K-ras Mutations in 1,2-Dimethylhydrazine-induced Colonic Tumors: Effects of Supplemental Dietary Calcium and Vitamin D Deficiency

Xavier Llor, Russell F. Jacoby, Ba-Bie Teng, Nicholas O. Davidson, Michael D. Sitrin, and Thomas A. Brasitus

Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637

ABSTRACT
Recent studies from our laboratory have demonstrated that dietary supplemental calcium had no significant effect on the incidence of 1,2-dimethylhydrazine-induced colonic tumors, but did decrease the number of rats with multiple tumors and reduced tumor size. Moreover, concomitant vitamin D deficiency appeared to abolish these protective effects of calcium on colonic tumors in this experimental model. To date, however, the mechanism(s) involved in these phenomena remain unclear.

In order to address these important issues, 1,2-dimethylhydrazine-induced colonic tumors from animals on control, Ca²⁺-supplemented, vitamin D-sufficient, and Ca²⁺-supplemented, vitamin D-deficient diets were examined for the presence of ras oncogene mutations. DNA was extracted from each of these tumors. Targeted areas of K-ras and H-ras genes were amplified by the polymerase chain reaction and analyzed for point mutations using allele-specific oligonucleotide hybridization and subsequent DNA sequencing.

The results of these studies demonstrated that: (a) one-third of 1,2-dimethylhydrazine-induced colonic carcinomas in the control group had K-ras G to A mutations; (b) no mutations, however, were detected in the cancers of the calcium-supplemented group; (c) concomitant vitamin D deficiency abolished the antimutagenic effect of dietary calcium supplementation (e.g., ~one-third of cancers in this group again had detectable K-ras mutations); and (d) no H-ras point mutations were detected in colonic tumors from any group. These findings suggest that alterations in K-ras mutations may be one possible mechanism by which calcium and vitamin D status influence colonic carcinogenesis in this experimental model.

INTRODUCTION
Colorectal cancer is a leading cause of death due to solid malignancies in the United States (1). Although it has generally been recognized that dietary factors, such as fat, may play an important promotional role in the development of these cancers (2, 3), until recently, it was not appreciated that certain dietary constituents might also have antitumorigenic effects in this organ (3). During the past few years, however, dietary calcium and vitamin D, in particular, have received increasing attention with respect to their possible chemopreventive actions in the colon (4–20). While controversial (2, 21–24), several lines of evidence have now accumulated from studies in humans (4–6, 10–13), proposes that calcium and/or vitamin D might have direct antiproliferative and anticarcinogenic effects. In this regard, it is now clear that genetic alterations may contribute substantially to the pathogenesis of colon cancer (26–32). While several types of genetic alterations have been reported in human colon cancers (26), one potentially important genetic alteration is ras gene mutation(s). Point mutations in ras protooncogenes, for example, have been demonstrated in 40 to 65% of malignant colorectal tumors (26–28). Recently, our laboratory has, in fact, also reported a high incidence of guanine to adenine K-ras gene mutations in DMH-induced tumors (32). This latter finding is of considerable interest since DMH, like other methylating agents, is thought to induce such point mutations via O⁶-methylguanine residue formation (33, 34), leading to G to A transitions as a consequence of its mispairing properties (35).

In view of the observation that calcium, presumably via calmodulin, may influence DNA repair (36), it was of interest to determine whether supplemental dietary calcium alone or in conjunction with vitamin D deficiency influenced the frequency or type of K-ras and H-ras mutations present in these DMH-induced tumors (20). In the present experiments, therefore, DNA was extracted from the tumors of rats fed three different diets (control, Ca²⁺ supplemented, and calcium-supplemented, vitamin D-deficient diets) which were previously reported (20). Targeted areas of K-ras and H-ras genes were then amplified by the polymerase chain reaction and analyzed for point mutations using allele-specific oligonucleotide hybridization and subsequent DNA sequencing. The results of these experiments as well as a discussion of the significance of these findings to colon carcinogenesis in this experimental model serve as the basis for this present report.

Received 1/11/91; accepted 6/4/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This investigation was supported by Grant CA36745 from the National Cancer Institute; Digestive Disease Research Center Grant DK42086 from the National Institutes of Diabetes and Digestive and Kidney Diseases; Grants HL 38180 and KO-4 HL 02166 from the Heart, Lung, and Blood Institutes; Clinical Nutrition Research Unit Grant DK 26678; and the Samuel Freedman Research Laboratories for Gastrointestinal Research. T. A. B. is the recipient of a Merit Award from the National Cancer Institute, NIH.

