Differential of Human Prostate Cancer PC-3 Cells Induced by Inhibitors of Inosine 5’-Monophosphate Dehydrogenase

Daniel Floryk, Sandra L. Tollaksen, Carol S. Giometti, and Eliezer Huberman

Gene Expression Group, Energy Systems Division and Bioscience Division, Argonne National Laboratory, Argonne, Illinois

ABSTRACT

To establish a system to study differentiation therapy drugs, we used the androgen-independent human prostate PC-3 tumor cell line as a target and mycophenolic acid (MPA), tiazofurin, or ribavirin, which are inhibitors of IMP dehydrogenase, as inducers. These inhibitors evoked replication arrest, caused an increase in cell size, and triggered vacuolization of the cytoplasm. By Northern and Western blotting and immunostaining, we demonstrated MPA-induced expression of 12 proteins reported to reside in prostasomes, organelles released by secretory luminal prostate cells. Additional MPA-induced proteins were identified by two-dimensional gel electrophoresis. Among these was keratin 17, a prostate cell differentiation marker. By Northern blotting, we also demonstrated the constitutive expression of keratins 8 and 18 and induced expression of keratin 19, three other prostate cell differentiation markers. In addition, we established that cells were committed to differentiate after the 2nd day of MPA treatment using guanosine, which can abrogate the effects of MPA. Based on the expression patterns of prostasomal proteins and keratins and the presence of tentative secretory vacuoles, we hypothesize that MPA dehydrogenase inhibitors induce androgen-independent PC-3 cells to mature into cells with a phenotype that resembles normal prostate luminal cells, but at their intermediate state of differentiation.

INTRODUCTION

Prostate cancer is the second-leading cause of tumor-related death in men (1). Despite the introduction of the prostate-specific antigen assay (2) and progress in androgen ablation therapy, only a limited improvement in survival benefit has been achieved. In particular, a problem persists with hormone-refractory tumors, which are resistant to an array of chemotherapeutic drugs with different modes of action (3). Although the precise reason for this resistance is unknown, it may be related to the fact that tumor formation, including prostate cancer, is caused by an accumulation of genetic alterations in a variety of genes that control malignancy (4).

A potential innovative cancer treatment that may eliminate some drawbacks of the current prostate cancer chemotherapy (e.g., systemic toxicity) is differentiation therapy (5). In this treatment approach, the desired drug causes the malignant cells to undergo terminal differentiation instead of killing the tumor cells. The concept behind this therapy is based on the observations that cells of most tumors, including prostate cancers, are blocked at an early stage of cellular differentiation and that certain agents can bypass or correct this block in vitro (6). On the basis of these studies, a number of drugs have been identified, tested, and found to show promise in the treatment of human myeloid leukemia (7).

An obstacle in identifying drugs for prostate cancer differentiation therapy is the absence of an appropriate in vitro cell maturation system and useful markers that truly define the normal mature prostate cell. A number of attempts have been made to develop such a system (8–11). These studies, however, used apoptosis indicators and/or a limited number of neuroendocrine markers rather than an array of indicators that define the particular function of normal prostate cells.

Secretory cells of the mature prostate contain highly organized organelles called prostasomes (12), which are released into the prostate fluid and semen (13). These organelles contain in their membrane a variety of proteins including complement regulators CD46 (14), CD55, and CD59 (15), an essential co-factor for coagulation, tissue factor (16), and several peptidase activities, including dipeptidyl-transpeptidase/CD26 (17), neutral endopeptidase/CD10 (18), aminopeptidase N/CD13 (19), γ-glutamyltranspeptidase (20), and aminopeptidase P (21). The prostasomes also contain neuroendocrine markers including chromogranin B, neuropeptide Y (NPY), and vasointestinal polypeptide (VIP) (22) and the common secretory granule protein granulophysin/CD63 (23). Although the physiologic function of the prostasomes is still not clearly defined, they display properties that are beneficial to sperm (24–29). As such, cultured prostate tumor cells that can be induced to produce prostasomes or their building blocks coupled with replication arrest should represent a useful system to study potential differentiation therapy drugs.

