Inhibitory effect of S-adenosylmethionine on the growth of human gastric cancer cells in vivo and in vitro

Ye Zhao¹, Jian-Sheng Li¹, Ming-Zhou Guo², Bai-Sui Feng¹, Jin-Ping Zhang¹

¹ Department of Gastroenterology, the First Affiliated Hospital of Zhengzhou University, Key-Disciplines Laboratory Clinical-Medicine, Zhengzhou, Henan 450052, P. R. China; ² Department of Gastroenterology, the General Hospital of the People’s Liberation Army, Beijing 100853, P. R. China

Abstract

Background and Objective: S-adenosylmethionine (SAM), the most important methyl donor in human body, is generally used to treat cholestasis in clinic. In recent years, SAM has been found to have inhibitory effects on breast cancer, liver cancer and colon carcinoma. This study was to investigate the inhibitory effects of SAM on human gastric cancer cells in vivo and in vitro, and the antitumor mechanisms. Methods: The effects of SAM on the proliferation of gastric cancer SGC-7901 and MKN-45 cells were determined by MTT assay. After SGC-7901 and MKN-45 cells were treated with 0, 2, and 4 mmol/L SAM for 72 h, the expression and methylation of c-myc and urokinase type plasminogen activator (uPA) were detected by reverse transcription-polymerase chain reaction (RT-PCR) and methylation-specific PCR (MSP). Tumor xenografts were established by injecting SGC-7901 cells subcutaneously in BALB/c nude mice. The mice were randomized into low concentration group [192 μmol/(kg·day)], high concentration group [768 μmol/(kg·day)], and control group [normal saline (NS)], and received peritoneal injection of relative reagents for 15 days. The tumor size was measured, the protein and mRNA expression of c-myc and uPA were detected by Immunohistochemistry and RT-PCR, and the methylation of c-myc and uPA genes was detected by MSP. Results: SAM inhibited the growth of SGC-7901 and MKN-45 cells obviously and the effects were enhanced with the increase of SAM concentration and treatment time. The mRNA expression of c-myc and uPA in SGC-7901 cells and that of uPA in MKN-45 cells significantly decreased. The c-myc and uPA genes in SGC-7901 cells and uPA gene in MKN-45 cells were partly or completely methylated after SAM treatment. The tumor volume was significantly lower in low concentration group [(618.51 ± 149.27) mm³] and high concentration group [(444,32 ± 118,51) mm³] than in control group [(1018,22 ± 223,07) mm³] (both P < 0,01). The inhibitory rates of tumor growth were 39,28% in low concentration group and 58,36% in high concentration group. The protein and mRNA expressions of c-myc and uPA were remarkably reduced (all P < 0,01), and the hypomethylation of c-myc and uPA genes were reversed after SAM treatment. Conclusions: SAM can inhibit the growth of human gastric cancer cells both in vivo and in vitro. The mechanism may be that SAM can reverse the hypomethylation of c-myc and uPA genes, reduce their expression, and then inhibit tumor growth.

Key words: S-adenosylmethionine, gastric neoplasm, nude mouse, DNA methylation, c-myc, uPA

S-adenosylmethionine (SAM), the active form of methionine, is widespread in plants and animals. SAM is the most important methyl donor in human body, and it undergoes DNA methylation catalyzed by DNA methyltransferase. Methylation of DNA plays an important role in maintaining chromosome structure, inactivation of X chromosome, genomic imprinting, and development of tumor. The change in methylation status is an important factor in oncogenesis. Abnormal methylation, which is widespread in malignant tumors, induces hypomethylation of the whole genome and oncogenes and hypermethylation of tumor suppressor genes. SAM has been widely used in the clinical treatment of cholestasis for many years.
In recent years, it has been reported that SAM can promote apoptosis and inhibit growth in breast cancer \cite{1}, liver cancer \cite{2,3}, and colon cancer \cite{4}, and can improve the hypomethylation of oncogenes such as c-myc, c-fos, c-H-ras, and c-K-ras and uPA gene (urokinase type plasminogen activator). The inhibitory effect of SAM is considered to be contributed by its being a donor of methyl to improve the hypomethylation of gene and inhibit the gene expression.

