Aspirin reduces the outcome of anticancer therapy in Meth A–bearing mice through activation of AKT-glycogen synthase kinase signaling

Antonella di Palma,1 Giuseppe Matarese,2 Vincenza Leone,1 Tiziana Di Matola,1 Fabio Acquaviva,1 Angela Maria Acquaviva,1 and Paolo Ricchi1

1Dipartimento di Biologia e Patologia Cellulare e Molecolare “L. Califano” and 2Istituto di Endocrinologia ed Oncologia Sperimentale “G. Salvatore” del Consiglio Nazionale delle Ricerche, Università “Federico II,” Naples, Italy

Abstract

Aspirin displays, at millimolar concentrations, several mechanisms independent from its ability to inhibit cyclooxygenases. Occasionally, the mechanisms displayed in vitro have been clearly related to an effect of clinical relevance in vivo. An expanding literature has been focusing on the cytoprotective effect of aspirin in neurodegenerative disorders and the activation of AKT pathway in neuroprotection and induction of resistance to anticancer drugs. In this work, we tested the ability of aspirin to activate the AKT survival pathway in methylcholanthrene-induced fibrosarcoma cells (Meth A) transplanted into BALB/c nude mice and the clinical effect of aspirin cotreatment during etoposide (VP-16)–based anticancer therapy. We found that cotreatment with aspirin reduced VP-16–induced apoptosis and activated AKT in vitro and in vivo. In Meth A–bearing mice, aspirin administration also activated glycogen synthase kinase-3 and reduced the activity and the efficacy of anticancer therapy in VP-16 cotreated animals. Our data suggest that the antia apoptotic effect of aspirin operates in vitro through the activation of AKT-glycogen synthase kinase pathway causing a decrease in the outcome of VP-16–based therapy. These findings could have clinical relevance in treatment of human malignancies. [Mol Cancer Ther 2006;5(5):1318–24]

Introduction

Aspirin, a widely used nonsteroidal anti-inflammatory drug, is a nonselective inhibitor of cyclooxygenase (COX)-1 and COX-2 (1). High doses of salicylates (4–10 g/d), including sodium salicylate and aspirin, have been used to treat inflammatory conditions, such as rheumatic fever and rheumatoid arthritis. There are several actions displayed by high doses of aspirin independent from its ability to inhibit COXs. These include the following: (a) inhibition of the activation of nuclear factor-κB by prevention of the phosphorylation and degradation of the inhibitory subunit IκB (2), (b) modulation of gene transcription (3), and (c) modulation of several protein kinases and other molecular signaling pathways (4, 5). Several effects have been shown in vitro: aspirin treatment inhibits cell growth (6–8) and protects against a variety of toxic stimuli, such as H2O2, free radicals, hypoxia, and chemical toxins (9–15). Occasionally, the wide spectrum of molecular mechanisms and targets displayed in vitro has been clearly related to an effect with clinical relevance in vivo. For instance, high doses of salicylates can lower blood glucose concentrations by reducing signaling of the IκB kinase pathway (16). In addition, in an in vivo model of focal brain ischemia, aspirin has been recently found to be neuroprotective by inhibiting the sustained activation of extracellular signal-regulated kinase 1/2 pathway (17).

We have reported previously that millimolar doses of aspirin protected Caco-2 cells from apoptosis both induced by anticancer drugs treatment and serum deprivation (18, 19). We showed that these effects were determined by the ability of aspirin to activate dose-dependently the phosphatidylinositol 3-kinase (PI3K)/AKT signal transduction survival pathway in Caco-2 cells (19). In this study, we aimed to test the ability of aspirin to activate PI3K/AKT pathway in vitro and in vivo in methylcholanthrene-induced fibrosarcoma cells (Meth A) transplanted into BALB/c nude mice. We found that aspirin treatment activates AKT also in vivo, thus reducing the effect of anticancer drug and the survival of transplanted mice cotreated with aspirin with respect to animals treated with anticancer drugs alone.