IMP dehydrogenase (IMPDH) is a key enzyme in guanine nucleotide biosynthesis, and as such, it is vital for the replication of all cells (30). In addition, inhibitors of this enzyme have been found to effectively induce differentiation in a variety of distinct human tumor cell types (31). For this reason, we tested IMPDH inhibitors for their ability to induce a mature phenotype in androgen-independent prostate tumor cells. Here, we describe IMPDH inhibitor-induced differentiation of PC-3 cells into cells with a phenotype resembling that of mature prostate secretory cells.

MATERIALS AND METHODS

Cells, Giemsa Staining and Cell Cycle Analysis. PC-3 cells were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in 8% CO<sub>2</sub> at 37°C in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 units/mL streptomycin (Invitrogen). MPA (Sigma, St. Louis, MO) was dissolved in dimethyl sulfoxide (Fisher Scientific, Hampton, NH) as a stock solution of 20 mg/mL, and ribavirin (Sigma) and tiazofurin (NSC 286193; National Cancer Institute Drug Synthesis and Chemistry Branch, Bethesda, MD) were dissolved in dimethyl sulfoxide as a stock solution of 50 mg/mL. Standard chemicals were purchased from Sigma. To determine cell numbers, PC-3 cells were plated in 60-mm plates at 3 × 10<sup>5</sup> cells per plate or in 100-mm plates at 1 to 2 × 10<sup>6</sup> cells per plate. Treatment with MPA, ribavirin, or tiazofurin was initiated a day after cell seeding. Treatment with guanosine was performed as indicated in the text. To demarcate vacuoles, cells were stained with a Giemsa solution (Sigma) for 1 minute and washed three times with PBS after being fixed in 3% paraformaldehyde in PBS for 10 minutes at room temperature. Images of stained cells were captured with a Zeiss inverted microscope and printed, and the cells with and without vacuoles were counted to determine their percentages. Cell cycle analysis was performed as described previously (32) using the WinMDI 2.8 software (Joe Trotter; Scripps Research Institute, La Jolla, CA).

IMP Dehydrogenase Activity. Enzyme activity was assayed as described previously (33). Specific IMPDH activity was calculated as μmol of NADH...
formed per milligram per milliliter (1 unit). The results represent the mean ± SD of three independent experiments.

**Chromatin Condensation.** Dissociated cells were washed twice in PBS and adhered to microscope slides by centrifugation (770 rpm, 5 minutes) using Cytospin 2 (Shandon, Pittsburgh, PA). The adhered cells were fixed with 3% paraformaldehyde in PBS for 10 minutes at room temperature, washed 3 times with PBS, permeabilized with 0.5% Triton X-100 in PBS, and stained with 100 ng/mL 4',6-diamidino-2-phenylindole hydrochloride (Boehringer Mannheim, Indianapolis, IN) for 30 minutes at room temperature. The fraction of cells displaying chromatin condensation was determined by fluorescent microscopy of the mounted slides. Cells treated with 50 μM/L ZnSO4 in serum-free medium for 1 day served as positive control.

**Immunostaining.** Cells were grown for 2 days on coverslips in 60-mm plates and treated with 20 μM/L MPA for 3 days. After treatment, cells were washed three times in PBS, fixed with 3% paraformaldehyde in PBS, washed again three times in PBS, and permeabilized with 1% Triton X-100 in PBS. To reduce the background, permeabilized cells were incubated with 5% bovine serum albumin in PBS for 10 minutes at room temperature and then stained with primary antibody mAb78 (1:2; a gift from Dr. G. Ronquist; Department of Medical Sciences, University Hospital, Uppsala, Sweden) or with a primary antibody to β-actin (1:200; Sigma, Hercules, CA), based on the method of Bradford (34). For electrophoresis, 20 μg of protein was loaded onto each of four isoelectric focusing gels. The gels were electrophoresed at 300 V for 3.5 hours to separate the proteins. The gels were then equilibrated with reducing SDS sample buffer for 10 minutes and blotted with semi-dry transfers to PVDF membranes (Immobilon, Millipore, Bedford, MA; 150 V, 90 minutes). Membranes were washed 3 times with cold PBS and lysed in ice-cold radioluminoluminoperoxidase assay buffer (50 mM/L Tris (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, and 150 mM/L NaCl) containing a mixture of protease inhibitors (Invitrogen) at 4°C for 10 minutes. Cell lysates were centrifuged at 13,000 rpm for 10 minutes at 4°C. Protein concentrations were measured with a Protein Assay Kit (Bio-Rad, Hercules, CA), based on the method of Bradford (34). For electrophoresis, solubilized proteins (100 μg per well) were separated on an 8% SDS-polyacrylamide gel and transferred onto a Hybond-C extra membrane (Amersham, Piscataway, NJ). The membrane was blocked with freshly prepared 5% dry milk in PBS/0.05% Tween (milk/PBS/Tween) for 10 minutes at room temperature and then incubated overnight at 4°C with a primary antibody against fibronectin (1:100; Sigma). After the membrane was washed three times with milk/PBS/Tween at room temperature, it was incubated with goat anti-rabbit secondary antibody labeled with horseradish peroxidase (1:5,000; Pierce, Rockford, IL) for 60 minutes at room temperature. Chemiluminescence was obtained by enhanced chemiluminescence detection kit (Amersham).