However, studies of SAM in the gastric cancer have not yet been reported. In this study, the effects of SAM in human gastric cancer cell lines SGC-7901 and MKN-45 were observed, and SAM was used in the nude mice xenograft model of SGC-7901 cells to study the effect of SAM on the growth of gastric cancer cells, methylation status, and expressions of c-myc and uPA genes and to investigate the possible anti-tumor effects of SAM on gastric cancer and its mechanisms.

Materials and Methods

Cells and reagents

SGC-7901 cells were purchased from Type Culture Collection cell bank of the Chinese Academy of Sciences. MKN-45 cell line was a gift from Professor Wu Kaichun in Xijing Hospital, the Fourth Military Medical University. BALB/c female nude mice of 4–6 weeks of age, weighing 14–16 g, were purchased from Shanghai experimental animal Center of Chinese Academy of Sciences [Number of certificate of conformity: SCXK (Shanghai) 2007–0005] and kept under SPF conditions. SAM was purchased from Sigma. RPMI-1640 medium was purchased from Gibco Company. The 96-well plates were purchased from Costar Corporation. Total RNA extraction kit Trizol was purchased from Invitrogen Corporation. RT-PCR kit was purchased from Tiangen Company. The 2 $\times$ Taq PCR MasterMix was purchased from Tiangen company. General genomic DNA extraction kit was purchased from Shanghai Sangon Company. EZ DNA Methylation Kit-Gold (No. D5005) was purchased from ZYMO RESEARCH Company. Ex Taq hot start enzyme was purchased from TaKaRa Company. Human lymphocyte separation medium was purchased from TBD Company. CpG methyltransferase was purchased from NEB Company. The primary antibodies of c-myc and uPA were mouse anti-human monoclonal antibodies and purchased from Lab Vision Corporation. SP kit was purchased from Beijing Zhong Shan Company. Primers were synthesized by Shanghai Sangon Company. uPA MSP primers were designed by Professor Guo Mingzhou, People’s Liberation Army General Hospital. c-myc MSP primer sequences were cited from Fang et al.\cite{5}

Determination of cell proliferation with MTT assay

A total of 200 $\mu$L of cells in logarithmic phase were seeded into 96-well plates at a density of 2.5 $\times$ 10$^4$/mL, each concentration with 3 parallel holes. After 24 h, the supernatant was decanted and cell medium containing SAM at the concentrations of 0.5, 1, 2, 4, 8, 16, or 32 mmol/L was added. The control group without any treatment was set up. The incubation was terminated after 24, 48, and 72 h, one plate at each time. A total of 20 $\mu$L of freshly prepared MTT solution (5 g/L) was added into each well, and the cells were incubated for another 4 h. After centrifugation at 1200 r/min for 10 min, supernatants were removed and 150 $\mu$L of dimethyl sulfoxide (DMSO) was added. A value of each well at a wavelength of 492 nm ($A_{492}$) was detected after shaking for 10 min. The proliferative capacity was determined by the average absorbance. Each experiment was repeated 3 times.

Establishment of human gastric cancer nude mouse model and treatment with SAM

SGC-7901 cells in logarithmic phase were digested and suspended in PBS. A total of 200 $\mu$L of PBS with approximately 1 $\times$ 10$^7$ cells was injected into the subcutaneous tissue of the left axillary of female nude. Seven days later, 15 mice with subcutaneous tumor over 60 mm$^3$ were divided randomly into three groups: low concentration group [192 $\mu$mol/(kg·day)], high concentration group [768 $\mu$mol/(kg·day)], and control group [normal saline, (NS)], with 5 mice in each group. According to body weight of nude mice, they were intraperitoneally injected with appropriate drugs, one time a day for 15 days. Mice were killed on the 16th day.

