Methyl Groups in Carcinogenesis: Effects on DNA Methylation and Gene Expression

Elvise Wainfan² and Lionel A. Poirier

Abstract

Lipotrope-deficient (methyl-deficient) diets cause fatty livers and increased liver-cell turnover and promote carcinogenesis in rodents. In rats prolonged intake of methyl-deficient diets results in liver tumor development. The mechanisms responsible for the cancer-promoting and carcinogenic properties of this deficiency remain unclear. The results of the experiments described here lend support to the hypothesis that intake of such a diet, by causing depletion of S-adenosylmethionine pools, results in DNA hypomethylation, which in turn leads to changes in expression of genes that may have key roles in regulation of growth. In livers of rats exposed to cancer-promoting chemicals and in hepatomas, Northern blot analysis of polyadenylated RNAs from livers of rats fed control or deficient diets showed that, after 1 week of MDD intake, there were decreased levels of expression of c-myc and c-fos oncogenes, somewhat smaller increases in c-Ha-ras mRNA, and virtually no change in levels of c-Ki-ras mRNA. In contrast, mRNAs for epidermal growth factor receptor decreased significantly. The elevated levels of expression of the c-myc, c-fos, and c-Ha-ras genes were accompanied by selective changes in patterns of methylation within the sequences specifying these genes. Changes in DNA methylation and in gene expression induced in livers of rats fed MDD for 1 month were gradually reversed after restoration of an adequate diet. In hepatomas induced by prolonged dietary methyl deficiency, methylation patterns of c-Ki-ras and c-Ha-ras were abnormal. Although human diets are unlikely to be as severely methyl deficient as those used in these experiments, in some parts of the world intake of diets that are low in methionine and choline and contaminated with mycotoxins, such as aflatoxin, are common. In industrialized nations, deficiencies of folate acid and vitamin B₁₂ are not uncommon and are exacerbated by some therapeutic agents and by substance abuse. Thus, it seems possible that interactions of diet and contaminants or drugs, by inducing changes in DNA methylation and aberrant gene expression, may contribute to cancer causation in humans.

Introduction

Diet and exposure to toxic chemicals are among the environmental factors that are believed to play roles in causing human cancer. There is extensive evidence that carcinogenesis in rodents is significantly influenced by dietary supplies of lipotropes, a group of nutrients that includes choline, methionine, folate acid, and vitamin B₁₂ (1–7). These substances interact in the regulation of the intracellular supply and metabolism of methyl groups, particularly in the form of AdoMet (⁸⁻¹⁰). Lipotrope-deficient or methyl-deficient diets cause extensive liver damage including fatty livers, induce cell turnover, and promote liver carcinogenesis in rats and certain strains of mice (1–7). In male Fischer 344 rats and C57BL/6 × C3H F₁ (thereafter called B6C3F₁) mice, prolonged intake of methyl-deficient diets, even without exposure to any known carcinogens, has been observed to result in development of liver tumors (1, 3, 9, 10). In contrast, diets enriched with nontoxic levels of choline and methionine can prevent or diminish the effects of some chemical carcinogens (4, 5, 11–13) and prolong the survival of spontaneously leukemic AKR mice (14).

Although the carcinogenic potential of methyl-deficient diets has been known for many years, the mechanisms responsible remain unclear. The studies to be described here represent efforts to test the hypothesis that dietary methyl deficiency promotes liver-cancer development by inducing hypomethylation of DNA, with consequent alterations in the expression of genes that have critical roles in the regulation of growth and differentiation (1, 4, 5). These effects on DNA methylation and gene expression are mediated through the decreased supply of AdoMet, which results from intake of such a diet. This putative mechanism may be considered as one facet of a more general hypothesis that states that alterations in patterns of DNA methylation play a key role in the process of carcinogenesis (5, 15–18). Such changes in DNA methylation may be due to a variety of causes, including loss of methylation sites by various types of mutations, steric blockage of methylation sites by binding of alkyl group donors to DNA, and/or a deficiency of methyl group donor molecules (15–18). We describe here studies carried out to determine whether the molecular effects of dietary methyl deficiency are consistent with this hypothesis. The early changes in gene methylation and expression in the livers of rats fed methyl-deficient diets have been compared with those seen in liver tumors resulting from long-term intake of such diets. In the design of these experiments, we have kept in mind that the early effects of promoters often can be reversed after exposure to the promoter is terminated (19–21)

