

Role of Methionine Adenosyltransferase 2A and S-adenosylmethionine in Mitogen-Induced Growth of Human Colon Cancer Cells

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Background & Aims: Two genes (*MAT1A* and *MAT2A*) encode for methionine adenosyltransferase, an essential enzyme responsible for S-adenosylmethionine (SAME) biosynthesis. *MAT1A* is expressed in liver, whereas *MAT2A* is widely distributed. In liver, increased *MAT2A* expression is associated with growth, while SAME inhibits *MAT2A* expression and growth. The role of *MAT2A* in colon cancer is unknown. The aims of this study were to examine whether *MAT2A* expression and SAME and its metabolite methylthioadenosine (MTA) can modulate growth of colon cancer cells. **Methods:** Studies were conducted using resected colon cancer specimens, polyps from *Min* mice, and human colon cancer cell lines RKO and HT-29. *MAT2A* expression was measured by real-time polymerase chain reaction and cell growth by the 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** In 12 of 13 patients and all 9 polyps from *Min* mice, the *MAT2A* messenger RNA levels were 200%–340% of levels in adjacent normal tissues, respectively. Epidermal growth factor, insulin-like growth factor 1, and leptin increased growth and up-regulated *MAT2A* expression and *MAT2A* promoter activity in RKO and HT-29 cells. SAME and MTA lowered the baseline expression of *MAT2A* and blocked the growth factor-mediated increase in *MAT2A* expression and growth in colon cancer cell lines. Importantly, the mitogenic effect of these growth factors was inhibited if *MAT2A* induction was prevented by RNA interference. SAME and MTA supplementation in drinking water increased intestinal SAME levels and lowered *MAT2A* expression. **Conclusions:** Similar to the liver, up-regulation of *MAT2A* also provides a growth advantage and SAME and MTA can block mitogenic signaling in colon cancer cells.

Methionine adenosyltransferase (MAT) is an essential cellular enzyme that catalyzes the formation of S-adenosylmethionine (SAME), the principal biological

methyl donor and the ultimate source of the propylamine moiety used in polyamine biosynthesis.¹ In mammals, 2 different genes, *MAT1A* and *MAT2A*, encode for 2 homologous MAT catalytic subunits, $\alpha 1$ (forms either a dimer MAT III or tetramer MAT I) and $\alpha 2$ (forms MAT II), while a third gene, *MAT2 β* , encodes for a regulatory subunit β that regulates MAT II.^{1,2} *MAT1A* is expressed mostly in liver and is a marker for normal differentiated liver.¹ *MAT2A* is widely distributed. *MAT2A* also predominates in the fetal liver and is progressively replaced by *MAT1A* during liver development.¹ In hepatocytes, increased *MAT2A* and *MAT2 β* expression is associated with increased growth and malignant degeneration.^{1,3-5} Even though the MAT isoenzymes catalyze the same reaction, they are regulated differently by the product SAME. In liver cells, SAME maintains *MAT1A* expression and suppresses *MAT2A* expression.^{6,7} In addition, SAME and its metabolite methylthioadenosine (MTA) can inhibit liver cell growth and induce apoptosis in liver cancer cells while protecting normal hepatocytes against apoptosis.^{5,8,9} Outside of the liver, the only cell type studied is the lymphocyte, where *MAT2A* expression and SAME levels have been shown to increase during T-lymphocyte activation.¹⁰ The increase in SAME level was believed to be necessary to support polyamine synthesis during rapid growth.¹⁰ The influence of *MAT2A* and SAME levels on cell growth in other cell types has not been studied.

One small study examined MAT protein expression and activity in colorectal carcinoma and normal colon. The ratio of MAT activity in tumor tissue versus normal

Abbreviations used in this paper: EGF, epidermal growth factor; GSS, glutathione synthetase; IGF-1, insulin-like growth factor 1; IGF-1R, insulin-like growth factor 1 receptor; MAT, methionine adenosyltransferase; MTA, methylthioadenosine; MTT, 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide; PCR, polymerase chain reaction; RNAi, RNA interference; SAME, S-adenosylmethionine; siRNA, small interfering RNA.

tissue correlated well with the stage of the colorectal tumor. The higher activity correlated with higher immunohistochemical staining for MAT II.¹¹ However, the mechanism of MAT II up-regulation in colon cancer was not investigated, and whether this may play a role in colon cancer pathogenesis is also unknown.

Epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1) and leptin are 3 well-known growth factors that have been implicated in colon cancer growth and invasion. Higher levels of both IGF-1 and IGF-1 receptor (IGF-1R) have been shown in colon cancer,^{12,13} and IGF-1R signaling plays an important role in tumor growth, angiogenesis, and metastasis.¹² Increased EGF receptor signaling has also been shown to provide growth advantage and correlate with colon cancer progression and metastatic potential.^{14,15} Likewise, elevated leptin levels were found to be a risk factor for colon cancer in men,¹⁶ and leptin has been shown to promote invasiveness of colon cancer cells.¹⁷ Leptin levels correlate with body mass index, and obesity is a well-recognized risk factor for colon cancer.¹⁶

The aims of the current study were to examine (1) expression of *MAT2A* in resected colon cancer specimens, (2) the effects of mitogens on expression of *MAT2A* genes and growth in colon cancer cell lines RKO and HT-29, and (3) the influence of SAME and MTA on *MAT2A* expression and effects of mitogens. We found that similar to the liver, *MAT2A* expression is important for cell growth and is required for the mitogens to induce growth in colon cancer cells. Furthermore, SAME and MTA also lower *MAT2A* expression and modulate the growth response of colon cancer cells to mitogens. These findings may have important implications in the pathogenesis and treatment of colon cancer.

Materials and Methods

Materials

Cell culture media, fetal bovine serum, and primers were obtained from Gibco BRL Life Technologies (Grand Island, NY). The Luciferase Assay System was obtained from Promega (Madison, WI). All restriction endonucleases were obtained from either Promega or Gibco. RNA interference (RNAi) against *MAT2A* was obtained from Invitrogen (Carlsbad, CA). SAME in the form of disulfate p-toluenesulfonate spray dried powder (97.18% purity) was generously provided by Gnosis SRL (Cairate, Italy). MTA was purchased from Sigma (St Louis, MO). All other reagents were of analytical grade and were obtained from commercial sources.

Source of Normal and Cancerous Colon Tissue

We had available to us colon cancer and paired normal colon specimens from 13 patients, which were from a repository based on availability of both normal and cancerous colon specimens from the same patient.

Each patient signed an informed consent for analysis of resected tissue for molecular markers. These tissues were immediately frozen in liquid nitrogen for subsequent analysis of messenger RNA (mRNA), Western blot, and SAME levels as described in the following text.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Keck School of Medicine University of Southern California's human research review committee.

Experiments in Mice

Nine adenomatous polyps were obtained from three 3-month-old male *Min* mice (4 from different parts of the small intestine from one mouse and 5 from different parts of the colon from 2 other mice). Normal intestinal tissues adjacent to the polyps were included for comparison. These tissues were immediately snap frozen for subsequent analysis of mRNA and SAME levels as described in the following text.

Three-month-old male C57/B6 mice were fed ad libitum a standard diet (Harland Teklad irradiated mouse diet 7912; Madison, WI), were caged individually, and had free access to water supplemented with SAME (75 or 150 mg · kg⁻¹ · day⁻¹), MTA (75 mg · kg⁻¹ · day⁻¹), phosphate-buffered saline (vehicle for SAME), or dimethyl sulfoxide (0.2% vehicle for MTA) for 6 days. SAME and MTA were made fresh daily. The amount of water intake, body weight, and animal behavior were closely monitored. After 6 days, animals were killed, intestine was cut open along the longitudinal axis, and mucosa was stripped from the intestine as we described.¹⁸ Mucosa was weighed and a portion processed for SAME measurement as described in the following text, and the rest was snap frozen for subsequent RNA extraction.