Long diameter (a) and short diameter (b) were measured every 3 days to calculate tumor volume ($V = \frac{1}{6} \pi a b^2$), tumor growth curve was drawn and tumor inhibition rate calculated. The body weight of mice was measured. Part of the tumor tissue was stored in liquid nitrogen quickly and the rest of the tissue were fixed with 4% formaldehyde, embedded with paraffin, and sliced. Tumor growth inhibition rate = ($1 - \frac{\Delta V \text{ treatment group}}{\Delta V \text{ control group}}$) $\times$ 100%.

Detection of protein expression in sections with immunohistochemical SP method

After deparaffinage with xylene, the sections were rehydrated. Antigen was retrieved with 0.01 mol/L citrate under high-pressure, and the sections were incubated in 3%
H₂O₂ for 15 min. After block in serum for 30 min, the sections were incubated with the primary antibody overnight and with the secondary and third antibodies for 30 min. DAB color reaction was controlled under microscope. Afterward, the sections were counterstained with hematoxylin. Followed by dehydration and transparency, the sections were mounted. Instead of the primary antibody, PBS was used as the negative control. Brown staining was indicative of positive staining. c-myc was located in the nucleus and cytoplasm; uPA was located in the cytoplasm. According to the staining intensity of positive cell, no staining, yellow, brown, and dark brown staining were defined as 0, 1, 2, and 3, respectively (Color depth should be compared with the background color). Based on the percentage of positively stained cells, the results were graded as follows: 0, no staining; 1, < 10% positively stained cells; 2, 11%–50% positively stained cells; 3, 51%–75% positively stained cells; 4, > 75% positively stained cells. The product of the staining intensity and the percentage of positive cells over 3 were defined as positive (+).

**Detection of gene expression with RT–PCR**

Cells treated with 0, 2, and 4 mmol/L SAM for 72 h and frozen tumor tissues were collected. Total RNA was extracted with Trizol reagent according to the instructions. cDNA was synthesized according to instructions of the reverse transcription kit. cDNA (2 µL) was used as the template for PCR amplification. Primer sequences and PCR amplification conditions are shown in Table 1. PCR products were analyzed after electrophoresis with β-actin gene as an internal control to correct the gray value to get the relative values of gene expression.

**Detection of methylation status of the target gene with MSP**

Cells treated with 0, 2, and 4 mmol/L SAM for 72 h and frozen tumor tissues were collected. Total DNA was extracted according to the instructions of DNA extraction kit. EZ DNA glycosylation-Gold kit was used for bisulfite modification of DNA. Modified DNA (2 µL) was used as the template for PCR amplification with hot start enzymes. Normal peripheral blood lymphocyte DNA was extracted. DNA without treatment with CpG methyltransferase that was modified with bisulfate was used as a negative control for PCR amplification instead of templates. Primers and PCR amplification conditions are shown in Table 2. PCR products were analyzed after electrophoresis.

**Table 1 RT-PCR primer sequences and amplification conditions**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5'-3')</th>
<th>Antisense primer (5'-3')</th>
<th>Primer size</th>
<th>Reaction condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc</td>
<td>CTCTGGGAAAAGGTCAGAG</td>
<td>GGCTTITGTATGTITTCCA</td>
<td>355 bp</td>
<td>94°C 3 min, 94°C 30 s, 58°C 30 s, 72°C 1 min, 32 cycles; 72°C 5 min</td>
</tr>
<tr>
<td>uPA</td>
<td>TAGGTGGAADTCGTACCT</td>
<td>GACAGCATTTTGGTGAGAC</td>
<td>320 bp</td>
<td>94°C 3 min, 94°C 30 s, 58°C 30 s, 72°C 1 min, 34 cycles; 72°C 5 min</td>
</tr>
<tr>
<td>β-actin</td>
<td>AAGAGGGCATCCTACCCCT</td>
<td>TACATGCGTTGGGTTGAA</td>
<td>218 bp</td>
<td>Same as above</td>
</tr>
</tbody>
</table>