Materials and Methods

In vitro Studies

Cell Growth and Culture. The transplantable sarcoma Meth A cells were kindly provided from Dr. Pramod Srivastava. Original tumor cell lines were induced by a single s.c. injection of 0.1 mg 3-methylcholantrene dissolved in 0.1 mL sesame oil. Meth A cells have a well-defined immunogenicity and typically produce either solid tumors or ascites when injected s.c. or i.p., respectively.
The cells were maintained by continuous propagation in the peritoneal cavity of BALB/c mice (6–7 weeks old). In addition, Meth A cells can routinely grown in 100-mm plastic dishes at 37°C in a humidified atmosphere of 5% CO₂ in air in DMEM supplemented with 10% FCS, glutamine (2 mmol/L), penicillin (100 units/mL), and streptomycin (100 μg/mL) and buffered with HEPEs (20 mmol/L).

All experiments in vitro were done after one passage from peritoneal cavity. Aspirin (Sigma, Milan, Italy) was prepared as a 1 g/50 mL stock solution (111 mmol/L). The solution was buffered with Tris to obtain the final pH equal to that of control DMEM and prepared weekly; indomethacin (Sigma) was dissolved in DMSO and prepared as a 50 mmol/L stock solution. Etoposide (VP-16; 2 mg/mL) was obtained from Teva Pharma B.V. (Mijdrecht, the Netherlands) and prepared in its clinical formulation. 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)-phenyl-2(S)-furanone (DFU) was kindly provided by Merck Frosst (Kirkland, Quebec, Canada), dissolved in DMSO, and prepared as 10 mmol/L stock solution.

**Apoptosis Detection.** Apoptosis was evaluated by using Annexin V-FITC staining technique. Briefly, Meth A cells were collected and Annexin V-FITC stained by using a detection kit from Medical and Biological Laboratories Co., Ltd. (Nagoya, Japan) according to the manufacturer’s instructions. Fluorescence analysis was done by a flow cytometer apparatus (Becton & Dickinson, Mountain View, CA) and the CellQuest analysis software. For each sample, at least 30,000 events were stored. Quadrant settings were based on the negative control. Each experiment was repeated at least thrice.

**Western Blot Analysis.** Phosphorylated AKT and total AKT were detected by using rabbit polyclonal antibodies from Cell Signaling Technology, Danvers, MA. Phosphorylated glycogen synthase kinase (GSK)-3α/β (Ser21/Ser9) and GSK-3β were detected by using rabbit polyclonal antibody and mouse monoclonal antibody from Cell Signalling. Cells were washed in cold PBS and lysed for 10 minutes at 4°C with 1 mL lysis buffer [50 mmol/L Tris (pH 7.4), 0.5% NP40, 0.01% SDS] containing complete protease inhibitor cocktail (Roche, Mannheim, Germany) and phosphatases inhibitor (1 mmol/L orthovanadate). Lysates were centrifuged at 12,000 x g for 15 minutes at 4°C and the supernatant was collected. Protein concentration in cell lysates was determined by Bio-Rad protein assay (Bio-Rad, Richmond CA), and 50 μg total protein from each sample was analyzed. Proteins were separated by a 12% SDS-PAGE and transferred on nitrocellulose membrane (Hybond-ECL nitrocellulose, Amersham, Rainham, United Kingdom). After incubation with horseradish peroxidase–conjugated anti-mouse or anti-rabbit secondary antibodies (Bio-Rad) diluted 1:2,000 in PBS-0.2% Tween, the membranes were washed and protein bands were detected by an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ). Relative intensities of bands were quantified by densitometry with a desk scanner (Pharmacia Discovery System, Amersham Pharmacia Biotech) and RFLPrint software (PDI, New York, NY).

**In vivo Studies**

Female BALB/c mice (6–7 weeks old) were purchased from Charles River Laboratories (Milan, Italy). The research protocol was approved and mice were maintained in accordance to institutional guidelines of the University of Naples Animal Care and Use Committee. Mice were acclimatized at the University of Naples Medical School Animal Facility for 1 week before injection with cancer cells. All these animals received ~1 million Meth A tumor cells in 0.2 mL saline on day 0. VP-16 was used at 4 mg/kg, and aspirin was used at 15, 30, and 45 mg/kg. After 5 days, when established ascites were evident and animal weight started to increase, seven mice per group were treated i.p. at the indicated doses of aspirin alone or in combination with VP-16. Drugs were given (on days 1–3) for 3 days every 24 hours. Control group was injected with vehicle. All animals were injected i.p. with an equal volume of solvent as a control (300 μL). Then, mice were randomly divided into seven groups [1 control group, 1 VP-16-treated group, 2 aspirin-treated groups (15 and 45 mg/kg), and 3 aspirin and VP-16 cotreated groups], with each group containing seven animals. Of each group, six animals were observed daily to monitor their survival and their weight increase and one animal was killed 4 hours after completing treatment; ascitic fluid containing tumor cells was collected in PBS with proteases and phosphatases inhibitors and processed as described in Western Blot Analysis.