Animals were treated humanely, and all procedures were in compliance with our institutions' guidelines for the use of laboratory animals.

Cell Culture and Treatment With Growth Factors SAME and MTA

HT-29 and RKO cells were obtained from the Cell Culture Core of the University of Southern California Liver Disease Research Center and grown according to instructions provided by the American Type Culture Collection (Rockville, MD). Before treatment with growth factors SAME or MTA, medium was changed to 0.1% fetal bovine serum overnight. Medium was then changed to withhold serum and cells were treated with leptin, IGF-1, or EGF (all at 100 ng/mL), SAME (0.5–5 mmol/L), MTA (0.5–1 mmol/L), or respective vehicle controls for 1–24 hours for various assays as described in the following text. These growth factors were shown to exert mitogenic effects in colon cancer cell lines at the dose chosen.^{12,17,19}

RNAi

RNAi experiments were performed using Lipofectamine RNAiMax (Invitrogen) according to the manufac-

turer's instructions. Small interfering RNA (siRNA) oligonucleotides for *MAT2A* and scrambled siRNA were synthesized by the University of Southern California Norris Comprehensive Cancer Center Microchemical Core Laboratory and annealed to form duplexes. Stealth RNAi for *MAT2A* and stealth RNAi negative control duplexes were synthesized by Invitrogen. The following siRNA sequences were used: si-*MAT2A* #1, 5'-ACACAUUGGAUAUGAUGAUTT-3' (sense) and 5'-AUCAUCAUAUCCAAUGUGUTT-3' (antisense); si-control with scrambled sequence (negative control siRNA having no perfect matches to known human genes), 5'-UUCUCCGAACGUGUCACAUdTdT-3' (sense) and 5'-AUGUGACACGUUCGGAGAAAdTdT-3' (antisense); stealth RNAi-*MAT2A* #5, 5'-CCACCUACAGCCAAGUGGCAGAUUU-3' (sense) and 5'-AAAUCUGCCACUUGGCUGUAGGUGG-3' (antisense). Transfection was allowed to proceed 72 hours before collection for different assays.

RNA Isolation and Gene Expression Analysis

Total RNA was isolated by the EZgeno Total RNA Isolation Kit (Genemega, San Diego, CA) and subjected to reverse transcription by using Moloney murine leukemia virus reverse transcriptase (Invitrogen). A total of 2 μ L of reverse transcription product was subjected to quantitative real-time polymerase chain reaction (PCR) analysis. The primers and TaqMan probes for *MAT2A*, *MAT2 β* , and Universal PCR Master Mix were purchased from ABI (Foster City, CA). Hypoxanthine phosphoribosyl-transferase 1 and ubiquitin C were used as housekeeping genes as described.²⁰ The thermal profile consisted of 1 cycle at 95°C for 15 minutes followed by 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. The expression of *MAT2A* and *MAT2 β* was checked by normalizing the Ct of *MAT2A* and *MAT2 β* to that of the control housekeeping gene (hypoxanthine phosphoribosyl-transferase 1 or ubiquitin C).²¹ The Δ Ct obtained was used to find the relative expression of MAT genes according to the following formula: Relative Expression = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ represents Δ Ct of MAT genes in colon cancer or treated cells minus Δ Ct of MAT genes in normal colon or control cells.

Human MAT2A and Glutathione Synthetase Promoter Constructs

The human *MAT2A* promoter construct -571/+60-LUC and glutathione synthetase (GSS) promoter construct -1686/-46-LUC were previously described^{7,22,23} and subcloned in the sense orientation upstream of the luciferase coding sequence of the pGL-3 enhancer vector (Promega). Both promoter constructs contain maximal promoter activity.

Effect of Mitogens on MAT2A Promoter Activity in HT-29 Cells

To study the effect of mitogens on human *MAT2A* promoter activity in HT-29 cells, HT-29 cells (5×10^5

cells in 2 mL serum-free medium) were transiently transfected with 2 μ g *MAT2A* promoter firefly luciferase gene construct or promoterless pGL3-enhancer vector (as negative control) using the Superfect Transfection Reagent (Qiagen, Valencia, CA) as we described.²² To control for transfection efficiency, cells were cotransfected with the Renilla phRL-TK vector (Promega). Cells were treated with mitogens (100 ng/mL), SAME (5 mmol/L), MTA (1 mmol/L), or vehicle control during the last 3 (IGF-1 and EGF) to 6 hours (leptin, SAME, and MTA) of the transfection (18 hours total). The luciferase activity driven by the *MAT2A* promoter construct was normalized to Renilla luciferase activity. Each experiment was performed with triplicate samples.

Specificity of SAME and MTA on promoter activity was examined by transfecting HT-29 cells with the GSS promoter construct and treating cells with SAME or MTA as previously described.

Measurement of Cell Growth

Cell growth was measured by the cell growth determination kit 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma. The MTT assay measures the cell proliferation rate and reduction in cell viability. HT-29 or RKO cells (1×10^4 /well) were plated in 96-well plates and treated with growth factors (all 100 ng/mL) SAME (0.5–5 mmol/L) and MTA (0.5–1 mmol/L), alone or in combination for 16 hours in serum-free medium. To determine the effect of *MAT2A* RNAi on mitogen-induced changes in MTT, cells were first treated with RNAi for 48 hours, followed by mitogen treatment for another 24 hours.

Measurement of Apoptosis

Apoptosis was assessed by Hoechst staining as we described.²² Briefly, RKO cells were grown on coverslips and treated with RNAi#1 for 24 hours, and cells were fixed with paraformaldehyde and stained with 8 μ g/mL Hoechst 33258 dye for 30 minutes. Cells with bright, fragmented, condensed nuclei were identified as apoptotic cells using the Nikon Eclipse TE300 fluorescent microscope (Melville, NY). At least 5 random fields (at 300 \times) were counted.

SAME, MTA, and Polyamine Levels

Cellular SAME and MTA levels were measured as we described.²⁴ Polyamine levels were determined according to the method described.²⁵

Western Blot Analysis

Western blot analysis for MAT II in colon cancer and HT-29 cells treated with IGF-1 (100 ng/mL) for 8 hours was performed as we described⁹ using anti-MAT II antibodies (GenWay Biotech, Inc, San Diego, CA).

Table 1. MAT Expression in Normal Colon and Colon Cancer Specimens

| Patient no. | Sex | Age (y) | Cancer site | Stage | Histology | MAT2A ^a | MAT2B ^a |
|-------------|-----|---------|--------------|---------|---------------------|--------------------|--------------------|
| 1 | M | 65 | Rectosigmoid | T3N1M0 | Moderate | 222 | 31 |
| 2 | F | 47 | Rectum | TXN2M0 | Unknown | 171 | 90 |
| 3 | M | 53 | Rectosigmoid | T3N1M0 | Poor | 152 | 189 |
| 4 | M | 66 | Rectum | T3N1M1 | Moderate | 174 | 220 |
| 5 | M | 59 | Sigmoid | T3N2M1 | Moderate | 53 | 114 |
| 6 | F | 61 | Left colon | T3N2M1 | Moderate | 203 | 122 |
| 7 | F | 77 | Right colon | T2N2M1 | Well differentiated | 245 | 88 |
| 8 | M | 67 | Right colon | T3N2M1 | Unknown | 138 | 174 |
| 9 | M | 62 | Rectum | T3N2M0 | Moderate | 171 | 103 |
| 10 | F | 56 | Right colon | Unknown | Moderate | 187 | 60 |
| 11 | M | 52 | Rectum | T3N2M0 | Poor | 295 | 153 |
| 12 | M | 73 | Rectum | TXNXM1 | Moderate | 266 | 89 |
| 13 | F | 68 | Right colon | T4N1M1 | Poor | 284 | 234 |

^aPercentage relative expression, tumor/normal colon. MAT expression determined by real-time PCR.