**Table 2 MSP primer sequences and amplification conditions**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5'-3')</th>
<th>Antisense primer (5'-3')</th>
<th>Primer size</th>
<th>Reaction condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc (M)</td>
<td>TAGAATTGGATCGGCTGAA</td>
<td>CGAGCGAAAAATCAAGCGAAT</td>
<td>131 bp</td>
<td>94°C 30 s, 56°C 30 s, 72°C 1 min, 35 cycles; 72°C 5 min</td>
</tr>
<tr>
<td>c-myc (U)</td>
<td>TAGGGTGATCGGTGAA</td>
<td>CCAACGAAATCAAGCGAAT</td>
<td>132 bp</td>
<td>94°C 30 s, 56°C 30 s, 72°C 1 min, 35 cycles; 72°C 5 min</td>
</tr>
<tr>
<td>uPA (M)</td>
<td>GAATAGGTTTTTGCATTGTAGC</td>
<td>CGAACCGACGCCGGTAAAACC</td>
<td>112 bp</td>
<td>94°C 30 s, 56°C 30 s, 72°C 1 min, 35 cycles; 72°C 5 min</td>
</tr>
<tr>
<td>uPA (U)</td>
<td>GGAATAGGTTTTTGCATTGTAGC</td>
<td>CCAACCGACGCCGGTAAAACC</td>
<td>111 bp</td>
<td>94°C 30 s, 56°C 30 s, 72°C 1 min, 35 cycles; 72°C 5 min</td>
</tr>
</tbody>
</table>

**Statistical analysis**

The statistical software SPSS11.1 was used for the statistical analysis. Data are presented as mean ± standard deviation (SD). The results of immunohistochemistry were analyzed by Wilcoxon rank-sum test. The results of MSP were analyzed by contingency table χ² test. Variance analysis was performed for analyzing other results. Statistical significance was set as P < 0.05.

**Results**

**Effects of SAM on the proliferation of gastric cancer cells in vitro**

Compared with control group, the proliferation activities of cells in experimental groups were all inhibited significantly with the increase of the concentration and the prolonging of reacting time (P < 0.05) (Table 3).
Effects of SAM on c-myc and uPA mRNA expressions

(1) c-myc mRNA expression: In SGC-7901 cells, c-myc mRNA expression in 2 and 4 mmol/L SAM groups was significantly lower than that in control group (0.76 ± 0.08 and 0.56 ± 0.05 vs. 0.94 ± 0.03, P < 0.05 and P < 0.01). In MKN-45 cells, there was no significant difference in c-myc mRNA expression between 2 mmol/L SAM and control groups and between 4 mmol/L SAM and control groups (1.02 ± 0.07 and 0.97 ± 0.06 vs. 1.00 ± 0.13, P > 0.05) (Figure 1).

Table 3 Effect of SAM on proliferation of SGC-7901 and MKN-45 cells

<table>
<thead>
<tr>
<th>Concentration of SAM</th>
<th>SGC-7901</th>
<th>MKN-45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>0.81±0.026</td>
<td>0.885±0.021</td>
</tr>
<tr>
<td>0.5 mmol/L</td>
<td>0.79±0.027</td>
<td>0.822±0.025</td>
</tr>
<tr>
<td>1 mmol/L</td>
<td>0.69±0.022</td>
<td>0.737±0.022</td>
</tr>
<tr>
<td>2 mmol/L</td>
<td>0.62±0.024</td>
<td>0.614±0.017</td>
</tr>
<tr>
<td>4 mmol/L</td>
<td>0.59±0.021</td>
<td>0.562±0.021</td>
</tr>
<tr>
<td>8 mmol/L</td>
<td>0.56±0.013</td>
<td>0.529±0.024</td>
</tr>
<tr>
<td>16 mmol/L</td>
<td>0.52±0.017</td>
<td>0.456±0.023</td>
</tr>
<tr>
<td>32 mmol/L</td>
<td>0.48±0.015</td>
<td>0.429±0.019</td>
</tr>
</tbody>
</table>

All values are presented as mean ± standard deviation (SD). *P < 0.05, vs. control group.

