Phenylacetate in Chemoprevention: In Vitro and in Vivo Suppression of 5-Aza-2'-Deoxycytidine-induced Carcinogenesis

Premakala Prasanna, Sonsoles Shack, Vincent L. Wilson, and Dvorit Samid

Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892 [P. P., S. S., D. S.], and Laboratory of Molecular Genetics/Oncology, Department of Pathology, The Children’s Hospital, Denver, Colorado 80218-1088 [V. L. W.]

ABSTRACT

Differentiation inducers selected for their low cytotoxic and genotoxic potential could be of major value in chemoprevention and maintenance therapy. We focus here on phenylacetate, a naturally occurring plasma component recently shown to affect the growth and differentiation of established neoplasms in experimental models. The ability of phenylacetate to prevent carcinogenesis by the chemotherapeutic hypomethylating drug 5-aza-2'-deoxycytidine (5AzadC) was tested in vitro and in mice. Transient exposure of immortalized, but poorly tumorigenic ras-transformed 4C8 fibroblasts to 5AzadC resulted in neoplastic transformation manifested by loss of contact inhibition of growth, acquired invasiveness, and increased tumorigenicity in athymic mice. The latter was associated with elevation in ras expression and a decline in collagen biosynthesis. These profound phenotypic and molecular changes were prevented by a simultaneous treatment with phenylacetate. Protection from 5AzadC-induced carcinogenesis by phenylacetate was: (a) highly efficient despite DNA hypomethylation by both drugs, (b) free of cytotoxic and genotoxic effects, (c) stable after treatment was discontinued, and (d) reproducible in vivo. Whereas athymic mice bearing 4C8 cells developed fibrosarcomas following a single i.p. injection with 5AzadC, tumor development was significantly inhibited by systemic treatment with nontoxic doses of phenylacetate. Phenylacetate and its precursor suitable for oral administration, phenylbutyrate, may thus represent a new class of chemopreventive agents, the efficacy and safety of which should be further evaluated.

INTRODUCTION

The multistep nature of neoplastic transformation makes this disease process amendable to chemopreventive intervention. Several agents have been shown to inhibit carcinogenesis and thereby prevent the development of primary or secondary cancers (1–4). Of major interest are natural products and their analogues, including vitamins (A, B12, C, D3, and E), retinoids, and terpenes. These agents can suppress neoplastic transformation subsequent to a carcinogenic insult by regulating cell growth and differentiation. We focus here on the efficacy of the growth regulator phenylacetate.

Phenylacetate is a common metabolite of phenylalanine implicated in growth control and differentiation in diverse organisms throughout phylogeny (5–7). Phenylacetate was recently shown to induce tumor cytostasis and reversal of cancer in tissue culture and in animal models (8–11). Although high drug concentrations were required to produce antitumor effects in the experimental systems (1–5 mM, a 1000-fold above physiological human plasma levels), no cytotoxicity or genotoxicity was observed (8). Consistent with the laboratory findings, clinical experience obtained during phenylacetate treatment of patients with urea cycle disorders indicates that millimolar levels can be achieved in humans without significant adverse effects (12, 13). The demonstratable antitumor activities, lack of toxicity, and convenient p.o. administration prompted us to explore the role of phenylacetate in chemoprevention.

The efficacy of phenylacetate as a chemopreventive agent was tested using in vitro and in vivo models of 5AzadC-induced carcinogenesis. Despite the promise of 5AzadC in the treatment of cancer and of β-chain hemoglobinopathies, its clinical applications have been hindered by concerns regarding carcinogenic potential (14–16). Our preliminary studies with embryonic mouse C3H 10T1/2 mesenchymal cells showed that high concentrations of phenylacetate (5–10 mM) prevent 5AzadC-induced neoplastic transformation; these studies were however limited by the low frequency of transformation (7 × 10⁻⁴; see Ref. 8). The model used in the present studies involved premalignant murine fibroblasts (cell lines 4C8 and PR4) which express a transcriptionally activated c-Ha-ras proto-oncogene. These poorly tumorigenic cells are highly susceptible to malignant conversion by pharmacological doses of 5AzadC (17, 18). We show here that phenylacetate can protect such vulnerable cells from 5AzadC-induced carcinogenesis both in culture and in mice.