Statistical Analysis

Data are given as mean \pm SEM. Statistical analysis was performed using Student *t* test for comparison of paired samples and analysis of variance followed by Fisher test for multiple comparisons. Significance was defined by $P < .05$.

Results

MAT2A Expression Is Induced in Human Colon Cancer

We first examined MAT expression in colon cancer and adjacent normal tissue control by real-time PCR. We had available to us colon cancer and paired normal colon specimens from 13 patients, which were randomly selected from a repository based on availability of both normal and cancerous colon specimens from the same patient. Table 1 summarizes the clinical and molecular data. Note that MAT2A is induced in 12 of 13 colon cancer specimens. By comparison, the gene that encodes the regulatory subunit MAT2B is not significantly changed as compared with normal colon. MAT1A is not expressed in normal or cancerous colon. Increased MAT II ($\alpha 2$ and $\alpha 2'$) level was confirmed using Western blot analysis (Figure 1). The smaller $\alpha 2'$ subunit is believed to be derived from $\alpha 2$ by posttranslational modification.²⁶ Consistent with this, SAME levels were higher in all colon cancer specimens (normal colon, 0.26 ± 0.03 nmol/mg protein; colon cancer, 0.73 ± 0.11 nmol/mg protein; results are mean \pm SE from 6 specimens each; $P < .01$ by paired Student *t* test). Tissue MTA level was below detection limit.

MAT2A Expression Is Also Induced in Polyps of Min Mice

We also compared MAT2A mRNA levels in 9 adenomatous polyps with adjacent normal tissues from 3 *Min* mice, and all of the polyps had higher MAT2A mRNA levels ($338\% \pm 93\%$; range, 132%–909%; expressed as percentage of matched normal tissue mRNA level; $P < .05$).

SAME levels in 4 polyps (average MAT2A mRNA levels were $200\% \pm 50\%$ of normal) were also higher than adjacent normal tissues (normal, 0.28 ± 0.38 nmol/mg protein; polyps, 0.38 ± 0.22 nmol/mg protein; $P < .05$). MTA levels were unchanged (normal, 0.06 ± 0.03 nmol/mg protein; polyps, 0.08 ± 0.04 nmol/mg protein).

Leptin, IGF-1, and EGF Induce MAT2A But Not MAT2B Expression in RKO and HT-29 Cells

We next determined the effect of leptin on these 2 MAT genes in RKO and HT-29 cells and found that leptin treatment resulted in a time-dependent increase in MAT2A but not MAT2B mRNA levels, with near doubling of the MAT2A mRNA levels 5 hours after leptin treatment in both cell types (Figure 2A and C). IGF-1 and EGF treatment of both cell types also doubled the MAT2A mRNA levels (Figure 2B and 3B). Similar to leptin, these growth factors also did not affect MAT2B mRNA level in the 2 colon cancer cell lines (not shown). IGF-1 treatment also increased MAT II level in HT-29 cells (Figure 1).

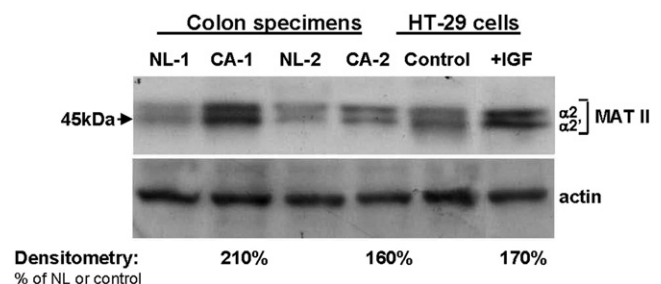


Figure 1. Steady-state MAT II protein levels in colon cancer and HT-29 cells treated with IGF-1. Human colon cancer specimens (CA; CA-1 is patient 12 and CA-2 is patient 13 in Table 1) and matched normal colon tissues (NL), and HT-29 cells treated with IGF-1 (100 ng/mL for 8 hours), were processed for Western blot analysis as described in Materials and Methods using 40 μ g protein/lane. Densitometric changes (% of NL or control) are shown below each blot.

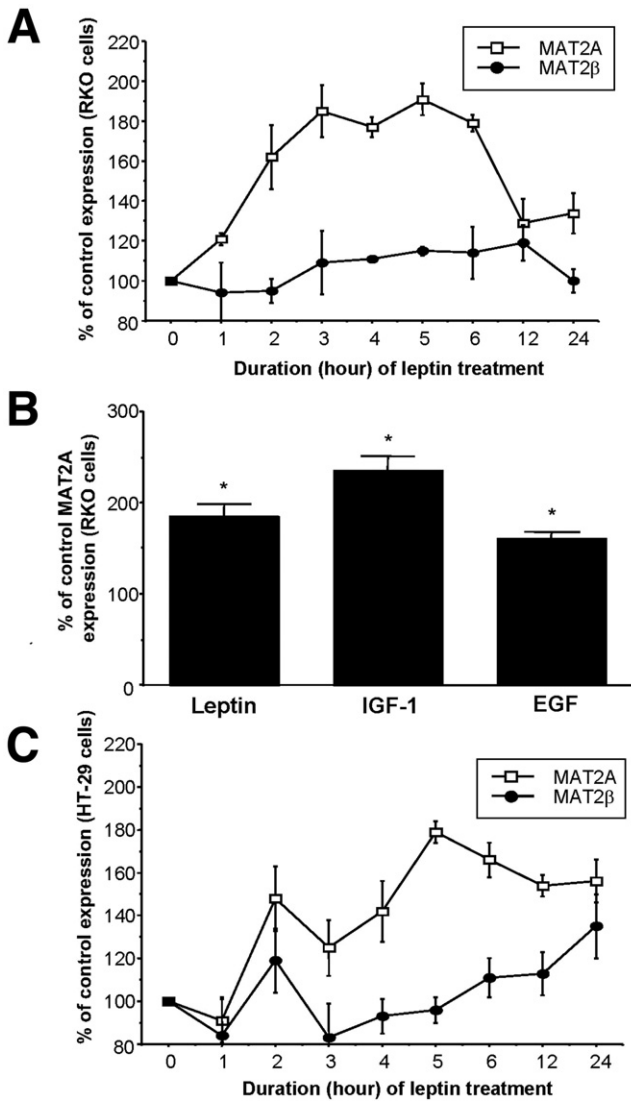


Figure 2. Effects of leptin, IGF-1, and EGF on expression of MAT genes in RKO and HT-29 cells. (A) RKO cells were treated with leptin (100 ng/mL) for 1–24 hours, and *MAT2A* and *MAT2β* mRNA levels were measured using real-time PCR as described in Materials and Methods. (B) RKO cells were treated with leptin (100 ng/mL, 6 hours), IGF-1, or EGF (both at 100 ng/mL, 3 hours), and *MAT2A* mRNA levels were measured as in A. (C) HT-29 cells were treated with leptin as in A. All results represent mean ± SEM from 3 independent experiments performed in duplicate. **P* < .05 vs control.

SAME and MTA Lower Baseline MAT2A Expression and Prevent Mitogen-Mediated Induction of MAT2A Expression

We have previously shown that SAME treatment lowers *MAT2A* expression in hepatocytes.^{6,7} Similar to hepatocytes, SAME also lowers basal *MAT2A* expression in HT-29 cells in a time-dependent fashion. With 6-hour treatment, SAME lowers *MAT2A* expression in a dose-dependent manner (0.5 mmol/L SAME, 81% baseline, 1 mmol/L SAME, 65% baseline, 2 mmol/L SAME, 55% baseline, 5 mmol/L SAME, 38% of baseline). MTA, an important metabolite of SAME, also lowers basal *MAT2A* ex-

pression to comparable levels even at a lower dose (Figure 3A). Next we determined whether SAME or MTA can modulate the effect of mitogens on *MAT2A* expression. Figure 3B shows that both agents are able to prevent (leptin and EGF) or significantly blunt (IGF-1) the mitogen-induced *MAT2A* expression. MTA is more efficient than SAME in blocking the effect of mitogen effect on *MAT2A* expression at one fifth the dose.