Materials and Methods

Animals and Diets. Male Fischer 344 rats were used in two types of feeding experiments, short term and chronic. The basic diet was AAD, an amino acid-defined semisynthetic diet that lacked choline, methionine, folic acid, and vitamin B₁₂ (22–24). To prepare the adequate diet (CS), the following nutrients were added to AAD: 5.0–5.2 g/kg DL-methionine, 2 g/kg choline chloride, 5 mg/kg folic acid, and 50–100 μg/kg vitamin B₁₂. MDD was prepared by supplementing AAD with 9 g/kg DL-homocysteine only. Another diet (SD), lacking only choline and methionine, was prepared by supplementing AAD with 9 g/kg homocysteine, 5 mg/kg folic acid, and 50 μg/kg vitamin B₁₂. Food and water were supplied ad libitum. The chronic feeding studies were carried out in the laboratories of Dr. L. A. Poirier, at the National Center for Toxicological Research and the National Cancer Institute, using weanling rats. CSD and SD were fed for these long-term studies. Most of the short-term experiments were carried out in the laboratory of Dr. E. Wainfan at the New York Blood Center, in collaboration with Dr. J. K. Christman of the Michigan Cancer Foundation. In these studies...
CSD and MDD were fed for periods of 1–4 weeks to rats that were 10–12 weeks old when the experiments were started.

AdoMet, AdoHcy, and 5-MC Analyses. The control (CSD) and deficient diets (SD or MDD) were fed for 22 weeks. Animals were killed at 4, 8, and 22 weeks. Livers were immediately excised and processed for extraction and analyses of AdoMet and AdoHcy or for preparation of DNA, as previously described (22, 23). AdoMet, AdoHcy, and 5-MC were determined by high performance liquid chromatography, by using the procedures described by Wilson et al. (23).

Methylation Assays. The assay for rat liver DNA methyltransferase activity measures the ability of the enzyme to catalyze the in vitro transfer of labeled methyl groups from AdoMet to heterologous acceptor DNA or hypomethylated liver DNA (25, 26). Similarly, the assay for tRNA methyltransferase measures the in vitro transfer of methyl groups to heterologous tRNAs or to hypomethylated liver tRNA (24, 26, 27). For enzyme-activity assays, incubation was carried out under conditions where only enzyme was limiting. To assess hypomethylation of liver tRNA and DNA, the isolated tRNA or DNA was used as the acceptor substrate for in vitro enzymatic methylation catalyzed by homologous enzymes, under conditions where only the quantity and acceptor capacity of the substrates were limiting. The methods for these assays were previously described in detail (26).

mRNAs for Specific Genes. Northern blot analysis was performed as previously described, with 5-μg samples of poly(A)+ RNA isolated from livers of animals fed control or methyl-deficient diets (28). Electroelution was carried out in 1% agarose gels containing 6% formaldehyde. Plasmid DNAs obtained from the American Type Culture Collection (Rockville, MD) for use as probes include pBS9 for c-Ha-ras, containing a 460-base pair EcoRI fragment of Ha-MuSV (29); pHiHi3 for c-Ki-ras, containing a 580-base pair SstI/EcoRI fragment of Ki-MuSV (29); pSVcmyc1 for c-myc, containing a 4.8-kilobase pair pMsgF-26F12 for EGF, containing a 960-base pair PstI fragment of mouse EGF (32); and pHER-A64-1 for EGFR, containing a 1.84-kilobase pair EcoRI fragment of human EGFR (33).