2 To whom requests for reprints should be addressed, at The University of Chicago Hospitals and Clinics, 5841 S. Maryland Avenue, Box 400, Chicago, IL 60637.

3 The abbreviations used are: DMH, 1,2-dimethylhydrazine; PCR, polymerase chain reaction; MGMT, O⁶-methylguanine-DNA methyltransferase.

The mechanism(s) involved in these phenomena, however, remain unclear. Supplemental calcium has been shown in some studies to reduce colonic proliferation in patients at high risk for the development of colon cancer (10–13) as well as inhibit the increased colorectal proliferation induced by bile salts and fatty acids, putative colon tumor promoters, in experimental animals (14–16). Based on these findings, it has been speculated that supplemental dietary calcium may lead to an increase in colonic luminal Ca²⁺ concentration which could reduce the promotional effects of free bile salts or fatty acids by converting them to insoluble Ca²⁺ soaps (25).

An alternative hypothesis, based on in vitro studies in human colon epithelial cells (11, 13, 17), proposes that calcium and/or vitamin D might have direct antiproliferative and anticarcinogenic effects. In this regard, it is now clear that genetic alterations may contribute substantially to the pathogenesis of colon cancer (26–32). While several types of genetic alterations have been reported in human colon cancers (26), one potentially important genetic alteration is ras gene mutation(s). Point mutations in ras protooncogenes, for example, have been demonstrated in 40 to 65% of malignant colorectal tumors (26–32). Recently, our laboratory has, in fact, also reported a high incidence of guanine to adenine K-ras gene mutations in DMH-induced tumors (32). This latter finding is of considerable interest since DMH, like other methylating agents, is thought to induce such point mutations via O⁶-methylguanine residue formation (33, 34), leading to G to A transitions as a consequence of its mispairing properties (35).

In view of the observation that calcium, presumably via calmodulin, may influence DNA repair (36), it was of interest to determine whether supplemental dietary calcium alone or in conjunction with vitamin D deficiency influenced the frequency or type of K-ras and H-ras mutations present in these DMH-induced tumors (20). In the present experiments, therefore, DNA was extracted from the tumors of rats fed three different diets (control, Ca²⁺ supplemented, and calcium-supplemented, vitamin D-deficient diets) which were previously reported (20). Targeted areas of K-ras and H-ras genes were then amplified by the polymerase chain reaction and analyzed for point mutations using allele-specific oligonucleotide hybridization and subsequent DNA sequencing. The results of these experiments as well as a discussion of the significance of these findings to colon carcinogenesis in this experimental model serve as the basis for this present report.
MATERIALS AND METHODS

Materials

Dietts were purchased from ICN Biochemicals (Cleveland, OH). Deoxynucleotides (dATP, dGTP, dCTP, dTTP) and T4 polynucleotide kinase (10,000 units/ml) were obtained from Pharmacia (Piscataway, NJ). DNA sequencing was performed using Sequenase from USB (Cleveland, OH). Anion-exchange columns (Quiagen tip-MATERIALS AND METHODS) and phase extraction columns (SEP-PAK C18 cartridges) were from Waters (Milford, MA). All other reagents and chemicals were of the highest grade commercially available.

Methods

Animals and Dietary Protocols. As recently described in detail (20), weaning male albino Sprague-Dawley rats, initially in the range of 40 to 60 g, were randomly assigned to one of three dietary groups with 14 to 15 rats in each group (Ca2+ and phosphorus proportions by weight as percentage): (a) control diet, containing Ca2+ and phosphorus at 0.87% and 0.60% phosphorus, respectively, and 2.2 IU of vitamin D3 per g of feed; (b) Ca2+ supplemented, as above, but containing Ca2+ and phosphorus at 1.80% and 0.80%, respectively; and (c) vitamin D deficient, Ca2+ supplemented, animals consuming a vitamin D-deficient diet, containing Ca2+ and phosphorus at 1.80% and 0.80%, respectively. All groups consumed their respective diets and water ad libitum for 33 wk. Details of the diet composition have been recently described (20). After an initial 6-wk period, all rats were given injections s.c. of 20 mg/kg of body weight 1,2-dimethylhydrazine weekly for 26 wk. Animals were sacrificed 1 wk following the last injection. All tumors were harvested individually, and sections of each tumor were fixed in formalin and subject to histological confirmation (20). DNA was extracted using the techniques of Perucho et al. (37) from 11 of 16 tumors in the control group, 9 of 11 tumors in the Ca2+-supplemented group, and 12 of 17 tumors in the Ca2+-supplemented, vitamin D-deficient group. In this regard, it should be noted that certain tumors in each group (see above) could not be analyzed because they were used for other analyses (20).