Effects of Mitogens SAME and MTA on the Human MAT2A Promoter in HT-29 Cells

To see whether the effect of the mitogens SAME and MTA on *MAT2A* lies at the transcriptional level, we examined their effects on the activity of the human *MAT2A* promoter. Figure 4 shows that these mitogens more than double the human *MAT2A* promoter activity and both SAME and MTA lower the promoter activity to comparable levels as their effects on the endogenous *MAT2A* expression. Importantly, SAME and MTA treatment had no influence on the human GSS promoter activity (not shown).

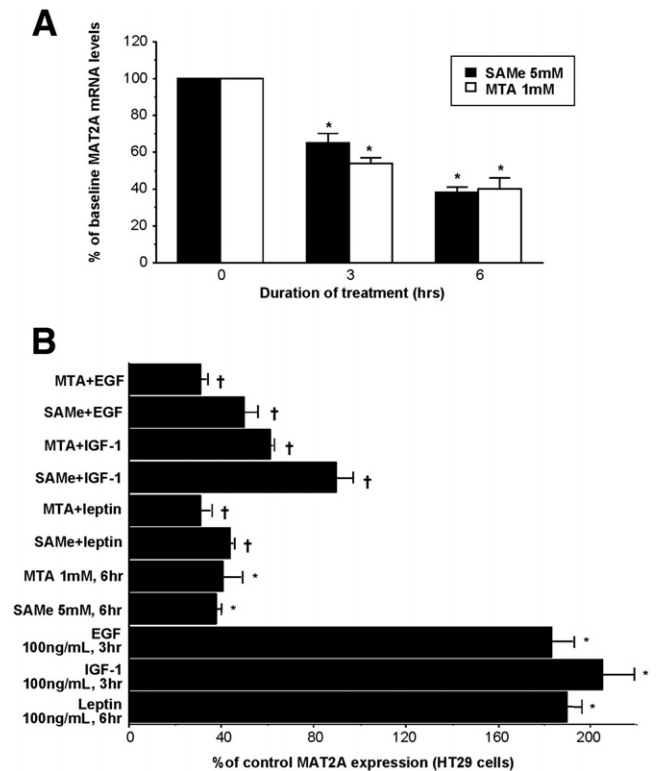


Figure 3. SAME and MTA (A) lower *MAT2A* mRNA levels and (B) prevent a growth factor-induced increase in *MAT2A* expression. HT-29 cells were treated with SAME (5 mmol/L) or MTA (1 mmol/L) or vehicle control for 3–6 hours, and *MAT2A* mRNA levels were determined by real-time PCR (A). To examine whether SAME and MTA can modulate the effect of the growth factors on *MAT2A* expression, HT-29 cells were treated with growth factors SAME or MTA alone or in combination. Cotreatment of SAME or MTA with leptin was for 6 hours, while cotreatment for IGF-1 or EGF was for 3 hours. All results represent mean ± SEM from 3–4 independent experiments performed in duplicate. **P* < .05 vs control, †*P* < .05 vs respective growth factors.

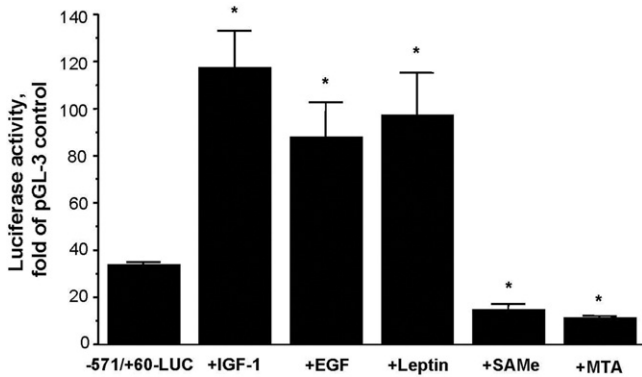


Figure 4. Effect of IGF-1, EGF, leptin, SAME, and MTA treatment on luciferase activity driven by the human *MAT2A* promoter. HT-29 cells were transfected transiently with the human *MAT2A* promoter construct -571/+60-LUC or pGL-3 enhancer vector and treated with IGF-1 (100 ng/mL, 3 hours), EGF (100 ng/mL, 3 hours), leptin (100 ng/mL, 6 hours), SAME (5 mmol/L, 6 hours), MTA (1 mmol/L, 6 hours), or vehicle control. Cells were cotransfected with Renilla luciferase for control of transfection efficiency. Results represent mean \pm SEM from 3 independent experiments performed in triplicate. Data are expressed as relative luciferase activity to that of pGL-3 enhancer vector control, which is assigned a value of 1.0. * $P < .05$ vs. -571/+60-LUC construct control.

SAME and MTA Can Block the Mitogenic Effect of Leptin, EGF, and IGF-1 in Colon Cancer Cells

We next examined whether SAME and MTA can modulate the mitogenic effects of these growth factors. All 3 growth factors increased growth of HT-29 cells (similar effects were seen with RKO cells; results not shown). Interestingly, while SAME (0.5–5 mmol/L) and MTA (0.5–1 mmol/L) by themselves had no effect on growth after 16 hours of treatment, they blocked the mitogenic action of all 3 growth factors (Figure 5). High-dose SAME (5 mmol/L) treatment for longer duration (24 hours) inhibited growth (71% of control).

MAT2A Expression Is Closely Correlated With Growth and Cell Death

In hepatocytes, *MAT2A* expression correlates with growth.^{1,4,5} To see if the increase in *MAT2A* expression induced by mitogens can be responsible for the increase in growth in colon cancer cells, we lowered the *MAT2A* expression using RNAi. Several RNAi constructs were examined. The most efficient RNAi (RNAi#1) lowered *MAT2A* expression to 9% of scrambled control by 48 hours (Figure 6A). Even by 24 hours, where the expression was down to 25% of scrambled control, a significant increase in apoptosis was detected (Figure 6B) and became more pronounced at 48 hours (Figure 6C).

Because RNAi#1 caused cell death, we chose a different RNAi construct that would only lower the *MAT2A* expression by 50% at 72 hours (RNAi#5). No apoptosis was detected with this construct after 24

hours (data not shown). We used this construct to blunt the ability of the mitogens to induce *MAT2A* expression. Figure 7A shows that RNAi#5 lowered *MAT2A* mRNA level to 50% of control and mitogens were able to increase *MAT2A* mRNA levels in the presence of RNAi#5 but only back to untreated or scrambled RNAi levels. Figure 7B shows the effect of RNAi#5 on growth. RNAi#5 lowered the MTT value by about 30% and blocked the ability of leptin, IGF-1, and EGF to fully exert their mitogenic effect. All 3 growth factors were able to increase the MTT value in RNAi#5-treated cells back to baseline.