**Two-Dimensional Gel Electrophoresis.** Two-dimensional gel electrophoresis of lysates was performed as described previously (35). Aliquots of 200 μg of protein were loaded onto each of four isoelectric focusing gels. Protein bands in the two-dimensional gel electrophoresis gels were stained with 0.25% Coomassie Blue R-250 (Serva, Heidelberg, Germany) in 2.5% phosphoric acid and 50% EtOH and stained until the bands were visible. The resulting images were scanned into a tiff format and analyzed using Progenesis software for two-dimensional gel electrophoresis data analysis (Nonlinear USA, Durham, NC) on a personal computer. The differences in protein expression among the different groups was evaluated using Student’s two-tailed t test (P < 0.05). Proteins found to be induced or reduced after treatment by >3-fold were excised from the gels and identified by
peptide mass determination at the W. M. Keck Facility, Yale University (New Haven, CT).

RESULTS

Mycophenolic Acid-Induced Replication Arrest. Incubation of PC-3 cells for 3 days with 0.3 to 40 μmol/L MPA resulted in replication arrest, which, up to 3 μmol/L MPA, was dose dependent; higher doses yielded a plateau effect (Fig. 1A). The plateau effect was also observed after 1 day of treatment with 20 to 40 μmol/L MPA (data not shown). Also, MPA at 1 μmol/L reduced IMPDH activity in PC-3 cell homogenates from 0.18 ± 0.03 milliunit to <0.02 ± 0.01 milliunit. Because of these observations, we used 20 μmol/L MPA in all subsequent experiments to characterize differentiation of PC-3 cells.

The MPA-evoked replication arrest was also validated by cell cycle analysis using flow cytometry. During a 3-day exponential growth of control PC-3 cells, the percentage of cells in the different cell cycle fractions was relatively constant, with about 70% of cells in the G0-G1 phase and about 15% of cells in each of the S and G2-M phases. MPA treatment caused a major shift in this composition; by the 3rd day, the percentage of cells in the G0-G1 phase was reduced to about 20%, and the fraction of S-phase cells increased to about 70%, whereas the percentage of G2-M–phase cells was constant at about 10% (Fig. 1B). These changes were also associated with an enlargement of cell nuclei (Fig. 1C). These results demonstrate the MPA-evoked replication arrest and indicate that this arrest is due to a cell cycle block causing treated PC-3 cells to accumulate in S phase.

Despite the replication arrest, treatment of PC-3 cells with 20 μmol/L MPA for up to 5 days failed to induce chromatin condensation, a hallmark of apoptosis. As a positive control, we used zinc, an inducer of apoptosis in these cells (36). Unlike MPA, treatment with 50 μmol/L zinc for 1 day caused >90% of the cells to display this apoptotic marker (Fig. 1C).

Vacuole Production. In addition to replication arrest, MPA caused PC-3 cells to increase in size and to display vacuoles (Fig. 2A), which failed to stain with the lipid stain Oil Red in viable cells or with Giemsa in paraformaldehyde-fixed cells. The percentage of cells with vacuoles changed with time, reaching a maximum on the 4th day of MPA treatment. Thereafter, their fraction decreased (Fig. 2B). These results indicate that MPA induces PC-3 cells to produce vacuoles...
containing as yet unknown substances, which most likely are released into the culture medium, at least after 4 days of MPA treatment.

