rpm for 20 minutes at room temperature. The solution was filtered through 70 µm Teflon mesh and keratinocytes were centrifuged at 160 × g for 7 minutes at 7°C. Cells were plated at a density of 1 × 10^6 cells per 100-mm dish (20).

Reporter gene assay

Confluent monolayers of JB6 P+ cells stably transfected with an AP-1 or NF-eB luciferase reporter plasmid were trypsinized, and viable cells (4 × 10^6) suspended in 1 mL of 5% FBS-MEM were added to each well of a 24-well plate. Plates were incubated overnight at 37°C in a humidified atmosphere of 3% CO2. Cells were incubated in serum-free medium for another 24 hours and then treated for 2 hours with kaempferol (0–50 µmol/L). Cells were then exposed to SUV (60 kJ/m²) and harvested after 3 hours. The cells were finally disrupted with 100 µL of lysis buffer (0.1 mol/L potassium phosphate, pH 7.8, 1% Triton X-100, 1 mmol/L dithiothreitol, and 2 mmol/L EDTA) and luciferase activity was measured using a luminometer (Luminoskan Ascent, Thermo Electro).

Mouse skin tumorigenesis study

Female SKH-1 hairless mice were purchased from Charles River and maintained under "specific pathogen-free" conditions according to guidelines established by Research Animal Resources, University of Minnesota (Austin, MN). The skin carcinogenesis experiments were conducted using mice of 6 to 8 weeks of age with a mean body weight of 25 g. Skin carcinogenesis in mice was induced using a SUV irradiation system. The SUV irradiation source (Q-Lab Corporation) emitted at wavelengths of 295 to 365 nm and the peak emission was 340 nm. SKH-1 mice were divided into five groups of 12 or 24 animals each. In the control group, the dorsal skin was topically treated with 150 µL of acetone or 1.0 mg kaempferol in 150 µL of acetone only. In the SUV-treated group, the dorsal skin was topically treated with 150 µL of acetone 1 hour before exposure to SUV. The mice in groups 3 and 4 received topical application of kaempferol (0.5 or 1 mg, respectively) in 150 µL of acetone 1 hour before SUV irradiation. At week 1, the SUV dose was 30 kJ/m² UVA and 1.8 kJ/m² UVB, three times per week. The dose of SUV was progressively increased (10% each week). At week 6, the dose was 48 kJ/m² UVA and 2.9 kJ/m² UVB and this dose was maintained for weeks 6 to 12. Tumor number and volume were recorded every week until the end of the experiment. One-half of the samples were immediately fixed in 10% formalin and processed for hematoxylin and eosin (H&E) staining and HIC. The other samples were frozen and used for Western blot analysis.

Statistical analysis

All quantitative data are expressed as mean values ± SD or ± SE and significant differences were determined by the Student t test or one-way ANOVA. A P value of <0.05 was used as the criterion for statistical significance.

Results

Phosphorylated RSK and MSK1 are highly expressed in SUV-exposed mouse skin and human SCCs

SUV light can be very harmful to human health, causing inflammation, erythema, sunburn, photoaging, and skin cancer (21). The MAPKs, including ERKs, JNKs, and p38, are involved in UV light-induced inflammation and related signal transduction (6). However, the primary signal transduction pathway(s) and key molecules involved in SUV-induced tumorigenesis are not yet completely elucidated. We previously reported that the ERKs and p38 signaling cascades are markedly activated by SUV and play important roles in SUV-induced skin carcinogenesis (8, 9). We found that the phosphorylation levels of the MEK and ERKs cascade were increased in a time- and dose-dependent manner by SUV, with the peak phosphorylation level occurring at 15 minutes after SUV (60 kJ/m²) exposure (Supplementary Fig. S1A and S1B). We also reported that RSK2 and MSK1 are key regulators in tumor promoter-induced cell transformation (11, 22). RSK2 and MSK1 are direct substrate kinases of ERKs or p38 and might be required for SUV-induced skin carcinogenesis. To confirm our hypothesis, we examined the level of phosphorylated RSK2 or MSK1 in SKH-1 mouse skin exposed to SUV for 12 weeks compared with normal unexposed skin. We observed that chronic SUV exposure dramatically induced RSK and MSK1 phosphorylation (Fig. 1A and B). To further investigate the function of RSK2 and MSK1 in SUV-induced skin cancer, we examined the level of phosphorylated RSK2 and MSK1 in human SCC samples compared with normal skin. Our data indicated that phosphorylated RSK and MSK1 are significantly increased in SCCs (Fig. 1C and D). This suggests that the RSK and MSK1 cascades could play an important role in SUV-induced skin cancer.