Effect of Mitogens SAME and MTA on Cellular SAME, MTA, and Polyamine Levels

To test the hypothesis that the mitogen-induced increase in *MAT2A* is to provide increased SAME necessary for polyamine synthesis in growing cells, SAME and polyamine levels were measured following treatment of HT-29 cells with mitogens. Table 2 shows that all mitogens increased intracellular SAME levels by 16 hours of treatment. However, SAME and MTA also increased SAME levels. MTA can increase the SAME level because it is converted back to SAME via the methionine salvage pathway.²⁷ Intracellular MTA levels increased dramatically after SAME treatment (18-fold with 5 mmol/L SAME for 16 hours). In fact, SAME treatment increased intracellular MTA levels by much higher magnitudes than SAME levels itself, especially

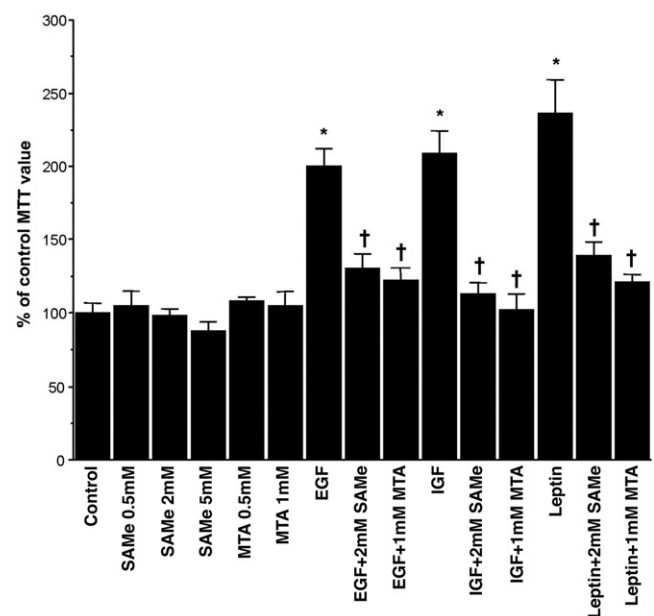


Figure 5. Effects of SAME, MTA, EGF, IGF-1, and leptin on cell growth. HT-29 cells were treated with various growth factors, SAME (0.5–5 mmol/L), and MTA (0.5–1 mmol/L) alone or in combination for 16 hours for cell growth determination by the MTT assay as described in Materials and Methods. Results represent mean \pm SEM from 3 independent experiments performed in triplicate. * $P < .05$ vs control, † $P < .05$ vs respective growth factors.

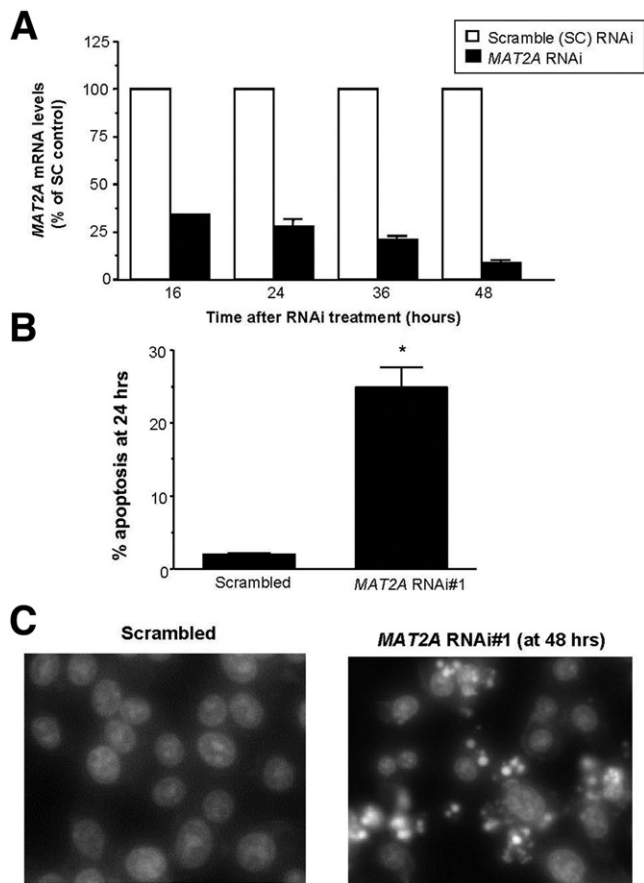


Figure 6. MAT2A RNAi#1 causes a profound decrease in MAT2A mRNA level and increased apoptosis. RKO cells were treated with MAT2A RNAi#1, and MAT2A mRNA levels were determined up to 48 hours after treatment (A). Apoptosis was determined by Hoechst staining after (B) 24 hours and (C) 48 hours of RNAi#1 treatment. Full time course study of RNAi#1 on MAT2A expression was performed in triplicate in 1 experiment, and apoptosis was determined in 3 separate experiments. **P* < .05 vs scrambled.

at high SAME concentration. This can be explained by the fact that SAME is highly unstable and can be converted to MTA spontaneously and also via the polyamine pathway.²⁷ Indeed, we determined the half-life of SAME (between 1 and 5 mmol/L) in culture medium without cells at 37°C, and it is 12 hours (Figure 8). On the other hand, the rate of conversion from SAME to MTA is 0.013 mmol/L per hour per mmol/L SAME under the same condition (Figure 8, rate determined using linear regression). In contrast, MTA is highly stable (Figure 8). SAME is much more stable in water at room temperature, with a half-life of 36 hours (data not shown).

Table 3 shows the effect of these treatments on the levels of polyamines. Treatment with mitogens for 6 hours increased putrescine levels significantly (Table 3). However, SAME- and MTA-treated groups had lower putrescine levels, and the MTA-treated group also had lower spermidine and spermine levels.

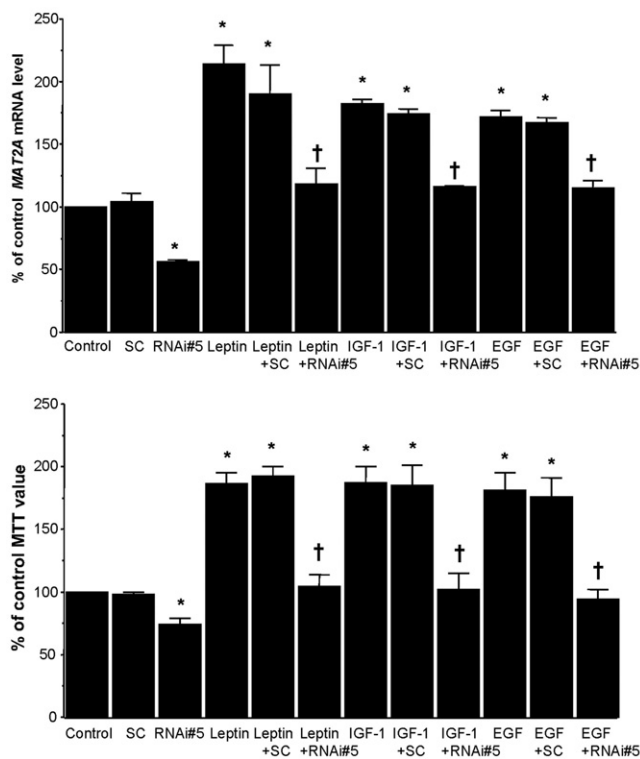


Figure 7. Effect of MAT2A RNAi#5 on MAT2A mRNA levels and cell growth in response to growth factors. (A) HT-29 cells were treated with RNAi#5 or scrambled (SC) RNAi control for 48 hours, and then growth factors were added for either 3 hours (IGF-1, EGF) or 6 hours (leptin) at 100 ng/mL. MAT2A mRNA levels were measured by real-time PCR. (B) HT-29 or RKO cells were treated with RNAi#5 or SC control for 48 hours, followed by addition of growth factors or vehicle control for another 24 hours for the MTT assay. **P* < .05 vs control, †*P* < .05 vs RNAi#5 and respective growth factors from 3 experiments.