MATERIALS AND METHODS

Cell Cultures and Reagents. The subclones of mouse NIH 3T3 fibroblasts, PR4N and 4C8-A10 (designated here PR4 and 4C8), have been described previously (19, 20). Both cell lines are phenotypic revertants isolated from long terminal

Received 8/24/94; revised 3/10/95; accepted 4/13/95.

1 This work was supported by funds from Elan Pharmaceutical Research Corporation through a Cooperative Research and Development Agreement (CACR-0139).

2 To whom requests for reprints should be addressed, at the Clinical Pharmacology Branch, National Cancer Institute, Building 10, Room 12C103, 9000 Rockville Pike, Bethesda, MD 20892.

3 The abbreviations used are: 5AzadC, 5-aza-2’-deoxycytidine; NaPA, sodium phenylacetate; NaPB, sodium phenylbutyrate; 5AzC, 5-azacytidine; 5mC, 5 methylcytosine.

4 S. Shack and D. Samid, unpublished data.
repeat/c-Ha-ras1-transformed 3T3 cells after long-term treatment with murine IFN-α/β. Cultures were maintained in DMEM supplemented with 10% heat-inactivated FCS (GIBCO) and antibiotics. The sodium salts of phenylacetic and phenylbutyric acids (Elan Pharmaceutical Corporation) were dissolved in distilled water. 5AzadC (Sigma, St. Louis, MO) was dissolved in PBS and stored in aliquots at −20°C until use. Exposure of 5AzadC to direct light was avoided at all times to prevent drug hydrolysis.

**Treatments with 5AzadC.** For treatment in culture, cells were plated at 1–2 × 10^5 cells in 100-mm dishes, and 5AzadC was added to the growth medium at 20 and 48 h later. The cells were subsequently subcultured in the absence of the nucleoside analogues and observed for phenotypic alterations. For our in vitro studies, we used 0.1 μM 5AzadC, a concentration within the pharmacological range that has previously been shown to efficiently transform the ras-expressing 3T3 cells (18). For in vivo treatment with 5AzadC, 6–9-week-old female athymic nude mice (Division of Cancer Treatment, National Cancer Institute Animal Program, Frederick Cancer Research Facility) were inoculated s.c. with 0.5 × 10^6 cells. Twenty-four h later 400 μg of freshly prepared 5AzadC in 200 μl PBS were administered i.p. into each animal (approximately 20 mg/kg). Systemic treatment with NaPA is described in the text.

**Growth and Invasion through Matrigel.** The ability of cells to degrade and cross tissue barriers was assessed by a qualitative in vitro invasion assay that utilized matrigel, a reconstituted basement membrane (Collaborative Research). Cells were exposed for 48 h in T.C. plastic dishes with 5AzadC alone or in combination with NaPA. 5AzadC treatment continued for an additional 1–2 weeks. Cells were then replated (at 5 × 10^5 point) onto 16-mm dishes (Costar), which were previously coated with 250 μl matrigel (10 mg/ml). NaPA was either added to the dishes or omitted in order to determine the reversibility of effect. Net-like formation characteristic of invasive cells occurred within 12 h; invasion into the matrigel was evident after 6–9 days. Quantitative analysis of invasion was performed using a Biocoat Matrigel invasion chamber (Becton Dickinson Labware, Bedford, MA) according to the manufacturer’s instructions. Briefly, cells pretreated with the tested drugs in culture were plated onto the upper chamber at a density of 3 × 10^5 cells/well and incubated at 37°C for 16–20 h. NIH 3T3-conditioned medium was used as a chemoattractant. Filters were then removed, fixed with methanol, and stained with Giemsa. Cells attached to the upper side of the matrigel membrane were removed, and the number of invading cells found on the inner side was determined by microscopy.

**Tumor Formation in Athymic Mice.** Cells were injected s.c. (5 × 10^5 cells/site) into 4–6-week-old female athymic nude mice (Division of Cancer Treatment, National Cancer Institute animal program, Frederick Cancer Research Facility). The number, size, and weight of tumors were recorded after 3–4 weeks. For histological examination, tumors were excised, fixed with methanol, and stained with hematoxylin and eosin. Cells attached to the upper side of the matrigel membrane were removed, and the number of invading cells found on the inner side was determined by microscopy.