PCR Amplification of ras Oncogene Sequences. The PCR was used to amplify K-ras exon 1 or exon 2 and H-ras exon 1 gene between nucleotides 172 to 287, 295 to 464, and 174 to 241, respectively (38, 39), as previously described (32). The amplified products were analyzed on agarose gels to confirm the size of the amplified fragments; for K-ras exon 1 and exon 2 and H-ras exon 1, the fragments were 116 base pair pairs, 169 base pairs, and 68 base pairs, respectively.

Analysis of ras Mutations by Oligonucleotide Probe Hybridization. PCR-amplified DNAs were immobilized on nitrocellulose membranes and hybridized to 32P-labeled oligonucleotide probes corresponding to the potential activating ras mutations as described previously (32). The membranes were washed at low and high stringency, with optimum high stringency wash temperatures of 58°C–60°C and 43°C–45°C for 20 mer and 15 mer wash oligonucleotide probes, respectively. Membranes were exposed to Kodak XAR films with intensifier screens at −70°C for 1 h and also overnight (32).

Direct Sequencing of Amplified DNA. Amplified DNA was purified over Quiagen columns and sequenced by the dideoxynucleotide chain termination method using Sequenase and a 32P-labeled internal primer (40–42). Purified DNA was denatured in annealing buffer [40 mM Tris-HCl (pH 7.5)-20 mM MgCl2;50 mM NaCl] for 3 min at 90°C and then annealed to the internal primer for 15 min at 55°C. The subsequent products were analyzed by 8% polyacrylamide-urea gel electrophoresis.

Statistical Analysis. All statistical analyses were performed in the present experiments using Fisher’s exact test (43). P values are presented in the text.

RESULTS

General Observations. As recently described in detail (20), there were no significant differences in final body weights of the three dietary groups of rats administered DMH. There were also no significant differences in the incidence of colonic tumors, but the Ca2+-supplemented diet significantly decreased the number of tumors/tumor-bearing animal (1.8 tumor for control versus 1.1 tumor for supplemented Ca2+, vitamin D-sufficient diet). Concomitant vitamin D deficiency negated this protective effect of the Ca2+-supplemented diet (1.9 tumor/tumor-bearing rat) (20). Tumors in the Ca2+-supplemented group also tended to be smaller than those in the other two groups (20). The majority of tumors in all groups were moderately well to poorly differentiated colonic adenocarcinomas, as assessed by light microscopy (20). Adenomas or carcinomas in situ represented 2 of 16, 3 of 11, and 1 of 17 tumors in the control, Ca2+-supplemented, and Ca2+-supplemented, vitamin D-deficient dietary groups, respectively (20).

ras Mutations. As noted above, 11, 9, and 12 tumors from control, Ca2+-supplemented, and Ca2+-supplemented, vitamin D-deficient groups were analyzed for ras mutations. All of the tumors analyzed in this study were adenocarcinomas.

Analysis for ras-activating point mutations was performed by allele-specific oligonucleotide hybridization after amplification of targeted areas of exon 1 and exon 2 of K-ras and exon 1 of H-ras. The DNA from 4 of 11 cancers analyzed in the control group and 4 of 12 cancers analyzed in the Ca2+-supplemented, vitamin D-deficient group was found to possess mutations involving codons 12 and 13 of K-ras exon 1. No such K-ras mutations, however, were detected in the DNA analyzed from 9 cancers of the Ca2+-supplemented group. The difference in the prevalence of K-ras mutations between the dietary control and Ca2+-supplemented groups was significant at P = 0.06 by the two-tailed Fisher’s exact test. Between Ca2+-supplemented and Ca2+-supplemented, vitamin D-deficient groups, the difference was significant at P = 0.08. As shown in Fig. 1 and Table 1, all mutations detected were exclusively G to A substitutions of the second nucleotide of codons 12 and 13 of the K-ras gene. No mutations were found in either exon 2 of K-ras or in exon 1 of H-ras in tumor DNA from all three groups. Subsequently, exon 1 of K-ras was sequenced in all samples, providing direct confirmation of the differential hybridization results (Fig. 2). The presence and nature (G to A mutations) of these K-ras mutations as well as the absence of H-ras gene nucleotide substitutions, in general, mimic the pattern of ras gene alterations described in human colorectal tumors, although in human colon cancers G to T and G to C mutations have also been reported (26, 44, 45). Additionally, two of the K-ras mutations found in the control diet group were from two different tumors arising in the same rat and involved different codons, suggesting that they arose independently.