Methylation Patterns of Specific Genes. DNA was prepared from rat livers as described in Current Protocols in Molecular Biology (34). The purified DNAs were digested with various restriction endonucleases and subjected to electrophoresis in 1% agarose gels, followed by Southern blotting performed under standard conditions. Probes were labeled with [3H]AdoMet into acid-precipitable material was measured as described in Refs. 22 and 23. For assay of methyl group acceptance by DNA, each assay tube contained 2 μg rat liver DNA and 1 unit DNA methyltransferase from Friend erythroleukemia cells. Incorporation was for 1 h at 37°C. Incorporation of methyl groups from [methyl-3H]AdoMet into acid-precipitable material was measured as described in Refs. 22 and 23. To measure methyl group acceptance by tRNA, tubes containing 25 μg rat liver tRNA and excess rat liver enzymes were incubated for 1 h at 37°C under conditions described previously (24). After appropriate washing, incorporation of methyl groups from [methyl-3H]AdoMet into acid-precipitable material was determined (12, 24).

MDD for periods of 4–28 days were assayed for hypomethylation with highly sensitive methods. These assays measure the capacity of the nucleic acids to accept methyl groups from AdoMet in reactions catalyzed by homologous enzymes in vitro. Increased acceptor capacity is indicative of decreased methylation in vivo (24–27). As shown in Table 2, in livers of rats given MDD, hypomethylated tRNA was found as early as 4 days after feeding of this diet was started. Hypomethylated DNA became detectable after 7 days. The extent of DNA hypomethylation increased when the period of MDD feeding was extended beyond 1 week (Table 2) (26). As shown earlier, there is a simultaneous increase in thymidine incorporation into newly synthesized DNA in the livers of MDD-fed rats (26). The liver DNA of rats fed SD, a diet lacking choline and methionine, for 2 months or longer also was seen to be hypomethylated (Table 1) (4, 23). These combined results indicate that exposure to dietary methyl deficiency results quickly in production of hypomethylated DNA and that hypomethylation is continuously maintained when such a deficient diet is con-

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>CSD</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>AdoMet (nmol/g)^a</td>
<td>96.9 ± 5.3</td>
<td>49.8 ± 2.3</td>
</tr>
<tr>
<td>AdoHcy (nmol/g)^a</td>
<td>23.4 ± 0.7</td>
<td>34.7 ± 1.8</td>
</tr>
<tr>
<td>AdoMet:AdoHcy</td>
<td>4.1 ± 0.3</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>5-MC (%)^d</td>
<td>3.42 ± 0.05</td>
<td>3.32 ± 0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>8 wk</th>
<th>22 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdoMet</td>
<td>86.2 ± 2.8</td>
<td>56.1 ± 1.5</td>
</tr>
<tr>
<td>AdoHcy</td>
<td>22.4 ± 1.0</td>
<td>21.2 ± 0.8</td>
</tr>
<tr>
<td>AdoMet:AdoHcy</td>
<td>4.3 ± 0.5</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>5-MC (%)^d</td>
<td>3.33 ± 0.03</td>
<td>3.13 ± 0.05</td>
</tr>
<tr>
<td>22 wk</td>
<td>3.27 ± 0.04</td>
<td>2.81 ± 0.04</td>
</tr>
</tbody>
</table>

^a nmol/g tissue.
^b Mean ± SE.
^c Significantly different from CSD, P < 0.02.
^d Percentage of total deoxycytidine in DNA.

### Table 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>MDD</th>
<th>CSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (cpm [3H]CH, accepted/2 μg)</td>
<td>21,120</td>
<td>6,670</td>
</tr>
<tr>
<td>tRNA (cpm [3H]CH, accepted/25 μg)</td>
<td>1,075</td>
<td>275</td>
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<table>
<thead>
<tr>
<th>Diet</th>
<th>MDD</th>
<th>CSD</th>
</tr>
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<tbody>
<tr>
<td>4 days</td>
<td>11,560</td>
<td>6,700</td>
</tr>
<tr>
<td>7 days</td>
<td>11,720</td>
<td>6,700</td>
</tr>
<tr>
<td>14 days</td>
<td>16,750</td>
<td>8,700</td>
</tr>
<tr>
<td>21 days</td>
<td>17,750</td>
<td>8,700</td>
</tr>
<tr>
<td>28 days</td>
<td>20,180</td>
<td>8,700</td>
</tr>
<tr>
<td>28 days</td>
<td>21,120</td>
<td>8,700</td>
</tr>
</tbody>
</table>