**Measurement of DNA Methylation.** To determine the 5-methylcytosine content, samples of cultures were taken 24 h after the second 5AzadC treatment. The cell pellets were lysed in 0.5% SDS, 0.1 M NaCl, 10 mM EDTA (pH 8.0), added with 400 μg/ml proteinase K (Boehringer Mannheim), and stored at −70°C until DNA isolation and analysis. The content of methylated/unmethylated cytosine residues in the cellular DNA was measured by a 32P-postlabeling technique as described previously (19).

**RESULTS**

In Vitro Carcinogenesis Induced by 5AzadC and Its Prevention by Phenylacetate. Untreated 4C8 and PR4 formed contact-inhibited monolayers composed of epithelial-like cells. In agreement with previous observations (17, 18), transient exposure of these cultures to 0.1 μM 5AzadC during logarithmic phase of growth resulted in rapid and massive neoplastic transformation. Within 1 week of 5AzadC treatment, the great majority of the cell population became refractile and spindly in shape and formed multilayered cultures with increased saturation densities (Table 1), indicative of loss of contact inhibition of growth. These phenotypic changes could be prevented by the addition of NaPA. The effect was dose dependent: while 10 mM NaPA completely blocked neoplastic transformation (Table 1), lower doses had an intermediate effect (5 mM) or no significant effects (1 mM), as determined by cell morphology and contact inhibition of growth. The profound effect of the aromatic fatty acid observed with the higher dose could not be explained by cytostasis or cytotoxicity per se, as 10 mM NaPA caused only 45 ± 5% inhibition of 4C8 cell proliferation, and cell viability remained over 95% (reminiscent of its effect on other nonmalignant cells; Ref. 8). Therefore, all further studies used NaPA at 10 mM. Several different regiments of NaPA treatment were found to be similarly effective. These included: (a) pretreatment with NaPA, starting 1 day prior to the addition of 5AzadC; (b) simultaneous exposure to both drugs; and (c) addition of NaPA 1 day after 5AzadC. In all cases, cells were subsequently subjected to continuous treatment with NaPA for at least 1 week. Cells cultured under these conditions, like those treated with NaPA alone, formed contact-inhibited monolayers resembling untreated controls. These cells maintained the benign growth pattern for at least 3 weeks after NaPA treatment was discontinued.

That NaPA can prevent neoplastic transformation was further indicated by the inability of cells to invade reconstituted...
basement membranes (matrigel) and form tumors in athymic mice. When plated on matrigel, 5AzadC-transformed 4C8 and PR4 cells developed net-like structures characteristic of highly malignant cells, and eventually degraded the extracellular matrix components (Fig. 1). In marked contrast, NaPA-treated cultures formed small, noninvasive colonies on top of the matrigel, as previously observed with normal fibroblasts (Fig. 1 and Ref. 10). Untreated parental cells exhibited an intermediate phenotype, as their colonies were slow growing and noninvasive, yet irregular in shape possibly due to increased cell motility. The invasive capacity of cells was confirmed by a quantitative assay using invasion chambers with matrigel-coated filters (Table 1). Finally, the chemopreventive effect of phenylacetate could be mimicked by its precursor, phenylbutyrate. Cells exposed to 5AzadC and NaPB maintained contact-inhibited growth (Table 1).

The in vitro growth characteristics of cells correlated with their behavior in athymic mice. 5AzadC-treated 4C8 cells developed rapidly growing fibrosarcomas within 2 weeks of s.c. transplantation into mice. Consistent with their behavior in vitro, the parental cells were far less aggressive, forming small lesions after 3–4 weeks in three of eight recipient animals. However, no tumors developed in animals given injections of 4C8 cells that have been pretreated for 1 week in culture with the combination of 0.1 μM 5AzadC and 10 mM NaPB (Table 1). There was also no tumor formation in mice given injections of 4C8 cells treated with NaPB alone. It appears therefore that NaPB-induced phenotypic reversion of the premalignant fibroblasts and prevented their malignant conversion by the cytotoxic analogue.