(2) uPA mRNA expression: Both in SGC-7901 and MKN-45 cells, uPA mRNA expression in 2 and 4 mmol/L SAM groups was significantly lower than that in control group (SGC-7901 cells: 0.54 ± 0.08 and 0.37 ± 0.11 vs. 0.94 ± 0.06, both P < 0.01; MKN-45 cells: 0.69 ± 0.08 and 0.46 ± 0.05 vs. 0.87 ± 0.07, P < 0.05 and P < 0.01) (Figure 1).

Effect of SAM on promoter methylation status of c-myc and uPA in vitro

The 131-bp band was amplified with methylated c-myc positive control primers and the 132-bp band with unmethylated c-myc positive control primers. The 112-bp band was amplified with methylated uPA positive control primers and 111-bp band with unmethylated uPA positive control primers. No band was amplified with U primers, suggesting that 5’ CpG islands of gene promoters all underwent methylation; if no band was amplified with M primers and the corresponding bands can be amplified with U primers, it suggested that no gene underwent methylation; if the corresponding bands can be amplified with M or U, it suggested that part of target genes underwent methylation.

1) Results of c-myc methylation: No SGC-7901 cells in the control group and part of SGC-7901 cells in 2 and 4 mmol/L groups underwent methylation; however, no MKN-45 cells in the control group, 2 and 4 mmol/L groups underwent methylation (Figure 2).

![Figure 1](image-url)  Effect of SAM on expression of c-myc and uPA mRNA in SGC-7901 and MKN-45 cells

Lanes 1-3, cells treated with 0, 2, and 4 mmol/L SAM, respectively. β-actin (218 bp) was used as internal control. Compared with control group, the expression of c-myc and uPA mRNA in SGC-7901 cells as well as uPA mRNA in MKN-45 cells treated with 2 and 4 mmol/L SAM were remarkably reduced (P < 0.05 and P < 0.01).
(2) Results of uPA methylation: No SGC-7901 cells in the control group, part of SGC-7901 cells in 2 mmol/L group, and all the SGC-7901 cells in 4 mmol/L group underwent methylation; no MKN-45 cells in control group and part of MKN-45 cells in 2 and 4 mmol/L groups underwent methylation (Figure 2).

**Figure 2** Effect of SAM on methylation of c-myc and uPA gene in SGC-7901 and MKN-45 cells

Lanes 1–3, cells treated with 0, 2, and 4 mmol/L SAM, respectively. MP, positive control; UP, negative control; M, methylation production; U, non-methylation production. The methylation of c-myc and uPA gene in SGC-7901 cells as well as uPA gene in MKN-45 cells was reversed after treated with SAM.

**Effects of SAM on the body weight of nude mice and the tumor volume**

There was no death in 15 nude mice. The body weight gradually increased. When executed, the average body weights of mice were in (16.48 ± 0.83) g in the control group, (16.16 ± 1.14) g in the low concentration group, and (16.36 ± 0.98) g in the high concentration group. No significant difference was found among these 3 groups (P > 0.05). The average tumor volumes were (1018.22 ± 223.07) mm³ in the control group, (618.51 ± 149.27) mm³ in the low concentration group, and (444.32 ± 118.51) mm³ in the high concentration group. Compared with control group, the average tumor volume of both experimental groups significantly reduced (P < 0.01), but there was no significant difference between two experimental groups (P > 0.05). The inhibition rates of tumor volume in low concentration and high concentration groups were 39.26% and 56.36% .

According to the tumor growth curve, SAM can significantly inhibit the growth of SGC-7901 tumor xenografts (Figures 3 and 4).

**Effects of SAM on of c-myc and uPA protein expressions in tumor xenografts**

(1) c-myc protein expression: The staining scores in control group, low concentration group, and high concentration group were 8.2 ± 0.4, 4.4 ± 1.5, and 1.8 ± 1.3. The c-myc protein expression was significantly lower in both experimental groups than in the control group (both P < 0.01) (Figure 5).