RSK2 and MSK1 play an important role in anchorage-independent skin cancer cell growth and proliferation

SUV irradiation rapidly activates the EGFR through the induction of EGFR ligands and the inactivation of cytoplasmic protein tyrosine phosphatases (23). EGFR is activated by UV radiation and is overexpressed in UV-induced human skin cancers (24). A431 cells comprise an EGFR-amplified human squamous carcinoma cell line. After knocking down expression of RSK2 or MSK1, A431 anchorage-independent cell growth was inhibited. Moreover, colony formation was more substantially decreased in double knockdown RSK2 and MSK1 cells (Fig. 2A). Similar effects...
were observed in proliferation of A431 cells transfected with mock, sh-RSK2, sh-MSK1, or sh-RSK2/sh-MSK1 (Fig. 2B). Double knockdown of RSK2 and MSK1 showed the most significant inhibitory effect. Mutations in ras, particularly H-Ras, are frequent in SCCs (25, 26). To further examine the role of endogenous RSK2 and MSK1 signaling in Ras-induced cell transformation, we conducted a RasG12V-mediated foci-formation assay in NIH3T3 cells. We introduced various combinations of RasG12V, RSK2, and MSK1 into NIH3T3 cells and foci formation was measured. The results indicated that RasG12V induced foci formation and RasG12V/RSK2 or RasG12V/MSK1 substantially enhanced foci formation (Supplementary Fig. S2). Furthermore, the number of foci induced by the introduction of RasG12V/RSK2 or RasG12V/MSK1 was increased more with cotransduction of RasG12V/RSK2/MSK1. However, size was not affected. These results indicate that RSK2 and MSK1 are potential targets for prevention of SUV-induced skin cancer.

Kaempferol inhibits RSK2 and MSK1 activity by competing with ATP for binding

Flavonoids found in many foods exhibit antioxidant, antitumor, and anti-inflammatory effects (27). We previously demonstrated that kaempferol, a flavonol, is a natural compound that inhibits RSK2 kinase activity and attenuates JB6 P+ cell transformation induced by EGF. To determine potential targets of kaempferol, kinase assays were conducted by Millipore’s Kinase Profiler Service according to their established protocols. Scores represent the percentage of control, which was derived from the following formula: % of control = [(sample – mean no enzyme)/(mean plus enzyme – mean no enzyme)] × 100. The results revealed that RSK2 and MSK1 were the most relevant potential targets of kaempferol compared with other members of the MAPK family (Supplementary Fig. S3A). To further confirm this finding, we conducted an in vitro kinase assay with active RSK2 and histone H3 as substrate with ATP. RSK2 activity was strongly suppressed by kaempferol (Fig. 3A). As expected, kaempferol also had a similar effect on MSK1 activity (Fig. 3B). These results indicate that kaempferol might be a dual inhibitor of both RSK2 and MSK1. To further examine this idea, we conjugated kaempferol with CNBr–Sepharose 4B beads and conducted a pull-down assay. We confirmed that active RSK2 (100 ng) binds with kaempferol–Sepharose 4B beads, but not with Sepharose 4B beads alone (Fig. 3C, top). Using JB6 P+ mouse

Figure 1. Phosphorylated RSK (p-RSK) and phosphorylated MSK1 (p-MSK1) are highly expressed in SUV-exposed mouse skin and human SCCs. IHC analysis of p-RSK (A) and p-MSK1 (B) in normal mouse skin and SUV-treated mouse skin samples. IHC analysis of p-RSK (C) and p-MSK1 (D) in normal human skin and human SCC samples. Tissue slides from each group were prepared in paraffin sections after fixation with formalin and then stained with the indicated antibodies. Expression of p-RSK or p-MSK1 was visualized by microscopy. Stained cells were counted from five separate areas on each slide and an average of three samples was calculated per group. Data, the mean percentage of control ± SD. The asterisk indicates a significant increase in p-RSK or p-MSK1 expression (*, P < 0.05; **, P < 0.01).
epidermal cell lysates, we performed another pull-down assay and results revealed that kaempferol also binds with RSK2 in JB6 \( P^+ \) mouse epidermal cells (Fig. 3C, middle). Results of an ATP competition assay showed that the binding ability of kaempferol with RSK2 (Fig. 3C, bottom) was altered in the presence of ATP. Similar results were obtained for MSK1 \textit{in vitro} (Fig. 3D, top) and in cells (Fig. 3D, middle). Also, kaempferol bound with MSK1 competitive with ATP (Fig. 3D, bottom). These data were supported by our computer modeling results (Supplementary Fig. S3B and C). We also observed that kaempferol does not bind with JNK2 or p38 in JB6 \( P^+ \) cells (Supplementary Fig. S3D). All these data confirmed our hypothesis that kaempferol binds and inhibits RSK2 or MSK1 activity in an ATP-competitive manner.