Modulation of Gene Expression by NaPA. The NIH 3T3-derived cells lines 4C8 and PR4 carry an long terminal repeat-activated c-Ha-ras proto-oncogene. Northern blot analysis of 5AzadC-treated 4C8 cells (Fig. 2) revealed a significant increase in ras mRNA levels and a decline in the differentiation marker collagen α (type I) transcripts. No such changes in gene expression occurred in cultures to which 10 mM NaPB was added (Fig. 2). Withdrawal of NaPB after 1 week of continuous treatment did not cause restoration of ras expression, confirming that the therapeutic benefit of NaPB is stable in the absence of further treatment.

Effect of Phenylacetate and 5AzadC on DNA Methylation. 5AzadC is a potent inhibitor of DNA methylation, an epigenetic mechanism implicated in the control of gene expression and cell phenotype. Hypomethylation may underlay the therapeutic effect of 5AzadC in cancer and in severe inborn anemias (14–16, 21); however, changes in DNA methylation could also be responsible for its carcinogenic potential. It was of interest therefore to determine the degree of DNA methylation in cells protected by phenylacetate. As would be expected, 5AzadC caused a significant decrease in the content of 5mC (Table 2). There was, however, a comparable decline in 5mC in cells treated with 5AzadC in combination with NaPB as well as in those treated with NaPB alone (Table 2).

In Vivo Chemoprevention by NaPA. To determine the efficacy of NaPB in vivo, studies were extended to include an animal model involving athymic mice bearing the nontransformed 4C8 cells transplanted s.c. A single i.p. injection of mice with 5AzadC (20 mg/kg) resulted in tumor development at the site of 4C8 cell inoculation. However, when mice were pretreated with 400 mg/kg NaPB 1.5 h prior to 5AzadC injection, and NaPB treatment was continued for 22 days thereafter, the incidence of tumor formation was significantly decreased (Table 3 and Fig. 3). There were no adverse effects associated with NaPB treatment as indicated by animal weight and behavior. Furthermore, despite causing DNA hypomethylation, NaPB did not induce neoplastic transformation of transplanted 4C8 cells. The animal data are consistent with the in vitro findings, indicating that NaPB can prevent 5AzadC-induced carcinogenesis without producing significant toxicities.

### Table 1 In vitro prevention by phenylacetate of 5AzadC-induced carcinogenesis

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Saturation Densitya (cells/cm² × 10⁻²)</th>
<th>Invasivenessb</th>
<th>Tumorigenicity in micec</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.7 ± 0.3</td>
<td>-</td>
<td>3/8</td>
</tr>
<tr>
<td>5AzadC</td>
<td>7.3 ± 0.5</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td>5AzadC + NaPB</td>
<td>1.9 ± 0.4</td>
<td>-</td>
<td>0/8</td>
</tr>
<tr>
<td>5AzadC + NaPB</td>
<td>1.1 ± 0.2</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>NaPB</td>
<td>2.1 ± 0.3</td>
<td>-</td>
<td>0/8</td>
</tr>
<tr>
<td>NaPB</td>
<td>1.4 ± 0.1</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Cells were treated simultaneously with the indicated drugs and kept in culture for 5 days after confluence, at which time they were detached and counted. Exposure to 0.1 μM 5AzadC was transient, as described in "Materials and Methods," while treatment with 10 mM NaPB and 1.5 mM NaPA continued throughout the experiment. Similar results were obtained when NaPB treatment was initiated 1 day prior or after cell exposure to 5AzadC (data not shown). Data indicate mean ± SD (n = 3) of two experiments.

* Cells were plated on top of a matrigel layer and observed for malignant growth pattern, i.e., development of characteristic processes and degradation of the reconstituted basement membrane and invasion toward the plastic surface below. Quantitative analysis of invasion was performed using a Biocoat Matrigel invasion chamber, as described in "Materials and Methods." Data indicate mean ± SD of the number of invading cells (n = 3).

* Cells pretreated in culture were injected s.c. (5 × 10⁵ cells/site) into 2-month-old female athymic nude mice. Results determined after 3 weeks indicate tumor incidence (tumor-bearing/injected animals) and size. The values of tumor size are mean (range).