Finally, while as noted above, there was a tendency for tumors in the Ca2+-supplemented group to be smaller than their counterparts in the other groups (20), there were no significant differences in the size of carcinomas with or without mutations in either the control or Ca2+-supplemented, vitamin D-deficient adenocarcinomas. Specifically, tumor sizes in control animals (6.9 ± 2.2 mm; range, 3 to 10 mm; n = 7 without mutation versus 6.0 ± 1.8 mm; range, 4 to 8 mm; n = 4 with mutation, P > 0.05) were compared with Ca2+-supplemented, vitamin D-deficient animals (6.8 ± 2.5 mm; range, 4 to 11 mm; n = 7 without mutations versus 11.3 ± 9 mm; range, 3 to 23 mm; n = 4 with mutation, P > 0.05). These data suggest, however, a trend toward larger tumor size in this Ca2+-supplemented, vitamin D-deficient group bearing ras mutation, but this will require further study and additional confirmation.

Downloaded from cancerres.aacrjournals.org on March 30, 2020, © 1991 American Association for Cancer Research.
confirmed by DNA sequencing as shown in Fig. 2 and are summarized in Table 1. PCR-amplified DNAs for K-ras exon 1 were immobilized on nitrocellulose in duplicate dot blots and hybridized to either normal or mutant allele-specific probes end labeled with $^{32}$P. The autoradiograms in a demonstrate one carcinoma with a codon 12 (GGT-GAT) mutation and in b demonstrate three different carcinomas with codon 13 (GGC-GAC) mutation. Other autoradiograms (not shown) demonstrated three more carcinomas with codon 12 (GGT-GAT) mutation and in b demonstrate three different carcinomas with codon 13 (GGC-GAC) mutation. Other autoradiograms (not shown) demonstrated three more carcinomas with codon 12 (GGT-GAT) mutation and in b demonstrate three different carcinomas with codon 13 (GGC-GAC) mutation. No mutations were detected in similar hybridizations to 10 other K-ras codon 12 or codon 13 probes corresponding to the other possible mutations at these sites. Similarly, no mutations were detected in codon 59 or codon 61 of K-ras or in codon 12 or codon 13 of the H-ras genes.

**DISCUSSION**

In basic agreement with earlier studies from our laboratory (32), the present results demonstrate that approximately one-third of DMH-induced carcinomas in rats consuming the control diet have K-ras mutations, as assessed by differential oligonucleotide probe hybridization and confirmed by DNA sequencing. All were G to A mutations in the second base of codons 12 and 13. Additionally, no H-ras mutations were detected in these tumors. As noted earlier (32), these G to A transitions are consistent with the production of O$^\beta$-methylguanine adducts by DMH (33-35). Such adducts have been shown to result in G:C to A:T transitions by mispairing with thymidine during the replication of DNA (35). The selective nature of these mutations in codons 12 and 13 may be due to either differences in the accessibility of these guanine residues to DMH, as has been shown for other methylating agents' effects on ras oncogenes (35), or alternatively to differential repair rates of these adducts (46) as well as other possible effects.

In contrast to the control group, no mutations were detected in any of the tumors analyzed from the DMH-treated animals given supplemental dietary calcium. The mechanism(s) responsible for this apparent phenomenon remain unclear at this time, but include the possibility that dietary calcium might directly interfere with the production of O$^\beta$-methylguanine adducts. No experimental evidence, to date, however, would support such a contention. Calcium might alternatively enhance DNA repair of O$^\beta$-methylguanine residues, thereby preventing such mutations. Such adducts in DNA are removed by a repair protein, MGMT (47, 48). Repair of O$^\beta$-methylguanine by MGMT may be modulated by several factors including: (a) various cations (47); (b) cell cycle-dependent transcription (48); (c) degradation by proteases (49); and (d) electrostatic and noncovalent effects on DNA binding (47). While each of these possibilities will have to be addressed in future studies, it should be noted that in vitro addition of calcium has not been found to influence the properties of purified MGMT from the rat liver (47), making the first possibility less likely. Alternatively, since cells in S phase are more susceptible to mutagens (50), it is possible that the antimutagenic effects of intracellular calcium may be due to its direct antiproliferative actions on DMH-induced colonic hyperplasia (51). Regardless of the mechanism(s) involved, however, the present data would suggest that supplemental calcium in the diet may reduce the incidence of K-ras mutations in DMH-induced cancers.