Table 2. SAME and MTA Levels in HT-29 Cells

| Treatment | SAME | MTA |
|--------------------|--------------------------|----------------------------|
| Duration: 6 hours | | |
| Control | 0.40 ± 0.03 | 0.050 ± 0.007 |
| EGF | 0.54 ± 0.04 ^a | 0.054 ± 0.001 |
| IGF-1 | 0.40 ± 0.03 | 0.054 ± 0.003 |
| Leptin | 0.48 ± 0.04 | 0.058 ± 0.010 |
| SAME 2 mmol/L | 1.44 ± 0.13 ^a | 0.192 ± 0.036 ^a |
| SAME 5 mmol/L | 2.19 ± 0.21 ^a | 0.354 ± 0.096 ^a |
| MTA 1 mmol/L | 0.81 ± 0.09 ^a | 0.652 ± 0.123 ^a |
| Duration: 16 hours | | |
| Control | 0.42 ± 0.04 | 0.049 ± 0.005 |
| EGF | 0.63 ± 0.06 ^a | 0.061 ± 0.010 |
| IGF-1 | 1.03 ± 0.03 ^b | 0.087 ± 0.025 |
| Leptin | 0.63 ± 0.02 ^a | 0.064 ± 0.015 |
| SAME 2 mmol/L | 1.28 ± 0.01 ^b | 0.261 ± 0.035 ^a |
| SAME 5 mmol/L | 1.83 ± 0.01 ^b | 0.934 ± 0.105 ^a |
| MTA 1 mmol/L | 0.54 ± 0.01 ^a | 0.689 ± 0.108 ^a |

NOTE. The unit for all metabolites is nmol/mg protein. Results represent mean ± SEM from 3–4 experiments. ^a*P* < .05, ^b*P* < .005 vs respective controls.

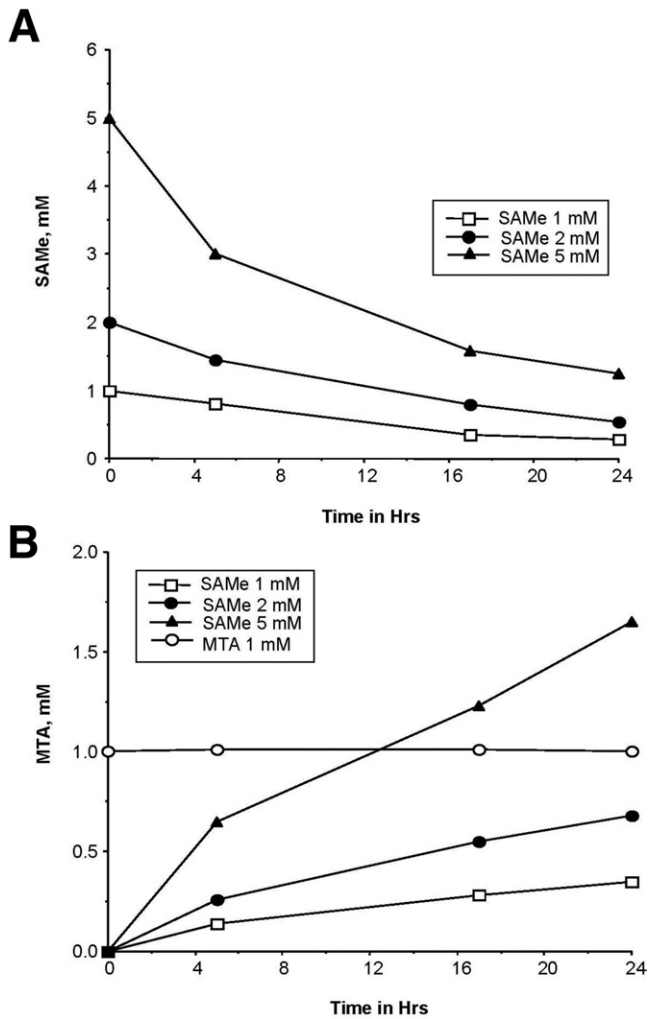


Figure 8. Stability of SAME and MTA in culture medium at 37°C. (A) SAME and (B) MTA levels were measured as described in Materials and Methods in culture medium without cells with starting SAME concentrations ranging from 1 to 5 mmol/L and MTA concentration at 1 mmol/L.

Effects of Oral SAME and MTA Supplementation on Intestinal SAME Levels and MAT2A Expression

We tested the effect of SAME (75 or 150 mg · kg⁻¹ · day⁻¹) and MTA (75 mg · kg⁻¹ · day⁻¹) supplementation

in water for 6 days on the intestinal MAT gene expression and SAME/MTA levels in wild-type mice. The dose of SAME was chosen based on the fact that 200 mg · kg⁻¹ · day⁻¹ is the dosage shown to prevent formation of hepatocellular carcinoma in rats,²⁸ and the adult human therapeutic dose is 1.2–3.6 g/day taken orally.²⁹ SAME and MTA were well tolerated, and animals drank comparable amounts of water and gained similar amounts of weight as controls. We examined changes in proximal small intestine and colon because we reasoned these regions should receive the highest and lowest amounts of SAME and MTA, respectively. Table 4 shows that intestinal levels of SAME increased in a dose-dependent fashion by SAME supplementation, especially in proximal small intestine (800% of control). Even colonic mucosa had a nearly 200% increase in SAME level. Intestinal MTA levels were below the detection limit. Importantly, both high-dose SAME and MTA decreased MAT2A expression in proximal small intestine by 20% after only 6 days.

Discussion

MAT is a critical cellular enzyme because it catalyzes the only reaction that generates SAME, the biological methyl donor and precursor for polyamines. In polyamine synthesis, SAME is decarboxylated to form S-adenosylmethionine. The propylamine moiety of S-adenosylmethionine is then donated to putrescine to form spermidine and a first molecule of MTA. Spermidine is then converted into spermine by the addition of one more propylamine group from S-adenosylmethionine, which generates a second molecule of MTA.¹ In mammalian liver, MAT1A is a marker for the differentiated or mature liver phenotype, while MAT2A is a marker for rapid growth and dedifferentiation.¹ Recently, MAT2β has also been shown to be induced in human liver cancer and to provide a growth advantage.³⁰ While much is known about the role of dysregulation of MAT genes in liver disease and cancer, very little is known about regulation of these MAT genes outside of the liver, and virtually nothing is known about the regulation or dysregulation of these genes in colon cancer. Although MAT is responsible for generation of SAME, which may enhance growth through the polyamine pathway, SAME

Table 3. Effect of Mitogens SAME and MTA on Polyamine Levels in HT-29 Cells

| ID | Putrescine | Spermidine | Spermine |
|-----------------------------|--------------------------|--------------------------|--------------------------|
| Treatment duration: 6 hours | | | |
| Control | 0.14 ± 0.02 | 2.59 ± 0.17 | 12.15 ± 1.50 |
| EGF | 0.32 ± 0.01 ^a | 2.19 ± 0.23 | 12.55 ± 2.04 |
| IGF-1 | 0.22 ± 0.02 ^a | 2.82 ± 0.09 | 12.55 ± 2.50 |
| Leptin | 0.27 ± 0.02 ^a | 2.62 ± 0.02 | 14.62 ± 2.70 |
| SAME 2 mmol/L | 0.06 ± 0.01 ^a | 2.37 ± 0.02 | 12.39 ± 2.86 |
| SAME 5 mmol/L | 0.01 ± 0.01 ^a | 1.90 ± 0.18 | 13.21 ± 2.58 |
| MTA 1 mmol/L | Not detectable | 1.30 ± 0.04 ^a | 6.73 ± 0.95 ^a |

NOTE. The unit for all metabolites is nmol/mg protein. Results represent mean ± SEM from 3–4 experiments.

^aP < .05 vs respective controls.

Table 4. Intestinal SAME Levels and *MAT2A* Expression With SAME or MTA Treatment

| ID | Proximal small intestine | | Colon | |
|---|--------------------------|-------------------------|--------------------------|-------------------------|
| | SAME level | <i>MAT2A</i> expression | SAME level | <i>MAT2A</i> expression |
| Control | 0.28 ± 0.01 | 100 ± 0 | 0.47 ± 0.02 | 100 ± 0 |
| SAME (75 mg · kg ⁻¹ · day ⁻¹) | 0.58 ± 0.16 | 99 ± 2 | 0.97 ± 0.10 ^a | 128 ± 20 |
| SAME (150 mg · kg ⁻¹ · day ⁻¹) | 2.29 ± 0.55 ^b | 81 ± 4 ^c | 1.21 ± 0.14 ^a | 85 ± 10 |
| MTA (75 mg · kg ⁻¹ · day ⁻¹) | 0.63 ± 0.09 ^b | 82 ± 2 ^c | 0.72 ± 0.04 ^a | 87 ± 20 |

NOTE. SAME levels are in nmol/mg protein, and *MAT2A* expression is expressed as percent of control *MAT2A* mRNA levels. Wild-type C57/B6 mice were given SAME or MTA in drinking water daily for 6 days. Results are mean ± SE from 4 mice per group.