* ND, not determined.
Fig. 1 Growth on Matrigel. Cells pretreated with 0.1 μM 5AzadC alone or in combination with 10 mM NaPA were replated onto dishes coated with matrigel. Photographs of 5AzadC-treated 4C8 cells taken 5 days later (B) show formation of net-like structures characteristic of malignant fibrosarcoma cells. After 9 days, these cells degraded the matrigel barrier and formed monolayers on the plastic surface beneath (D). In a marked contrast, 4C8 cells exposed simultaneously to 5AzadC and NaPA (C), like untreated controls (A), failed to form “nets” and developed isolated small colonies incapable of matrigel degradation. The chemopreventive effect of NaPA was stable after treatment was discontinued, as indicated by the benign growth pattern on matrigel (not shown).

DISCUSSION

There is considerable interest in the use of nontoxic differentiation inducers in cancer chemoprevention. Drug toxicity is particularly important considering the overall health condition and variable life span of candidate populations, i.e., high-risk individuals and patients in remission. We show here that the differentiation inducer phenylacetate can prevent 5AzadC-induced carcinogenesis both in vitro and in vivo when used at nontoxic doses.

Chemoprevention can be accomplished by either blocking the “initiation” step of carcinogenesis (i.e., mutagenesis), or by suppressing “promotion” and progression to malignancy. The current studies, using premalignant cells with an activated ras oncogene as a model, examined the efficacy of phenylacetate as an antipromotional drug. Other well-characterized chemopreventive agents that block promotion include vitamin A and its synthetic retinoids; like phenylacetate, these compounds are also regulators of cell growth and differentiation (1–4).

The current studies exploited in vitro and in vivo models involving fibroblasts (designated 4C8 and PR4) that are highly vulnerable to malignant conversion by the DNA hypomethylating agents 5AzadC and 5AzaC (17, 18). Transient exposure of these cells to 5AzadC, either in culture or in recipient athymic mice, caused rapid transformation. Malignant conversion was associated with an increase in ras mRNA levels and downregulation of collagen type I expression, indicating loss of cell differentiation. These profound biological and molecular changes brought about by 5AzadC could be prevented by a simultaneous treatment with noncytotoxic concentrations of phenylacetate and its precursor, phenylbutyrate. Phenylacetate’s antitumor activity and lack of toxicity were confirmed in athymic mice. In the in vivo model, mice bearing the susceptible 4C8 cells transplanted s.c. were given injections i.p. of 5AzadC. All mice so treated developed rapidly growing fibrosarcomas; however, the incidence of tumor formation was markedly reduced by systemic treatment with NaPA.

The mechanism by which NaPA prevented the 5AzadC-induced malignant conversion is unclear. Like other chemopreventive agents that block promotion (1, 22), phenylacetate may act by inducing cytostasis and tumor maturation. There is a growing body of evidence indicating that phenylacetate can cause selective growth arrest and tumor differentiation in vitro and in rodent models (8–11). In some cases, e.g., promyelocytic leukemia, differentiation induced by phenylacetate was linked to...
a decline in v-my c oncogene expression (8). In NaPA-treated 4C8 cells, protection from dedifferentiation (evidenced by growth characteristics and collagen expression) was associated with inhibition of ras overexpression. Down-regulation of oncogene expression may thus be responsible in part for the chemopreventive activity of NaPA. In addition to affecting ras at the mRNA levels, phenylacetate, an inhibitor of the mevalonate pathway of cholesterol synthesis (11, 23), could also block the posttranslational modification of the ras-encoded protein p21. Limonene, an inhibitor of p21 prenylation, is a chemopreventive agent as well (24).

Phenylacetate blocked carcinogenesis by 5azaC despite the decline in 5mC content. In fact, NaPA itself was found to inhibit DNA methylation; yet, in contrast to 5azaC, NaPA was not carcinogenic (this study and Ref. 8). Correlations between carcinogenic potential and DNA hypomethylating activities of chemical agents have been previously documented in tissue culture models (25, 26), and alterations in DNA 5mC patterns were proposed to contribute and enhance the initiation of carcinogenesis (27-29). However, the present data indicate that quantitative changes in DNA methylation alone are not sufficient to affect cell phenotype, and, thus, hypomethylating activity is not sufficient to induce the tumorigenic phenotype in our in vitro and animal models.