**Figure 3** Growth curves of gastric carcinoma xenografts in nude mice after different treatment

After treated with different concentrations of SAM, the growth of the tumor xenografts in nude mice were greatly inhibited.
(2) uPA protein expression: The staining scores in control group, low concentration group, and high concentration group were $7.8 \pm 1.1$, $4.4 \pm 1.5$, and $2.0 \pm 1.4$. The uPA protein expression was significantly lower in both experimental groups than in the control group (both $P < 0.01$) (Figure 5).
Effects of SAM on c-myc and uPA mRNA expressions in tumor xenografts

(1) c-myc mRNA expression: The corresponding ratios of c-myc mRNA in control group, low concentration group, and high concentration group were 0.99 ± 0.10, 0.73 ± 0.15, and 0.26 ± 0.08. Compared with control group, the c-myc mRNA expression in both experimental groups was significantly lower (P < 0.01) (Figure 6).

(2) uPA mRNA expression: The corresponding ratios of uPA mRNA in control group, low concentration group, and high concentration group were 0.53 ± 0.14, 0.34 ± 0.05, and 0.11 ± 0.04. The uPA mRNA expression was significantly lower in both experimental groups than in control group (P < 0.01) (Figure 6).

Effects of SAM on promoter methylation status of c-myc and uPA

(1) c-myc methylation status: None of the 5 mice in control group, 3 of 5 mice in low concentration group, and all the 5 mice in high concentration group underwent c-myc methylation. The difference in c-myc methylation status among these three groups was significant (χ² = 10.179, P < 0.01) (Figure 7).

(2) uPA methylation status: None of the 5 mice in the control group, 4 of 5 mice in low concentration group, and all the 5 mice in high concentration group underwent uPA methylation. The difference in uPA methylation status among these three groups was significant (χ² = 11.667, P < 0.01) (Figure 7).

Figure 6 Expression of c-myc and uPA mRNA in xenografts
Lane 1–5, 5 cases of every group. β-actin (218 bp) was used as internal control. Compared with those in control group, the expression of c-myc and uPA mRNA in low and high concentration groups were remarkably reduced (P < 0.01). Low, low concentration of SAM; High, high concentration of SAM.

Figure 7 States of methylation of c-myc and uPA gene in xenografts
Lanes 1–5, 5 cases of every group. M, methylation production; U, Un-methylation production. Methylation of c-myc was not detected in control group, but was detected in 3 cases in low concentration group and 5 cases in high concentration group (χ² = 10.179, P < 0.01). Methylation of uPA was not detected in control group, but was detected in 4 cases in low concentration group and 5 cases in high concentration group (χ² = 11.667, P < 0.01). Low, low concentration of SAM; High, high concentration of SAM.
Discussion

Gastric cancer is the most common gastrointestinal malignancy, accounting for the second cause of death in cancer. With the development in epigenetics, people began to realize that the abnormal change of DNA methylation plays an important role in the tumorigenesis and development of tumor[8]. Studies have found that unreasonably low-level methylation of genes would induce high expressions of some genes such as oncogenes which were inhibited in normal status and related factors[6]. In addition, unreasonably low-level methylation would increase the instability of the whole genome[8]. All of these are related to tumorigenesis. DNA methylation is a reversible process. Therefore, reversing the hypomethylation of promoters of tumor-promoting genes such as oncogenes by re-methylation to silence their expression would treat tumors.