^a*P* < .01, ^b*P* < .05, ^c*P* < .001 vs control group.

treatment of normal and malignant hepatocytes actually inhibits growth.^{5,31} Whether SAME or its metabolite MTA can influence growth in colon cancer cells has not been examined.

Although no study has examined the role of SAME in the pathogenesis of colon cancer, by comparison, there is an abundant literature on the role of folate on colon cancer risk. It is well recognized that folic acid deficiency correlates with increased risk of colorectal cancer.³² One of the consequences of folic acid deficiency is SAME deficiency because folic acid is involved in the remethylation of homocysteine to methionine, the precursor of SAME.¹ However, folic acid supplementation can either prevent or exacerbate intestinal tumorigenesis, depending on the timing of supplementation.³² This may be related to the fact that folic acid is also a precursor for nucleotides. This also makes the supplementation of folic acid in colon cancer complicated. Interestingly, low methionine intake in the diet, which would be expected to lower SAME levels, is also a recognized risk factor for colon cancer.³³ SAME is now widely available in the United States as a nutritional supplement and is largely free of side effects.¹ The goals of the present study were to examine whether the expression of *MAT* genes is altered in colon cancer, whether mitogens implicated in the pathogenesis of colon cancer can influence *MAT* gene expression, and whether SAME can modulate this response.

MAT2A mRNA levels were higher in 12 of 13 colon cancer specimens. This resulted in higher *MAT* II protein levels. In contrast to liver cancer, where *MAT2β* is also induced, it is mostly unchanged in colon cancer. To see if this pathway is important in the early state of intestinal neoplasia, we examined adenomatous polyps from *Min* mice, which are heterozygous for the multiple intestinal neoplasia (*Min*) mutation of the *APC* gene.^{18,34} The *Min* mouse is generally agreed to be a useful model for the study of early stages of intestinal cancer because the *APC* gene is usually inactivated early in the carcinogenic process in humans, both in sporadic colorectal cancer and in familial polyposis.³⁴ Indeed, *MAT2A* mRNA levels are higher in all polyps.

We next examined the effect of 3 well-known growth factors on the expression of *MAT* genes in 2 different

colon cancer cell lines to make sure the response is not unique to a particular cell line. Each of these growth factors has been implicated in the pathogenesis of colon cancer. All have been shown to be mitogenic in colon cancer cell lines.^{12,15,17} Higher levels of the growth factor and/or its receptor have also been found in colon cancer.¹²⁻¹⁷ We found that all 3 growth factors induced *MAT2A* gene expression but had little influence on *MAT2β* in both colon cancer cell lines. SAME and its metabolite MTA lowered *MAT2A* expression and largely prevented the induction of *MAT2A* by these growth factors. We extended the investigation to MTA because MTA is a product of SAME metabolism in the polyamine pathway as well as nonenzymatic hydrolysis.²⁷ We have shown that the effect of SAME on cell growth and gene expression can be mimicked by MTA.^{8,9,35,36} In contrast to SAME, MTA inhibits methylation and polyamine synthesis.^{27,37} Thus, additional insights can be gained by comparing the effect of these 2 agents. The fact that MTA is more potent than SAME for many of the effects of SAME suggests that many of the actions of SAME may in fact be mediated via MTA.

We have characterized transcriptional regulation of human *MAT2A* in human liver cancer cells and found 4 functional cis-acting elements and their corresponding transcription factors to contribute to the up-regulation of *MAT2A* in human liver cancer.^{22,38} In colon cancer cells, all 3 growth factors induced the *MAT2A* promoter activity to levels comparable to their effects on endogenous *MAT2A* expression. Both SAME and MTA lowered the *MAT2A* promoter activity, also to levels comparable to their effects on the endogenous gene expression. These results support the notion that the effect lies at the transcriptional level. Specificity is assured because SAME and MTA had no effect on the human *GSS* promoter activity.

To address whether the increase in *MAT2A* is causally linked to increased growth, we took 2 approaches. One is to block mitogen-mediated *MAT2A* induction by SAME and MTA. Indeed, both agents prevented the mitogenic effect of these growth factors. However, given that these agents may have other effects besides modulating *MAT2A* expression, we also took a more direct approach, namely modulating the level of *MAT2A* expression using RNAi. A

growth factors closely parallels their ability to induce *MAT2A* expression. SAME and MTA down-regulate *MAT2A* expression when used at pharmacologic doses and block the ability of the growth factors to induce *MAT2A* and exert the mitogenic response. Collectively, these results support an important role for *MAT2A* in the pathogenesis of colon cancer and suggest future studies examining whether SAME and MTA may be effective as chemopreventive agents in colon neoplasia.