Expression of Prostasomal Proteins Induced by Mycophenolic Acid. Previous studies have reported that PC-3 cells contain prostatesome-like granules, which can be detected with the mAb78 monoclonal antibody (37). Using this antibody, we found that a 3-day treatment of PC-3 cells with MPA resulted in increased mAb78 immunostaining (Fig. 2C). To determine whether this increase was coupled with a wide-range increase in other prostasomal proteins, we examined the expression of genes that encode these proteins using Northern blotting. Prostasomal proteins have been reported to contain NH2-terminal signal peptide (38), which allows their translocation into prostasomes. Using an on-line algorithm,3 we searched for NH2-terminal signal peptide-containing proteins, of which 21 were selected for our studies. The results indicate that MPA caused a time-dependent increase in the expression of CD46, CD55, CD59, clusterin, granulophysin/CD63, glucose-regulated protein 78 (GRP78), NPY, and zinc-α-2-glycoprotein and a transient increase in the expression of aminopeptidase N/CD13 and α-glucosidase (Fig. 3A). Unlike these results, the other prostasomal proteins, chromogranin B, fibrillin 3, γ-glutamyltranspeptidase, lactotransferrin, lipoprotein lipase, prostate-specific antigen, and synaptophysin, were not detected in untreated or MPA-treated cells, whereas secretory actin-binding protein/PIP, gastricsin, tissue factor, and VIP were already expressed in control cells, and MPA had little to no detectable impact on their expression. In control cells, and MPA had little to no detectable impact on their expression. In contrast, MPA induced a time-dependent increase in the expression of keratins 8, 18, and 19 (Fig. 4B). The results indicate that MPA induces PC-3 cells to express a considerable number of prostasomal proteins.

Two-Dimensional Gel Electrophoresis Analysis of Mycophenolic Acid-Treated Cells. To identify additional proteins associated with MPA-induced differentiation, we performed two-dimensional gel electrophoresis of lysates from control and 5-day MPA-treated PC-3 cells. Analysis of Coomassie Blue-stained gels revealed MPA-induced changes in the proteome of PC-3 cells (Fig. 4A). Proteins displaying pronounced changes in their levels were cut out of the gels and subjected to matrix-assisted laser desorption ionization examination. The analysis identified the following five known proteins: high mobility group protein-1 (HMG-1) was down-regulated; whereas APG-2, calreticulin, keratin 17, and IMPDH II were up-regulated (Fig. 4A).

To substantiate these results, we performed Northern blot analysis with specific DNA probes. The results indicated that the changes observed by the two-dimensional gel electrophoresis approach corresponded to changes in the expression of the genes coding for these proteins. Furthermore, MPA-induced altered gene expression was detected as early as 1 day after treatment (Fig. 4A).

The identification of keratin 17, a protein associated with normal prostate cell differentiation (40), as a MPA-induced protein raised the possibility that other related keratins may also be expressed as a result of this treatment. Therefore, by means of Northern blotting, we tested the ability of MPA to induce the expression of three other keratins, namely, keratins 8, 18, and 19 (Fig. 4B). The results indicate that keratins 8 and 18 were constitutively expressed, and MPA had little to no effect on their expression. In contrast, MPA induced the expression of keratin 19; the level of keratin 19 increased by about 3-fold after 3 days of treatment. These results indicate that MPA induces the expression of two keratins that are recognized indicators of maturing normal prostate cells.

Commitment to Mycophenolic Acid-Induced Differentiation. Guanosine, a guanine derivative, which is readily metabolized into IMPDH end products, is commonly used to neutralize the impact of MPA and other IMPDH inhibitors (41). Because of this property, we

---

used guanosine to determine the time frame required for PC-3 cells to commit to MPA-induced differentiation.

The results indicated that 100 μmol/L guanosine, when added together with MPA, negated the MPA-evoked replication arrest (Fig. 5A), vacuole production, and NPY gene expression (data not shown). Moreover, this neutralizing impact of guanosine was effective up to about 2 days after MPA treatment (Fig. 5B and C). After this time, the addition of guanosine had little or no effect on the manifestation of MPA-induced vacuole production (Fig. 5B) and NPY gene expression (Fig. 5C). These results demonstrate that PC-3 commitment to MPA-induced terminal differentiation occurs within the initial 2 days of MPA treatment.