**Kaempferol attenuates SUV-induced phosphorylation of CREB and histone H3 in mouse skin cells**

RSK2 and MSK1 are activated by growth factors, peptide hormones, or SUV. Numerous proteins, such as the CREB, histones, activating transcription factor 1 (ATF1), and transcription factor activator protein-1 (AP-1), are phosphorylated by active RSK2 and MSK1 (28–30). To examine the effect of kaempferol on SUV-induced skin carcinogenesis, we first determined the effect of kaempferol on MAPK signaling in JB6 \( P^+ \) mouse epidermal skin cells. Results indicated that SUV (60 kJ/m²) substantially induced phosphorylation of MEK, ERKs, RSK, and MSK1 (Fig. 4A, left). However, phosphorylation of these kinases was not changed by increasing doses of kaempferol (Fig. 4A, right). To further confirm our findings, we performed the same experiment in primary keratinocytes isolated from SKH-1 mice. Similarly, phosphorylation of CREB, ATF1, and histone H3, which are well-known substrates of RSK2 and MSK1, was suppressed by kaempferol in dose-dependent manner (Fig. 4A, right). To further confirm our findings, we performed the same experiments in primary keratinocytes isolated from SKH-1 mice. Similarly, phosphorylation of CREB, ATF1, and histone H3 was attenuated by kaempferol (Fig. 4B, right), whereas phosphorylation of MEK, ERKs, RSK, and MSK1 was not affected (Fig. 4B, left). AP-1 and NF-κB are
activated through the MAPK pathways upon stimulation with UV (31, 32). RSK2 and MSK1 are known to transmit signals downstream to regulate the transcriptional activity of proteins such as AP-1 and NF-κB (33). To examine the effect of kaempferol on SUV-induced transactivation of AP-1 and NF-κB, we exposed JB6 P+ cells stably transfected with an AP-1 or NF-κB luciferase reporter plasmid to kaempferol. Results indicated that kaempferol suppressed SUV-induced transactivation of AP-1 (Fig. 4C, left) and NF-κB (Fig. 4C, right) in a dose-dependent manner. On the basis of these results, we hypothesized that kaempferol might effectively reduce SUV-induced carcinogenesis in vivo.

Kaempferol significantly suppresses SUV-induced skin carcinogenesis in a mouse skin tumorigenesis model

To study the antitumorigenic activity of kaempferol in vivo, we evaluated the effect of kaempferol in a SUV-induced mouse skin tumorigenesis model. SUV irradiation consists of UVA and UVB and thus, more closely resembles the natural environment. Topical application of kaempferol on mouse skin resulted in a substantial inhibition of SUV-induced tumor incidence (Fig. 5A) and also decreased the average tumor volume per mouse (Fig. 5B). At 25 weeks after SUV exposure, results showed that topical treatment with 0.5 or 1 mg kaempferol reduced tumor volume by 56% or 68%, respectively, compared with the vehicle-treated group. Furthermore, treatment with 0.5 or 1 mg kaempferol also reduced tumor incidence by 78% or 91%, respectively, compared with the vehicle-treated group. Skin and tumor samples were processed for H&E staining at the end of the study (25 weeks). Epidermal thickness, caused by edema and epithelial cell proliferation, represents typical skin histologic inflammatory alterations (34). UV-induced skin inflammation is usually quantified by measurement of the epidermal thickness (35). The protective effect of compounds against UV-induced skin inflammation is confirmed by the observation of decreases in epidermal thickness (35–37). After treatment with solar UV, epidermal thickness was increased in the vehicle/SUV-treated group. Notably, kaempferol significantly decreased epidermal thickness (Fig. 5C, top). IHC data showed that Ki-67, which is a well-known cellular marker for proliferation, dramatically increased in the vehicle/SUV-treated group compared with the vehicle group. However, in the kaempferol-treated groups, Ki-67 expression was decreased compared with the vehicle/SUV-treated group (Fig. 5C, bottom). Western blot analysis of the mouse skin showed that phosphorylation of CREB, c-Fos, and histone H3 induced by SUV was dramatically suppressed in the kaempferol-treated group (Fig. 5D). These results clearly showed that kaempferol exerts a strong...
Discussion