References

- Lu SC, Mato JM. Role of methionine adenosyltransferase and SAME in alcohol-associated liver cancer. *Alcohol* 2005;35:227–234.
- Kotb M, Mudd SH, Mato JM, Geller AM, Kredich NM, Chou JY, Cantoni GL. Consensus nomenclature for the mammalian methionine adenosyltransferase genes and gene products. *Trends Genet* 1997;13:51–52.
- Cai J, Sun WM, Hwang J, Stain S, Lu SC. Changes in S-adenosylmethionine synthetase in human liver cancer: molecular characterization and significance. *Hepatology* 1996;24:1090–1097.
- Huang Z, Mao Z, Cai J, Lu SC. Changes in methionine adenosyltransferase during liver regeneration in the rat. *Am J Physiol* 1998;38:G14–G21.
- Cai J, Mao Z, Hwang J, Lu SC. Differential expression of methionine adenosyltransferase genes influences the rate of growth of human hepatocellular carcinoma cells. *Cancer Res* 1998;58:1444–1450.
- Garcia-Trevijano ER, Latasa MU, Carretero MV, Berasain C, Mato JM, Avila MA. S-Adenosylmethionine regulates *MAT1A* and *MAT2A* gene expression in cultured rat hepatocytes: a new role for S-adenosylmethionine in the maintenance of the differentiated status of the liver. *FASEB J* 2000;14:2511–2518.
- Yang HP, Huang ZZ, Zeng ZH, Chen CJ, Selby RR, Lu SC. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. *Am J Physiol* 2001;280:G184–G190.
- Ansorena E, García-Trevijano ER, Martínez-Chantar ML, Huang ZZ, Chen LX, Mato JM, Iraburu M, Lu SC, Avila MA. S-adenosylmethionine and methylthioadenosine are anti-apoptotic in cultured rat hepatocytes but pro-apoptotic in human hepatoma cells. *Hepatology* 2002;35:274–280.
- Yang HP, Sadda MR, Li M, Zeng Y, Chen LX, Bae WJ, Ou XP, Runnegar MT, Mato JM, Lu SC. S-Adenosylmethionine and its metabolite induce apoptosis in HepG2 cells: role of protein phosphatase 1 and Bcl-x_s. *Hepatology* 2004;40:221–231.
- Tobeña R, Horikawa S, Calvo V, Alemany S. Interleukin-2 induces γ -S-adenosyl-L-methionine synthetase gene expression during T-lymphocyte activation. *Biochem J* 1996;319:929–933.
- Ito K, Ikeda S, Kojima N, Miura M, Shimizu-Saito K, Yamaguchi I, Katsuyama I, Sanada K, Iwai T, Senoo H, Horikawa S. Correlation between the expression of methionine adenosyltransferase and the stages of human colorectal carcinoma. *Surg Today* 2000;30:706–710.
- Reinmuth N, Fan F, Liu W, Parikh AA, Stoeltzing O, Jung YD, Bucana CD, Radinsky R, Gallick GE, Ellis LM. Impact of insulin-like growth factor receptor-1 function on angiogenesis, growth, and metastasis of colon cancer. *Lab Invest* 2002;82:1377–1389.
- LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. *Cancer Lett* 2003;195:127–137.
- Roberts RB, Min L, Washington MK, Olsen SJ, Settle SH, Coffey RJ, Threadgill DW. Importance of epidermal growth factor receptor signaling in establishment of adenomas and maintenance of carcinomas during intestinal tumorigenesis. *Proc Natl Acad Sci U S A* 2002;99:1521–1526.
- Yokoi J, Thaker PH, Yazici S, Rebhun RR, Nam DH, He J, Kim SJ, Abbruzzese JL, Hamilton SR, Fidler IJ. Dual inhibition of epidermal growth factor receptor and vascular endothelial growth factor receptor phosphorylation by AEE788 reduced growth and metastasis of human colon carcinoma in an orthotopic nude mouse model. *Cancer Res* 2005;65:3716–3725.
- Stattin P, Lukanova A, Biessy C, Söderberg S, Palmqvist R, Kaaks R, Olsson T, Jellum E. Obesity and colon cancer: does leptin provide a link? *Int J Cancer* 2004;109:149–152.
- Attoub S, Noe V, Pirola L, Bruyneel E, Chastre E, Mareel M, Wymann MP, Gespach C. Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase, Rho- and Rac-dependent signaling pathways. *FASEB J* 2000;14:2329–2338.
- Sodir NM, Chen X, Park R, Nickel AE, Conti PS, Moats R, Bading JR, Shibata D, Laird PW. Smad3 deficiency promotes tumorigenesis in the distal colon of Apc^{Min/+} mice. *Cancer Res* 2006;66:8430–8438.
- Hardwick JCH, Van Den Brink GR, Offerhaus GJ, Van Deventer SJH, Peppelenbosch MP. Leptin is a growth factor for colonic epithelial cells. *Gastroenterology* 2001;121:79–90.
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002; 3:34.1–34.11.
- Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods* 2001;25:386–401.
- Yang HP, Sadda MR, Yu V, Zeng Y, Lee TD, Ou XP, Chen LX, Lu SC. Induction of human methionine adenosyltransferase 2A expression by tumor necrosis factor alpha: Role of NF- κ B and AP-1. *J Biol Chem* 2003;278:50887–50896.
- Lee TD, Yang HP, Whang J, Lu SC. Cloning and characterization of the human glutathione synthetase 5'-flanking region. *Biochem J* 2005;390:521–528.
- Farrar C, Clarke S. Altered levels of S-adenosylmethionine and S-adenosylhomocysteine in the brains of L-isoaspartyl (D-aspartyl) O-methyltransferase-deficient mice. *J Biol Chem* 2002;277:27856–27863.
- Brown ND, Strickler MP. Femtomolar ion-pair high-performance liquid chromatographic method for determining Dns-polyamine derivatives of red blood cell extracts utilizing an automated polyamine analyzer. *J Chromatogr* 1982; 245:101–108.
- Halim AB, Legros L, Geller A, Kotb M. Expression and functional interaction of the catalytic and regulatory subunits of human methionine adenosyltransferase in mammalian cells. *J Biol Chem* 1999;274:29720–29725.
- Clarke SG. Inhibition of mammalian protein methyltransferases by 5'-methylthioadenosine (MTA): a mechanism of action of dietary SAME? *Enzymes* 2006;24:467–493.
- Pascale RM, Simile MM, Satta G, Seddaiu MA, Daino L, Pinna G, Vinci MA, Gaspa L, Feo F. Comparative effects of L-methionine, S-adenosyl-L-methionine and 5'-methylthioadenosine on the growth of preneoplastic lesions and DNA methylation in rat liver during the early stages of hepatocarcinogenesis. *Anticancer Res* 1991;11:1617–1624.
- Mato JM, Lu SC. S-adenosylmethionine. In: Coates PM, Blackman MR, Levine M, Moss J, White JD, eds. *Encyclopedia of dietary supplements*. New York: Marcel Dekker, 2005. Available at: <http://www.dekker.com/sdek/abstractdb=enccountant=a713479736>.
- Martínez-Chantar ML, Garcia-Trevijano ER, Latasa MU, Martín-Duce A, Fortes P, Caballeria J, Avila MA, Mato JM. Methionine adenosyltransferase II β subunit gene expression provides a

- proliferative advantage in human hepatoma. *Gastroenterology* 2003;124:940–948.
31. Martínez-Chantar ML, Vázquez-Chantada M, Garnacho-Echevarria M, Latasa MU, Varela-Rey M, Dotor J, Santamaria M, Martínez-Cruz LA, Parada LA, Lu SC, Mato JM. S-adenosylmethionine regulates cytoplasmic HuR via AMP-activated kinase. *Gastroenterology* 2006;131:223–232.
 32. Kim YI. Role of folate in colon cancer development and progression. *J Nutr* 2003;133:3731S–3739S.
 33. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* 1995;87:265–273.
 34. Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, Li E, Weinberg RA, Jaenisch R. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* 1995;81:197–205.
 35. Latasa MU, Boukaba A, García-Trevijano ER, Torres L, Rodríguez J, Caballería L, Lu SC, López-Rodas G, Franco L, Mato JM, Avila MA. Hepatocyte growth factor induces *MAT2A* expression and histone acetylation in rat hepatocytes: role in liver regeneration. *FASEB J* 2001;15:1248–1250.
 36. Veal N, Hsieh CL, Xiong S, Mato JM, Lu SC, Tsukamoto H. Inhibition of lipopolysaccharide-stimulated TNF α promoter activity by S-adenosylmethionine and 5'-methylthioadenosine. *Am J Physiol* 2004;287 G352–G362.
 37. Dante R, Anaud M, Niveleau A. Effects of 5'-deoxy-5'-methylthioadenosine on the metabolism of S-adenosylmethionine. *Biochem Biophys Res Commun* 1983;114:214–221.
 38. Yang HP, Huang ZZ, Wang JH, Lu SC. The role of c-Myb and Sp1 in the up-regulation of methionine adenosyltransferase 2A gene expression in human hepatocellular carcinoma. *FASEB J* 2001;15:1507–1516.
 39. Tobeña R, Horikawa S, Calvo V, Alemany S. Interleukin-2 induces γ -S-adenosyl-L-methionine synthetase gene expression during T-lymphocyte activation. *Biochem J* 1996;319:929–933.
 40. Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer* 2004;4:143–153.
 41. Martínez-Chantar ML, Corrales FJ, Martínez-Cruz A, García-Trevijano ER, Huang ZZ, Chen LX, Kanel G, Avila MA, Mato JM, Lu SC. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002;16:1292–1294.

Received November 21, 2006. Accepted March 22, 2007.

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Supported by National Institutes of Health grants DK51719 (to S.C.L.), AT1576 (to S.C.L. and J.M.M.), CA075090 (to P.W.L.), and GM026020 (to S.C.); Plan Nacional of I+D SAF 2005-00855; and HEPADIP-EULSHM-CT-205 (to J.M.M.). HT-29 and RKO cells were provided by the Cell Culture Core, and polyamine levels were measured by the Cell Biology Core of the University of Southern California Research Center for Liver Diseases (P30DK48522). Normal and cancerous colon tissues were obtained from the University of Southern California/Norris Cancer Center (P30 CA14089).

The authors have no conflicts of interest to disclose.

H.C. and M.X. contributed equally to this report.