Ribavirin- and Tiazofurin-Induced Differentiation. To further confirm that MPA-induced differentiation of PC-3 cells is due to the function of MPA as an IMPDH inhibitor, we tested tiazofurin and ribavirin, IMPDH inhibitors that differ from MPA in their chemical structure (42). These inhibitors caused a dose-dependent replication arrest (Fig. 6A) and an increase in cell size (Fig. 6B). However, the doses required to achieve these changes were considerably higher than those used for MPA; a 70% to 80% reduction in cell number required a 3-day treatment with 400 μmol/L tiazofurin or ribavirin compared with 20 μmol/L MPA. This treatment also caused the cells to display vacuoles, with ribavirin being less effective than tiazofurin (Fig. 6B). These two IMPDH inhibitors also induced the expression of a series of prostasomal proteins and keratins including APG-2, CD55, clusterin, granulophysin/CD63, GRP78, and keratin 17 (Fig. 6C). These results indicate that tiazofurin and ribavirin are inducers of PC-3 cell differentiation; however, on a dose basis, they are considerably less effective than MPA.

DISCUSSION

The present studies were initiated to establish a model cell system to study differentiation therapy drugs for the treatment of prostate cancers that are refractory to standard chemotherapy. Here, we describe such a system with PC-3 cells, an androgen-independent prostate cancer cell line (43). In this system, PC-3 cells were induced to differentiate into cells that display traits associated with mature prostate secretory cells. This differentiation was achieved by treating PC-3 cells with MPA, tiazofurin, or ribavirin, which are inhibitors of IMPDH, an enzyme crucial for cell replication (30). These inhibitors were chosen because they were developed as anticancer drugs (42) and have the ability to induce differentiation in human tumor cell types belonging to distinct cell lineages (41, 44). Interestingly, single oral administration of 1 g of mycophenolate mofetil, a MPA pro-drug, resulted in the appearance of 76 μmol/L MPA in plasma of healthy volunteers (45). An oral dose of 1.5 g of mycophenolate mofetil administered to hepatic or cardiac transplant patients for longer than 6 months was well tolerated and yielded 62 μmol/L MPA in their plasma (46). In our studies, we used MPA at doses of up to 40 μmol/L.

The central function of the mature prostate epithelium is to secrete fluids into the seminal plasma to support the viability and activity of sperm. These fluids contain, among others, small membranous vesi-
cles termed prostasomes (12). To characterize the protein composition of these organelles, Renneberg et al. (47) performed a two-dimensional gel electrophoresis analysis and established the presence of 80 distinct protein entities, which were not well characterized. Another analysis of prostasomes from seminal fluid, which involved gas phase fractionation and microcapillary high-performance liquid chromatography-tandem mass spectrometry (21), identified 139 proteins, which were divided into six categories, namely, enzymes, transport/structural proteins, signal transduction proteins, and others.

Fig. 5. MPA-induced replication arrest, vacuolization, and NPY gene expression in the presence and absence of guanosine. A. A day after cells were seeded at $3 \times 10^5$ cells per 60-mm plate in triplicate, they were treated with the solvent dimethyl sulfoxide ( ), 20 μmol/L MPA ( ), 100 μmol/L guanosine ( ), or 20 μmol/L MPA + 100 μmol/L guanosine ( ). Error bars represent the SD of three independent experiments. B. Commitment of PC-3 to MPA-induced cell vacuolization was determined by adding 100 μmol/L guanosine at different times after treatment with 20 μmol/L MPA. Two days after cells were seeded at $2 \times 10^5$ cells per 100-mm plate, they were treated with MPA; guanosine was added at different periods of time after this treatment. The control depicts the percentage of vacuoles in cells treated with MPA for 5 days. Error bars represent the SD of three independent experiments. C. Commitment of PC-3 cells to MPA-induced NPY gene expression was determined by Northern blotting. Treatments were performed as indicated in B. HSP70 was used to indicate equal RNA loading. The left panel shows representative Northern blots, whereas the right panel depicts the relative intensities of the bands.