In recent years, some studies have shown that SAM, as a methyl group donor, can induce hypermethylation of genes and reverse the overall hypomethylation to inhibit the expression of oncogene, therefore it could inhibit tumor growth, reduce tumor invasiveness, and slow down tumor metastasis[8]. Its role in the prevention and treatment of tumor is increasingly concerned. After a long time study, Pascale et al.[2] have confirmed that SAM has preventive effect in liver cancer and could reverse the hypomethylation of c-Ha-ras, c-Ki-ras, c-myc, and their overexpression. Bai et al.[10] used SAM as an inducer to re-methylate DNA and found that the level of methylation increased while the activity of DNMT did not increase, suggesting that the increase of methylation level was caused by SAM which provides excessive methyl and not by the change of DNMT catalytic capacity. In both in vivo and vitro studies, we observed that the inhibitory activity of SAM on cell proliferation in SGC-7901 and MKN-45 cells gradually enhanced along with the increase of SAM concentration and the prolonging of the reacting time. At the same time, the inhibitory effect of SAM on human gastric cancer cell lines in vitro was confirmed. Seen from the growth curve of nude tumor xenografts, SAM significantly inhibited the growth of tumor xenografts of human gastric cancer cell line SGC-7901. According to the morphology of the tumor xenografts under microscope, SAM significantly promoted apoptosis of tumor cells. SAM is the normal material which exists in plants and animals and has a large LD₅₀[11]. In in vivo experiments of nude mice, there was no significant difference in the changes of body weight among three groups after treatment with SAM for 15 days. The impact of drug toxicity was excluded. It was considered that SAM inhibited the growth and proliferation of tumor cells through methylation of itself. In order to confirm this view, we further studied the methylation status of oncogenes c-myc and uPA.

C-myc is a kind of highly conserved DNA-binding protein carcinoid gene and involved in the regulation of cell proliferation and differentiation. Its abnormal expression is closely related to tumorigenesis and development of tumor. Sharrard et al.[9] detected c-myc gene methylation in human gastric cancer and adjacent normal tissues. They found that the level of c-myc gene methylation in about 40% to 50% of gastric cancer decreased when compared with the adjacent normal tissues, and that the transcription of the gene changed as a result. In this study, the results of MSP confirmed that there was hypomethylation in the promoter regions of c-myc oncogene in control group in SGC-7901 cells in both in vivo and in vitro experiments. After treated with different concentrations of SAM, c-myc oncogene promoter regions which were in low level of methylation underwent re-methylation. At the same time, c-myc mRNA and protein expressions significantly decreased in experimental groups treated with different concentrations of SAM than in control group. The decrease was positively correlated with the concentrations of SAM, suggesting that SAM can inhibit the expression of genes by reversing the hypomethylation in the promoter regions of c-myc oncogene. However, we found that SAM could not improve the hypomethylation in the promoter regions of c-myc oncogene in MKN-45 cells, suggesting that SAM cannot inhibit the expression of c-myc. Although SAM had no effect on the hypomethylation of c-myc gene in MKN-45 cells, the proliferation of MKN-45 and SGC-7901 cells were both inhibited, suggesting that SAM may inhibit the proliferation of gastric cancer cells through other genes or pathways.

uPA is an important protease involved in extracellular matrix degradation. Although it is not an oncogene, it is considered to be a tumor-promoting gene related to metastasis and its functions are similar to oncogenes due to its ability to promote cell growth, invasion, metastasis, and tumor angiogenesis[12]. Shukeir et al.[14] treated aggressive prostate cancer cell lines with SAM and found that the expression of uPA significantly reduced and thus resulted in gene silence. The inhibition of gene expression induced by SAM was in a time- and dose-dependent manner. In addition, Pakneshan et al.[11] found that treatment with SAM in breast cancer also led to the silence of uPA gene expression and suggested that demethylation of uPA may be one of initiating mechanisms that caused the up-regulation of uPA gene and accelerated tumor invasion and metastasis. In this study, we have demonstrated that there is hypomethylation in the promoter regions of uPA gene in MKN-45 and SGC-7901 cells in vivo and vitro. After treated with different concentrations of SAM, uPA gene promoter regions that were in low level of methylation underwent re-methylation. At the same time, uPA mRNA...
and protein expressions significantly decreased and were positively correlated with the concentrations of SAM, suggesting that SAM inhibited the expression of uPA gene through reversing its hypomethylation.

We inhibited the growth of human gastric cancer cell lines with SAM both in vivo and in vitro. Through detecting the methylation status of c-myc oncogene and metastasis-related gene uPA and the mRNA and protein expressions of these two genes, we further identified that SAM could inhibit the transcriptional activity by reversing the hypomethylation to inhibit the growth of tumor cells, confirming that SAM could be used widely in preventing and treating gastric cancer.

References