Results

Rapid changes in processes involving transmethylation have been found to occur in the livers of rats fed an amino acid-defined, methyl-deficient diet. In animals fed such a diet for 1 week, levels of AdoMet, the methyl group donor for most transmethylation reactions (1, 8, 38), were found to be significantly lower than those seen in rats fed the same diet supplemented with adequate quantities of lipotropes (Table 1) (22). AdoMet levels were seen to decline further as the period of feeding of the methyl-deficient diet was prolonged. In addition, as seen in Table 1, the ratio of the concentration of methyl donor to the concentration of the methylation inhibitor AdoHcy was decreased in animals fed the deficient diet. These diminished levels of AdoMet, accompanied by decreased ratios of AdoMet to AdoHcy, suggested that, as had been seen earlier in livers of animals given ethionine, the intracellular conditions were conducive to hypomethylation of macromolecules. In rats given ethionine, a carcinogenic analogue of methionine (39), AdoMet levels decline and hypomethylated RNA and DNA accumulate in liver (27, 40–44).

Liver tRNA and DNA isolated from rats fed either CSD or MDD.
GENE EXPRESSION IN METHYL-DEFICIENT RATS

Table 3. Activities of liver DNA- and tRNA-methylating enzymes of rats fed CSD or MDD

<table>
<thead>
<tr>
<th>Diet</th>
<th>DNA methyltransferase activity (cpm/100 µg protein)</th>
<th>tRNA methylase activity (cpm/1.5 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD</td>
<td>7 days</td>
<td>2,000</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>6,320</td>
</tr>
<tr>
<td></td>
<td>21 days</td>
<td>7,250</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>11,000</td>
</tr>
<tr>
<td>CSD</td>
<td>7 days</td>
<td>1,125</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>4,175</td>
</tr>
</tbody>
</table>

The observed hypomethylation of both tRNA and DNA was not due to lack of active enzymes to catalyze these methyl-group transfers. When the methyltransferases for tRNA and DNA from livers of the same MDD-fed rats were assayed in vitro with appropriate substrates, their activities were found to be higher than those from CSD-fed animals (Table 3) (45–49). These results are analogous to those obtained in experiments in which hypomethylated tRNA and active methyltransferases were observed in the livers of rats given ethionine (40–43, 48, 49).

Northern blot analysis of poly(A)⁺ RNAs from livers of rats fed MDD or CSD was used to study the effects of dietary methyl deficiency on the expression of various genes (45, 46, 50). As seen in Fig. 1, mRNAs for the c-myc and c-fos genes showed altered electrophoretic patterns and large increases in levels within 1 week. Levels of c-Ha-ras mRNAs also showed significant but lesser increases. In contrast, mRNAs for EGFR (Fig. 1) and EGF (data not shown) were found to decrease in MDD-fed animals. The levels of poly(A)⁺ RNAs hybridizing with the c-Ki-ras gene showed no significant quantitative changes in the same liver samples from MDD-fed rats (data not shown). These rapid effects of MDD on mRNAs in rat liver closely resemble those that we observed earlier in livers of mice fed the same diet for 4–14 days (28). In experiments using SD, the less severely methyl-deficient diet, similar rapid increases in the expression of the c-myc, c-fos, and c-Ha-ras oncogenes in rat liver were observed (51).

The reversibility of the short-term effects of dietary methyl deficiency on nucleic acid methylation and on gene expression was also investigated (46, 50). Rats were fed the deficient diets for 4 weeks and then placed on an adequate diet (CSD) for the remainder of the experiment. As shown in Table 4, the extent of methylation of DNA and tRNA returned to almost normal levels within 1 week. The restoration of normal patterns of gene expression, as judged by Northern blot analysis, took somewhat longer. However, within 3 weeks after the animals were transferred from MDD back to the CSD, the qualitative and quantitative alterations induced by the deficient diet were no longer evident for either the myc or the EGFR genes (Fig. 2). The levels of mRNAs for the c-Ha-ras gene also had returned to normal levels at that time (data not shown). Preliminary evidence suggests that MDD-induced effects on mRNAs for some other genes may require a time longer than 3 weeks for complete reversal (50). Thus, it seems that the reversal rates are not the same for all variables.