Fig. 6. Ribavirin- and tiazofurin-induced replication arrest (A), vacuolization (B), and expression of prostasomal proteins. A. A day after cells were seeded in triplicate at $10^6$ cells per 100-mm plate, they were treated for 3 days with ribavirin or tiazofurin. Error bars represent the SD of three independent experiments. B. Vacuolization of cells after treatment for 3 days with 400 μmol/L ribavirin or tiazofurin ($\times 20$ magnification; scale bar, 50 μm). C. Induced expression of prostasomal proteins in cells treated for 3 days with 400 μmol/L ribavirin or tiazofurin or 20 μmol/L MPA. The expression was determined by Northern blotting. MCID software was used to analyze the intensities of the bands in the blots. HSP70 was used to normalize these intensities, and the relative increase in expression was calculated in arbitrary units (a.u.).
REFERENCES


ACKNOWLEDGMENTS

We thank Dr. G. Ronquist for providing monoclonal antibody mAb878. We thank Drs. M. Baccam, M. H. Bhattacharya, I. Gavrin, and M. Protic for reviewing the manuscript.

REFERENCES


19. Ariens F, Carlini E, Verdacci R, et al. Prostasome to sperm transfer of CD13/ -2-glycoprotein, aminopeptidase N/CD13, and GTP proteins, chaperone proteins, signal transduction proteins, GTP proteins, chaperone proteins, signal transduction proteins, and unannotated proteins. This analysis revealed 128 proteins that had not been previously associated with prostasomes.

Our data, together with those of other investigators, demonstrate the presence of prostasomes in PC-3 cells (48). Interestingly, prostasomal-like granules isolated from PC-3 cells were found to enhance sperm motility (48), a function typical of normal prostasomes (26). Therefore, prostate tumor cells such as PC-3 that can be induced to express a substantial number of prostasomal proteins should represent a useful prostate differentiation cell system.

To identify such proteins in our target PC-3 cells, we used two approaches. First, we tested the expression of a selected collection of prostasomal proteins by means of Northern and Western blotting or immunostaining. Because of the large number of reported prostasomal proteins (21), we decided to concentrate on proteins with an NH2-terminal signal peptide; these proteins would most likely be navigated into the prostasome. Our studies demonstrated that MPA induced a time-dependent or transient increase in expression of 10 proteins (CD46, CD55, CD59, clusterin, granulophysin/CD63, GRP78, NPY, zinc-n-a2-glycoprotein, aminopeptidase N/CD13, and a-galactosidase), whereas another 4 proteins (secretory actin-binding protein/PIP, gastrin, tissue factor, and vasoactive intestinal polypeptide) were expressed constitutively and were not affected by MPA.

To identify additional MPA-induced proteins, we used two-dimension gel electrophoresis technology. This approach yielded an interesting observation that among the induced proteins was keratin 17, a marker known to be involved in prostate cell differentiation (40). This led us to test for the presence of three other keratins, namely, keratins 8, 18, and 19, which are additional maturation markers of the normal prostate (40). We found that MPA induced the expression of keratin 19 but not keratin 8 or 18, which were constitutively expressed as also reported by others (49, 50).

Undifferentiated human basal prostate cells do not express keratin 8, 17, 18, or 19. These keratins are manifested as the cells progress from this state into an intermediate basal cell and turn into an intermediate differentiated and then into a well-differentiated luminal cell. The phenotype of the latter cell is characterized by the preservation of keratins 8 and 18, but not of keratin 17 or 19 (40).

Based on the fact that MPA induced the expression of about half of the tested prostasomal proteins, keratins 17 and 19, and vacuolization of the cytoplasm, we suggest that IMPDH inhibitors induce the differentiation of PC-3 cells into cells that resemble normal luminal prostate cells at their intermediate state of differentiation. Perhaps other, as yet unknown chemicals may be able to shift the PC-3 cells into the ultimate mature state. These agents may represent potential differentiation therapy drugs and, as such, should be tested in an animal model and thereafter in humans.
Differentiation of Human Prostate Cancer PC-3 Cells Induced by Inhibitors of Inosine 5′-Monophosphate Dehydrogenase

Daniel Floryk, Sandra L. Tollaksen, Carol S. Giometti, et al.

Cancer Res 2004;64:9049-9056.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/24/9049

Cited articles
This article cites 49 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/24/9049.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/64/24/9049.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.