**Statistical Analysis**

Statistical comparisons were done using the Mann-Whitney U test and log-rank test. *P* < 0.05 was considered a significant difference.

**Results**

**Effect of Aspirin Cotreatment on Anticancer Drug Responsiveness in Meth A Cells**

Meth A cells were exposed for 72 hours at different concentrations of VP-16 and aspirin alone and in combination. Aspirin was used at 1, 2, and 3 mmol/L and VP-16 was used at 34 and 68 μmol/L. Apoptosis was evaluated through the analysis of cells positive for Annexin V staining at flow cytometry. Spontaneous apoptosis of Meth A cells in culture medium was in the average 10%. Treatment with aspirin up to 3 mmol/L concentration did not increase significantly apoptosis in Meth A cells with respect to basal level (Fig. 1, top). The percentage of apoptosis increased up to 11% following treatment with 5 mmol/L aspirin (data not shown). Conversely, VP-16 at 34 and 68 μmol/L dose-dependently increased apoptosis from 26% to 46% (Fig. 1, top). In addition, the levels of apoptosis were significantly reduced (*P* < 0.05) for each concentration of VP-16 when given in the presence of all concentration of aspirin compared with single-agent treatment; Meth A cells cotreated with aspirin at 3 mmol/L were particularly resistant to VP-16-induced apoptosis (*P* < 0.002). Similar results were obtained when levels of apoptosis were evaluated with propidium iodide staining analysis (data
not shown). These results indicated that VP-16 was an active anticancer agent in Meth A cells and that aspirin treatment reduced the ability of the drug to induce apoptosis.

To evaluate whether the effects of aspirin cotreatment on anticancer drug responsiveness were also obtainable toward others anticancer agents, we tested the effect of aspirin treatment on cisplatin-induced apoptosis. Meth A cells were cotreated with aspirin at 1, 2, and 3 mmol/L and cisplatin at 2, 5, 10, and 20 μmol/L under the above-mentioned experimental conditions. Aspirin cotreatment dose-dependently reduced cisplatin-induced apoptosis for each concentration of cisplatin tested. The effect of aspirin on apoptosis was of the same range of magnitude of that observed in VP-16 and aspirin cotreated Meth A cells (data not shown).

To further confirm the involvement of PI3K/AKT survival pathway, we evaluated the effect of the PI3K inflammatory drug that shows a COX-1/COX-2 ratio called the inhibition of COX isoform similar to that of aspirin. We used two different concentration of indomethacin and DFU both at doses well recognized to inhibit COX-1 and/or COX-2. Neither indomethacin at 50 and 100 μmol/L nor DFU at 0.1, and 0.5 μmol/L, respectively, reduced VP-16-induced apoptosis in Meth A cells (Fig. 1, bottom). These results, therefore, indicated that the cytoprotective action was unique to aspirin and was not shared by indomethacin.

Aspirin Reduces the Outcome of Anticancer Drug In vivo

To show that aspirin-induced cytoprotective effect correlated with the activation of AKT in Meth A cells, we assayed AKT activation status following VP-16 treatment in the presence or absence of aspirin.

Lysates from cells treated with 1, 2, and 3 mmol/L aspirin alone or in the presence of VP-16 at 34 μmol/L for 72 hours were probed for phosphorylated AKT at Ser473. The bands were quantified and normalized to total AKT protein kinases, respectively.

As shown in Fig. 2, aspirin treatment, but not VP-16 treatment, induced activation of AKT protein with respect to control cells. The relative densitometric analysis showed 7.4-, 9.5-, and 6.9-fold increase for AKT protein phosphorylation status following aspirin treatment at 1, 2, and 3 mmol/L, respectively (Fig. 2). Moreover, phosphorylation status of AKT protein were 2-, 3.5-, and 4.2-fold increased also in cells cotreated with 1, 2, and 3 mmol/L aspirin and VP-16, respectively, compared with untreated cells.