Finally, concomitant vitamin D deficiency appeared to abolish the antimutagenic effects of dietary calcium supplementation in DMH-treated rats. These effects of vitamin D deficiency could be mediated by calcium. The present data would suggest that intracellular, rather than intraluminal, calcium may be a more important determinant of these effects. In support of this

Table 1 K-ras mutations in colon carcinomas from rats fed control, calcium-supplemented, or calcium-supplemented vitamin D-deficient diets

<table>
<thead>
<tr>
<th>Colon carcinoma</th>
<th>K-ras codon 12</th>
<th>K-ras codon 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (A)*</td>
<td>A-</td>
<td>A-</td>
</tr>
<tr>
<td>2 (B)</td>
<td>-A,-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4 (A)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9 (B)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$-supplemented diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$-supplemented vitamin D-deficient diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>A-</td>
<td>-</td>
</tr>
<tr>
<td>25 (C)</td>
<td>-A,-</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27 (C)</td>
<td>A-</td>
<td></td>
</tr>
<tr>
<td>28 (D)</td>
<td>-A,-</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>A-</td>
<td>-</td>
</tr>
<tr>
<td>31 (D)</td>
<td>-A</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Capital letters in parentheses, tumors derived from the same animal.
- Dashes (-), normal sequence.
possibility, vitamin D deficiency has previously been demonstrated to reduce small intestinal and colonic calcium absorption and to lead to net colonic calcium secretion in the rat (52).

Recently, it has also become increasingly evident that the major active metabolite of vitamin D₃, 1,25-dihydroxycholecalciferol, may have a myriad of effects in addition to its well-known role in mineral metabolism (53–55). For example, 1,25-dihydroxycholecalciferol may serve as an immunoregulatory hormone as well as a differentiating hormone (54). This seocsteroid also appears to play an essential regulatory role in the expression of certain oncogenes and lymphokines, as well as replication-linked genes (54). Based on these observations it is, therefore, possible that vitamin D deficiency per se may have served to offset the protective effects of calcium supplementation on colonic mutagenesis and carcinogenesis.

In summary, the present studies when taken together with our previous findings (20) indicate that dietary calcium supplementation may inhibit DHH-induced colonic tumorigenesis, whereas vitamin D deficiency appears to abolish this protective effect of calcium. These studies support the testable hypothesis that K-ras mutations may be one possible mechanism by which these nutrients influence colonic carcinogenesis in this model. Future studies to further confirm these observations and define the mechanism(s) involved in the molecular pathogenesis of colon cancer are of interest and are currently being performed in our laboratory.

ACKNOWLEDGMENTS

The authors thank Lynn Nelson for her excellent secretarial support.

REFERENCES


25. Newmark, H. L., Wargovich, M. J., and Bruce, W. R. Colon cancer and
C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L.
4689, 1989.
progressive stages of human colon carcinomas. Proc. Natl. Acad. Sci. USA,
29. Stanbridge, E. J. Identifying tumor suppressor genes in human colorectal
30. Weinberg, R. A. Oncogenes, antioncogenes, and the molecular basis of
31. Marx, J. Many gene changes found in cancer. Science (Washington DC),
Mutations in the K-ras oncogene induced by 1,2-dimethylhydrazine in pre-
neoplastic and neoplastic rat colonic mucosa. J. Clin. Invest., 87: 624–630,
33. Rogers, K. J., and Pegg, A. E. Formulation of O6-methylguanine by alkalytion
of rat liver, colon, and kidney DNA following administration of 1,2-di-
34. Pegg, A. E. Methylation of the O6 position of guanine in DNA is the most
likely initiating event in carcinogenesis by methylating agents. Cancer Invest.,
35. Mitra, S., Pauly, G. T., Kumar, R., Pei, G. K., Hughes, S. H., Moschel, R.
C., and Barbadic, M. Molecular analysis of O6-substituted guanine-induced
1989.
36. Rasmussen, C. D., and Means, A. R. Calmodulin is involved in regulation
37. Perucchini, M., Goldfarb, M., Shinizu, K., Lama, C., Fogh, J., and Wigler, H.
Human-tumor-derived cell lines contain common and different transforming
38. Cagion, D. J., Seebarg, P. H., McGrath, J. P., Hayflick, J. S., Edman, U.,
Levinson, A. D., and Golddel, D. V. Activation of Ki-ras-2 gene in human
colon and lung carcinomas by two different point mutations. Nature (Lond.),
K-ras Mutations in 1,2-Dimethylhydrazine-induced Colonic Tumors: Effects of Supplemental Dietary Calcium and Vitamin D Deficiency

Xavier Llor, Russell F. Jacoby, Ba-Bie Teng, et al.


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/51/16/4305

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, use this link http://cancerres.aacrjournals.org/content/51/16/4305.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.