UV light is a well-known environmental carcinogen and is highly associated with skin carcinogenesis (38, 39). SCCs of the skin occur predominantly on UV-exposed areas of the body and have been linked with chronic exposure to UV (40). Studies in various skin cell lines have demonstrated that EGFR and MAPKs are specific signaling molecules in UV-induced skin carcinogenesis (41, 42). SUV treatment markedly enhances ERKs and p38 signaling, and inhibition of ERKs or p38 results in fewer and smaller tumors in mice exposed to chronic SUV (8, 9). The ERKs/RSK2 pathway regulates cell proliferation, survival, growth, motility, and tumorigenesis (22). ERKs are important in regulating cellular functions and double knockout of ERK1 and ERK2, and result in mortality of newborn pups within 1 day (43). MSK1 is activated in response to EGF, TPA, and UV stimuli (44, 45). RSK2 and MSK1 act downstream of ERKs and p38 and are located between ERKs and its own target transcription factors, including CREB, ATF1, and histone H3. In mouse epithelial cells and keratinocytes exposed to SUV, MAPK signaling cascades and downstream transcription factors such as CREB, ATF1, and histone H3 are activated (Fig. 4A and B). Chronic SUV exposure markedly induced phosphorylation of RSK and MSK1 in human SCCs (Fig. 1C and D). Mouse skin exposed to long-term SUV also expresses high levels of phosphorylated RSK and MSK1 (Fig. 1A and B). In an NIH3T3 cell foci formation assay, the number of foci was increased more by co-introduction of Ras<sup>G12V</sup>/RSK2/MSK1 (Supplementary Fig. S2). After knocking down RSK2 and MSK1 expression, human A431 skin cancer cell colony formation and growth were significantly inhibited (Fig. 2A and B). These data indicate that targeting RSK2 and MSK1 might be sufficient to prevent skin cancer and also might avoid unwanted side effects associated with targeting upstream kinases (43, 46).

Many anticancer drugs exert adverse side effects, which can be severe and life-threatening. Thus, identification of novel anticancer compounds from natural products might...
be a safer alternative and a promising strategy for cancer prevention or treatment (47). Epidemiologic studies indicated that high dietary intake of flavonoids found in fruits and vegetables is associated with a lower cancer incidence (48). Kaempferol is present in a wide variety of fruits and vegetables. In a previous study, we showed that kaempferol inhibited EGF-induced cell transformation (22). However, data showing that kaempferol could act as a cancer...
prevention agent in vivo was still not available. In the present study, kaempferol markedly suppressed SUV-induced skin carcinogenesis in a mouse skin tumorigenesis model by targeting RSK2 and MSK1 (Fig. 3A and B; Fig. 5A and B). The data showed that topical application of kaempferol (1 mg) markedly delayed tumor growth until 21 (measurable size) weeks compared with vehicle control, which was first observable at 13 weeks (Fig. 5A). By the end of the study (25 weeks), 1 mg kaempferol significantly reduced tumor volume and incidence by 68% and 91% compared with the vehicle control.

Overall, our results showed that kaempferol exerted excellent inhibitory effects against UV-induced skin carcinogenesis by directly targeting RSK2 and MSK1. Thus, kaempferol could have highly beneficial effects in the prevention of skin carcinogenesis.

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

Authors’ Contributions

References
6. Bode AM, Dong Z. Mitogen-activated protein kinase activation in UV-induced skin carcinogenesis in a mouse skin tumorigenesis model by directly targeting RSK2 and MSK1. Thus, kaempferol could have highly beneficial effects in the prevention of skin carcinogenesis.


Cancer Prevention Research

Kaempferol Targets RSK2 and MSK1 to Suppress UV Radiation-Induced Skin Cancer
Ke Yao, Hanyong Chen, Kangdong Liu, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-14-0126

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2014/07/14/1940-6207.CAPR-14-0126.DC1

Cited articles
This article cites 47 articles, 21 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/7/9/958.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cancerpreventionresearch.aacrjournals.org/content/7/9/958.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.