To further confirm the involvement of PI3K/AKT survival pathway, we evaluated the effect of PI3K

Figure 1. A, effect of 1 mmol/L (A1), 2 mmol/L (A2), and 3 mmol/L (A3) aspirin and 34 μmol/L (Vp34) and 68 μmol/L (Vp68) VP-16 treatments on apoptosis in Meth A cells. Cells were incubated with aspirin and VP-16 alone or in combination at the indicated concentrations for 72 h. B, effect of Indomethacin (50 and 100 μmol/L) and DFU (0.1, and 0.5 μmol/L) and VP (34 μmol/L). Cells were incubated with Indomethacin and DFU and VP34 alone and in combination for 72 h. Apoptosis was calculated as the percentage of cells positive at Annexin V-FITC as described in Materials and Methods. Columns, mean of triplicate experiments; bars, SD.

Figure 2. Effect of 1, 2, and 3 mmol/L aspirin and 34 μmol/L VP-16 treatment alone and in combination on (Ser437) phosphorylated AKT and total AKT expression in Meth A cells. Western blot analysis was done on protein lysates from cells cultured as described in Fig. 1. The immunobots were stripped and rebotted with antibodies against total AKT. One of the three separate experiments yielded similar results.
inhibitor LY294002 on the activation of AKT in aspirin-treated and cotreated Meth A cells. The use of LY294002 suppressed the activation induced by aspirin, restoring the basal level of AKT activation status (data not shown).

Effect of Aspirin Cotreatment on Anticancer Drug Responsiveness in Meth A Bearing Mice

To investigate whether our in vitro finding occurred also in vivo, we evaluated the effect of aspirin and VP-16 treatments in nude mice injected i.p. with Meth A tumor cells.

BALB/c mice average starting weight was 24.75 g. Because the rapid growth of Meth A tumor cells induced ascites in control group, weight gain was used to monitor tumor burden in the animals. Figure 3 shows the percentage of increase in body weight with respect to initial weight in control group; after 5 days, when Meth A cells induced visible ascites, and after 14 days from the inoculum, an average 10% and 53% increase (38.2 g mean weight; data not shown) in weight was reached, respectively (Fig. 3). Later, surviving animals reaching a size not compatible with normal life and suffering for the great ascites developed were killed for bioethical reasons.

Treatments started after 5 days from inoculum; animals (six for each group) were treated i.p. with either aspirin (15 and 45 mg/kg) and VP-16 (4 mg/kg) alone or in combination with aspirin (at the dose of 15, 30, and 45 mg/kg) or with vehicle (control group). In case of coadministration, to mimic experimental condition obtained in vitro, aspirin and VP-16 were simultaneously injected at different sides of peritoneum.

During the first 7 days of treatment (days 1–7), when animal death was not yet observed, follow-up was based on the calculation of the percentage of increase in animal weight with respect to first day of treatment (Fig. 4); during the subsequent days (days 8–32), the effects of different treatments were evaluated recording animal survival and were displayed with Kaplan-Meier curves (Fig. 5).

Treatment with VP-16 completely arrested tumor growth and ascites generation, and the suppression of Meth A tumor cell growth was associated with an ~2% reduction in body weight at days 5 to 7 (Fig. 4).

After 8 days from VP-16 treatment, 33.3% of animals died for causes not directly linked with weight increase (bleeding ascites and cachexia). In other 50% of animals, after a variable lag time from treatment, Meth A cells resumed a growth rate comparable with control, and animals were harvested for bioethical reason when they reached an average weight of 35 g (data not shown). In remaining 16.6% of animals, no tumor growth was detected until 25 days of observation (Fig. 5) and they remained tumor free for a period of observation of 40 days after treatment (data not shown). Therefore, although VP-16 treatment was not able to definitively cure almost all animals, tumor growth was arrested and mouse survival duration was significantly increased with respect to control ($P < 0.001$) for all the duration of follow-up.

Aspirin treatment as single agent at 15 and 45 mg/kg had no effect on animal body weight and survival with respect to the control group (Figs. 4 and 5); no precocious mortality for gastric toxicity was observed in the aspirin-treated groups. The combination of aspirin at 45 mg/kg and VP-16 did not significantly inhibit xenograft ascitic growth and did not produce any effects on animal survival compared
with VP-16-treated group ($P < 0.001$), displaying a trend similar to control group (Figs. 4 and 5). These data suggest that the highest dose of aspirin abolished the activity and the efficacy of VP-16 in tumor-bearing mice.