These important differences in activities between 5azaC and NaPA need to be further addressed. There are unique features of these agents, particularly their effect on gene expression, which could underlie the differences in biological outcome. Although both agents may alter methylation patterns, they may not be equally capable of inducing the expression of some demethylated genes. The selective induction of specific genes by intracellular factors and chemical agents subsequent to demethylation has been reported by several laboratories (30-32). For example, an increase in human -beta-globin gene expression with subsequent accumulation of fetal hemoglobin in cultured erythroid progenitors and in humans (9, 33, 34). We now have evidence that, in addition to affecting DNA methylation, NaPA and NaPB activate the human peroxisome proliferator-activated receptor, a member of the nuclear steroid receptor superfamily that func-

![Image](image.png)

**Fig. 2** Alterations in gene expression. Northern blot analysis of cytoplasmic RNA (20 µg/lane) isolated from 4C8 cells 2 weeks after a transient exposure to 0.1 µM 5azaC. Lane 1, untreated control; lane 2, 5azaC; lane 3, 5azaC plus continuous treatment with 10 mM NaPA; lane 4, 5azaC plus 10 mM NaPA for 1 week only, to examine the reversibility of effect. The blot was reprobed sequentially with the indicated cDNA probes. Actin levels indicate the relative amounts of RNA loaded in each lane. col α1, collagen type 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatmenta</th>
<th>Tumor incidenceb</th>
<th>Tumor size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PBS</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>NaPA</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>5azaC + PBS</td>
<td>9/9</td>
<td>12 (2–29)</td>
</tr>
<tr>
<td>D</td>
<td>5azaC + NaPA</td>
<td>4/10</td>
<td>3 (0–10)</td>
</tr>
</tbody>
</table>

4C8 cells (5 × 10³/site) were transplanted s.c. into athymic mice. The next day, the animals were treated i.p. with 400 mg/kg NaPA and 1.5 h later with 20 mg/kg 5azaC. NaPA treatment was repeated at 4.5 h after 5azaC injection. Subsequent treatments involved NaPA (400 mg/kg) i.p. injections twice daily for 8 days, and once a day for another 2 weeks. PBS was used as a control.

Data indicate tumor growth at 4 weeks after 5azaC treatment. Spontaneous tumors developed thereafter in control animals receiving PBS and subsequently in those treated with NaPA.
regulating growth and differentiation have been conserved in evolution.

The outcome of combining NaPA with 5AzadC (or 5AzaC) is of particular interest. The cytosine analogues have been shown to benefit patients with severe disorders such as leukemia, melanoma, sickle cell anemia, and thalassemia (14–16, 37), presumably due to the activation of dormant, methylation-dependent genes (16, 21). Unfortunately, the clinical application of 5AzadC has been limited by concerns regarding carcinogenesis. Our data indicate that NaPA can minimize the carcinogenic risk while preserving and even potentiating the therapeutic effects of 5AzadC (8, 33).

It appears therefore that phenylacetate, a common amino acid derivative, may be of value as an antitumor and chemopreventive agent. NaPA, which has an unpleasant odor, can be substituted by its precursor, NaPB, for p.o administration (38). Upon ingestion by humans, phenylbutyrate undergoes β-oxidation to phenylacetate (39). Like NaPA, NaPB exhibits antitumor and chemopreventive activities in experimental models, and both drugs already proved safe for long-term p.o. treatment of children with urea cycle disorders (38). More recent clinical studies involving adults with cancer have confirmed that millimolar plasma levels of phenylacetate can be achieved with no significant adverse effects, and result in clinical improvement in patients with high-grade gliomas and hormone-refractory prostate cancer (40, 41). We speculate that NaPB or NaPA could potentially benefit high-risk individuals predisposed to cancer development, be applied in combination with other anticancer therapeutics to enhance efficacy and minimize adverse effects, and perhaps be used in maintenance therapy to prevent disease relapse. The role of phenylacetate and analogues in chemoprevention should be further evaluated.

REFERENCES


Phenylacetate in chemoprevention: in vitro and in vivo suppression of 5-aza-2'-deoxycytidine-induced carcinogenesis.

P Prasanna, S Shack, V L Wilson, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/1/8/865

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.