Investigations were carried out to determine whether the alterations in gene expression induced by methyl-deficient diets were accompanied by altered patterns of methylation in the...
Table 4  Reversibility of diet-induced hypomethylation of nucleic acids
Incubation conditions were the same as described for Table 2, except for the quantities of tRNA used as substrate.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Methyl group acceptance</th>
<th>tRNA (cpm [¹⁴C]CH₃ accepted/10 µg)</th>
<th>DNA (cpm [³²P]CH₃ accepted/2 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSD</td>
<td></td>
<td>80</td>
<td>5,085</td>
</tr>
<tr>
<td>MDD, 4 weeks</td>
<td></td>
<td>809</td>
<td>14,990</td>
</tr>
<tr>
<td>MDD for 4 weeks, followed by 1 week of CSD</td>
<td></td>
<td>153</td>
<td>5,401</td>
</tr>
</tbody>
</table>

DNA specifying these genes. DNA isolated from the livers of rats fed control or methyl-deficient diets for short periods or isolated from rat liver tumors was subjected to Southern blot analysis after extensive digestion with restriction endonucleases that are either sensitive or insensitive to the presence of 5-MeC (36, 37, 52). Analysis of restriction-endonuclease digestion patterns revealed that the c-Ha-ras, c-fos, and c-myc genes in livers of rats fed MDD, the extremely methyl-deficient diet, for 1–4 weeks were hypomethylated (Fig. 3) (37). It should be noted that MDD feeding induced alterations in the methylation patterns in the region of the c-myc gene coding for the second and third exons, but no changes were apparent when the digests were probed with complementary DNA hybridizing to the first exon. No significant hypomethylation of c-Ki-ras gene sequences was seen. Rapid alterations in the methylation patterns for the c-myc and c-Ha-ras genes in livers of rats fed SD, the diet deficient only in choline and methionine, were also seen (52). In liver tumors that developed in rats, diethylnitrosamine initiated or uninitiated, that were fed SD, the c-Ha-ras (Fig. 4) and the c-Ki-ras (data not shown) genes were found to be hypomethylated (36).

Discussion

The studies described here show that dietary deficiency of lipotropes results in the rapid disturbance of normal processes of transmethylation in rodent liver. Experiments independently carried out by two separate groups, using two diets that differed in the severity of lipotrope deficiency, i.e., MDD (lacking choline, methionine, folic acid, and vitamin B₁₂) and SD (lacking only choline and methionine), produced strikingly similar results. The feeding of either MDD or SD for as short a period as 1 week resulted in a significant decrease in the hepatic level of AdoMet, the one-carbon donor for most transmethylation reactions. AdoMet levels remained below normal when feeding of a deficient diet was continued for longer periods of time (22). The tRNA and DNA in the livers of rats fed MDD were found

Fig. 2. Reversibility of diet-induced changes in gene expression. Northern blot analysis of c-myc and EGFR expression in livers of rats fed the following: lane 1, CSD, 4 weeks; lane 2, MDD, 4 weeks; lanes 3, 4, and 5, CSD, 7 weeks (individual animals); lanes 6, 7, and 8, MDD for 4 weeks, followed by CSD for 3 weeks (individual animals).