The combination of aspirin at 30 mg/kg and VP-16 produced an increase in body weight of a lesser extent compared with that observed in control group. However, a more rapid increase in weight gain after 5 days of treatment was observed in cotreated animal when compared with animal treated with VP-16 alone ($P < 0.002$ at day 7; Fig. 4). After this lag of time, a statistically significant difference in survival was observed between these two groups of animals (Fig. 5); in fact, in group of cotreated animals, 33% of the animals died for causes not directly linked with weight increase, and after day 15 from treatment, no animal survived (Fig. 5). These results indicate that aspirin cotreatment at 30 mg/kg attenuates the activity and the efficacy of VP-16 although at a lesser extent than cotreatment at 45 mg/kg. Finally, in animals treated with the combination of aspirin at 15 mg/kg and VP-16, the body weight reduction was slightly minor ($P < 0.02$) compared with that observed in animal treated with VP-16 alone (Fig. 4). Similarly, a minimal but not statistically significant difference in survival probability was observed (Fig. 5). These results suggest that 15 mg/kg aspirin is able to reduce the activity but not the efficacy of VP-16-based therapy.

Aspirin Treatment Activates AKT-GSK Survival Pathway in Meth A–Bearing Mice

To analyze the molecular pathways associated with aspirin treatment in Meth A–bearing mice, one animal was harvested 4 hours after completing treatment and ascitic fluid containing tumor cells from previously considered experimental points was collected for biochemical evaluation. Consistently with our in vitro finding, treatment with aspirin at 15 and 45 mg/kg determined
a 3.8- and 6.5-fold increase in activation status of AKT compared with control group, respectively (Fig. 6). In addition, aspirin cotreated groups showed higher levels of activation of AKT with respect to vehicle-treated group (Fig. 6).

Between several downstream targets of AKT, GSK-3 represents a convergence site of multiple signaling pathways involved in cell fate (22, 23). Phosphorylation at Ser21 (isoform) or Ser3 (isoform) of GSK-3β by AKT leads to inhibition of its activity and reduces apoptosis. Inactivation of GSK-3 by AKT may thus contribute to antiapoptotic effects of PI3K/AKT signaling (23). Therefore, we evaluated the effect of different treatments on activation status of GSK-3.

As shown in Fig. 6B, phosphorylation status of GSK-3 in Ser3 was increased in aspirin-treated mice. The effect was of lesser extent with respect to that observed for AKT phosphorylation and persisted, at 45 mg/kg aspirin treatment, also in the presence of VP-16. These results indicate that aspirin activates the AKT-dependent survival pathway leading to increased phosphorylation of GSK-3 at Ser3.

**Discussion**

Accumulating evidence suggests that wide spectrum of pharmacologic effect of aspirin might not only be dependent on COX inhibition. The mechanisms of action by which the drug exert its effect is still a matter of debate. Numerous pharmacologic and biochemical effects have been described. In fact, aspirin may target distinct molecular pathways in cells; however, these effects are often cell type specific and sometimes produces opposite effects (24).

An expanding literature has been focusing on the mechanism of action of aspirin as cytoprotective agent in neurodegenerative disorders. Aspirin and its metabolites have been shown to protect against cellular injury in neuronal and nonneuronal systems by preventing nuclear factor-κB nuclear translocation. More recently, the AKT pathway has been involved in neuroprotection in vivo and induction of resistance to anticancer drugs (25–28). In particular, several investigators have been focused on the involvement of AKT pathway in multidrug resistance (29). Although there is a great number of studies on the ability of aspirin to protect against toxic stimuli, there are no reports on the effect of aspirin on AKT survival pathway in vivo. We found previously the involvement of PI3K/AKT pathway in cytoprotective property of aspirin in cultured colon cancer Caco-2 cells (19). In this study, we used the fibrosarcoma Meth A cells as a system to evaluate aspirin interference with anticancer drug-induced apoptosis in vitro and in vivo. Our data showed for the first time that the antiapoptotic effects of aspirin are not cell type specific and operated in vivo through the activation of PI3K/AKT pathway. Our data showed that aspirin exposure, but not other COX-2 selective and nonselective inhibitors, prevented apoptosis induced by VP-16 and cisplatin in Meth A cells.

The in vitro data were consistent with previous results showing that aspirin protected cells from apoptosis through activation of PI3K/AKT. Because the AKT protein is the downstream effector of PI3K, further studies are needed to clarify at molecular levels the site(s) of aspirin interference with the PI3K survival pathway and the mechanism(s) responsible for PI3K activation. Preliminary data from our group indicate that aspirin is able to induce acetylation status of p85 regulatory subunit of PI3K. It is possible to postulate that the structural change by acetylation increase the level of PIP3, as shown previously (19), and induce activation of PDK1 kinase.

Furthermore, the in vitro data reinforce the evidence coming from our and other previous experiments (6, 18, 19, 24) that high doses of aspirin slightly affected or may increase basal level of apoptosis but were protective from apoptosis induced by external toxic stimuli. This at the molecular level confirms the aspirin pleiotropic activity and suggests that the aspirin interference with different survival/death pathways determines a global prosurvival promoting effect.

One of the major purposes of this study was to evaluate whether combined treatment in vivo would produce effects that could be relevant in the clinical use. Our data indicated that aspirin as single agent did not affect Meth A cell growth while provided evidence that aspirin administration reduced in vivo the activity and the efficacy of VP-16 treatment in Meth A–bearing mice. Our data indicated also that in vivo aspirin operated in a dose-response fashion to reduce the outcome of anticancer treatment. This trend was evident behind the lowest dose and indicated that coadministration of minimum 30 mg/kg aspirin was required to produce a significant effect on animal survival. Furthermore, aspirin-induced increase of animal body weight and decrease of survival strictly correlates with the activation of AKT. Our results agree with previous data showing that AKT activation in vivo is a major factor in the resistance of tumor cells to apoptosis induced by chemotherapeutic drugs (29).

In addition, our in vivo data indicated that aspirin inhibited the activity of GSK-3, a key factor not only for survival and chemoresistance (30) but also for the attenuation of the cellular action of insulin (31). Because it has been shown that high-dose aspirin could improve glucose metabolism in humans (16) and that GSK-3 activity inhibitors may therapeutically benefit in treating insulin resistance and type 2 diabetes (32), the data of this report should encourage further evaluation of the in vivo mechanism for aspirin hypoglycemic effect.

Although an expanding literature is showing the role of nonsteroidal anti-inflammatory drugs used alone or in combination with conventional anticancer drugs and their ability to alter toxicity profile of anticancer drugs (33, 34), the use of aspirin in combination with anticancer drugs has never been investigated in vivo. In this study, we used doses of aspirin in vitro and in vivo approximately corresponding to that required for the treatment of arthritis.
Aspirin Reduces the Outcome of Anticancer Drug In vivo

and rheumatic fever. In fact, the clinical treatment of inflammation requires 2 to 5 g aspirin oral administration, which determines aspirin plasma level up to 1 to 3 mmol/L (35). In our experiments, the antiapoptotic effect of aspirin was observed starting at dose at least 10 times higher than those used for antiplatelet therapy (75 mg/d). Therefore, our finding could be of relevance in the clinical condition where treatment schedules involving both cytotoxic agents and high doses of aspirin are required, suggesting that cotreatment with aspirin could limit the outcome of anticancer therapy.

In conclusion, our data reinforce the emerging and wide observation coming from neuronal models (17) that aspirin operates also in vivo as cytoprotective agent. We also show that aspirin cytoprotective effect is mediated through the activation of AKT pathway. Because AKT pathway mediates survival against a wide variety apoptotic stimuli, it should be also verified the possible involvement of this pathway as mechanism by which aspirin exerts neuroprotection.

References
Molecular Cancer Therapeutics

Aspirin reduces the outcome of anticancer therapy in Meth A–bearing mice through activation of AKT-glycogen synthase kinase signaling

Antonella di Palma, Giuseppe Matarese, Vincenza Leone, et al.

*Mol Cancer Ther* 2006;5:1318-1324.

Updated version

Access the most recent version of this article at:

http://mct.aacrjournals.org/content/5/5/1318

Cited articles

This article cites 32 articles, 15 of which you can access for free at:

http://mct.aacrjournals.org/content/5/5/1318.full.html#ref-list-1

Citing articles

This article has been cited by 1 HighWire-hosted articles. Access the articles at:

/content/5/5/1318.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.