Fig. 3. Southern blot analysis of HpaII- and MspI-digested DNAs from livers of rats fed CSD or MDD. All fragments were gel-purified before radio-labelling with ^³²P. mw, molecular weight markers (XHindIII fragments). Sizes are indicated for each panel. A, c-fos a 3.5-kilobase pair subfragment of pc-fos (human)-1 cloned between EcoRI and BamHI sites in the SP65 polylinker. The fragment includes the 5' flanking region and exons of the human fos gene but lacks the 3' Alu repeats (50). Lanes 1 and 2, CSD, 4 weeks; lane 3, MDD, 1 week; lane 4, MDD, 4 weeks. B, c-Ha-ras, a 460-base pair EcoRI fragment of Ha-MuSV from pBS9 (39). Lanes 1 and 2, CSD, 4 weeks; lane 3, MDD, 1 week; lane 4, MDD, 4 weeks. Please note that the "band" at approximately 2.4 kilobases in lane 1 is an artifact. Complete MspI digestion yields one major band at ~0.3 kilobase. C, rat c-myc, a 1.8-kilobase pair EcoRI/SalI fragment of pHMYC-1.4 (51), covering the 5' flanking region and first exon of the mouse c-myc gene. Lanes 1 and 2, CSD, 4 weeks; lane 3, MDD, 1 week; lane 4, MDD, 4 weeks. D, mouse c-myc, a 4.8-kilobase pair XhoI-BamHI fragment containing the second and third exon and 3' flanking sequences of the mouse c-myc gene (23, 24). Lane 2, CSD, 1 week; lanes 1 and 3, CSD, 4 weeks; lane 4, MDD, 1 week; lane 5, MDD, 4 weeks. Also note that lane 1 was run in a different part of the gel and was moved for purposes of consistency. E, c-Ki-ras, a 580-base pair SstII/EcoRI fragment of Ki-MuSV from pHH3 (50) lane 2, CSD, 4 weeks; lanes 1 and 3, MDD, 4 weeks.
Gene Expression in Methyl-Deficient Rats

The early diet-induced changes in methylation and in gene expression were found to be reversible (46, 50). When animals were fed MDD for 1 month and then returned to adequate diets, a gradual reversal of the diet-induced alterations in methylation processes and gene expression was seen. The rates at which the studied variables returned to normal were not uniform. Three weeks after the restoration of adequate diet, most but not all of the patterns of gene expression had reverted to normal.

The DNA coding for some of the oncogenes showing altered levels of expression was studied for possible changes in patterns of DNA methylation. Hypomethylation of the c-Ha-ras, c-myc, and c-fos genes was evident at times coinciding with the early changes in mRNAs for these genes and with the appearance of global hypomethylation of DNA (26, 37). Altered patterns of methylation were seen after either MDD or SD was administered for 1 week or longer (37, 52). In the short-term feeding experiments, no evidence for either altered expression levels or changes in DNA methylation was seen with the c-Ki-ras gene (37). In hepatomas found in animals fed SD for long periods, both the c-Ha-ras and the c-Ki-ras genes showed increased levels of expression and altered DNA-methylation patterns (36).

These results indicate that, beginning very early, dietary methyl insufficiency can induce sequential alterations in patterns of DNA methylation that are accompanied by altered patterns of gene expression. Because the effects of dietary methyl deficiency on specific gene methylation and expression were independently observed in two separate laboratories, using slightly different diets and animals of different ages, we believe that the validity of these findings is enhanced. The two methyl-deficient diets used, MDD and SD, differ only in folate and vitamin B12. Both diets cause preneoplastic and neoplastic changes in the livers of rats, even in the absence of an initiating agent (1, 4, 9, 57). However, because of the severity of its deficiency, MDD can be administered to rats for only a limited period. The combined results reported here support the hypothesis that one of the mechanisms by which lipotrope deficiency promotes carcinogenesis is by inducing production of hypomethylated DNA, which results in alterations in the expression of genes that have roles in the regulation of growth and differentiation. The observations that the early molecular effects of dietary methyl deficiency are reversible also are consistent with the reversibility characteristic of some promoter-induced effects (19–21). However, because there are differences in the rates at which various processes return to normal, it seems possible that intermittent exposure to such a deficiency could result in cumulative effects.

Although the ability of lipotrope deficiency to promote hepatocarcinogenesis in experimental animals is well documented, its possible role in causing human cancer is more obscure. However, hypomethylated DNA, altered processes of transmethylation, and enhanced expression of oncogenes are commonly found in human and animal tumors (5, 15–18, 58–60). In some parts of the world, intake of diets that are at least marginally lipotrope deficient, combined with exposure to foods contaminated with aflatoxin and other toxic materials, is quite common and may play a significant role in promoting liver cancer in these populations (1, 61, 62). In industrialized nations...
additional factors, such as the intake of alcohol and of certain therapeutic drugs that are known to cause depression of AdoMet levels and to interfere with DNA methylation (57, 63–66), also may contribute to cancer in humans.

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References


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