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Gene silencing of *TSPYL5* mediated by aberrant promoter methylation in gastric cancers

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DNA methylation is crucial for normal development, but gene expression altered by DNA hypermethylation is often associated with human diseases, especially cancers. The gene *TSPYL5*, encoding testis-specific Y-like protein, was previously identified in microarray screens for genes induced by the inhibition of DNA methylation and histone deacetylation in glioma cell lines. The *TSPYL5* showed a high frequency of DNA methylation-mediated silencing in both glioma cell lines and primary glial tumors. We now report that *TSPYL5* is also inactivated by DNA methylation and could be a putative epigenetic target gene in gastric cancers. We found that the expression of *TSPYL5* mRNA was frequently downregulated and inversely correlated with DNA methylation in seven out of nine gastric cancer cell lines. *TSPYL5* mRNA expression was also restored after treating with a DNA methyltransferase inhibitor. In primary gastric tumors, methylation-specific PCR results in 23 of the 36 (63.9%) cases revealed that the hypermethylation at CpG islands of the *TSPYL5* was detectable at a high frequency. Furthermore, TSPYL5 suppressed the growth of gastric cancer cells as demonstrated by a colony formation assay. Thus, strong associations between TSPYL5 expression and hypermethylation were observed, and aberrant methylation at a CpG island of *TSPYL5* may play an important role in development of gastric cancers.
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Epigenetic events are heritable modifications that regulate gene expression and can contribute to cancer development.[1] In particular, changes in DNA methylation are among the most common molecular alterations in human neoplasia, because the hypermethylation of a tumor suppressor gene at a promoter can lead to transcriptional silencing and the loss of gene function.[1–4] Gastric cancer is one of the most common malignancies in the world.[5] The promoter hypermethylation of multiple tumor suppressor genes resulting in gene silencing has been recognized as an important mechanism in gastrointestinal carcinogenesis.[6–15]

In a previous study, *TSPYL5* was identified as one of the genes induced after treatment with DNA methylation and histone deacetylation inhibitors in glioma cell lines using microarray analysis.[16] The *TSPYL5* showed a high frequency of DNA methylation-mediated silencing in both glioma cell lines and primary glial tumors. A human *TSPYL5* encoding testis-specific Y-like protein 5 (TSPYL5) is located on chromosome 8q22.1, but its role in human cancer has not been fully understood. The TSPYL5 protein is a member of TSPYL family that includes TSPYL1, TSPYL2, TSPYL3, TSPYL4, and TSPYL6. TSPYL proteins are also members of the nucleosome assembly protein (NAP) superfamily. The TSPYLs show sequence homology to NAPs, which have a highly conserved domain (the NAP domain) of approximately 180 amino acids.[17,18] The NAP domain is known to be required and sufficient for histone binding.[19] Members of the NAP family of proteins were identified in different systems and were classified with the NAPs based on their sequence homology and the possession of the NAP domain rather than their functions. The expression of NAP proteins is highly specific for many cell types and tissues.[16–18,20,21] In humans, the NAP family members include NAP1, NAP1-like protein (NAP1L), SE translocation (SET), testis-specific protein, Y-encoded (TSPY), and testis-specific protein, Y-encoded like (TSPYL).[17,20,22–26] It is known that proteins in the NAP family participate in chromatin structure and diverse cellular events, and may be involved in regulating gene expression as a result of histone accessibility.[17] NAP1, which is the best characterized member of the NAP family, plays a role in the exchange process of histone H2A and H2B dimers from assembled nucleosomes in DNA replication as a histone

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chaperone, and NAP1 may assist nucleosome sliding.[24] NAP1 is also known to be involved in nucleocytoplasmic shuttling in mitotic progression in yeast.[27] TSPY functions in early spermatogenesis and testicular tumorigenesis,[21] and potentiates cell proliferation and tumorigenesis by promoting cell-cycle progression in some cancer cells.[28] TSPYL2 (also known as CDA1, CINAP, or DENTT) facilitates chromatin assembly by binding to core histones *in vitro* and arrests cell growth.[29–31] Only a few studies have reported inactivation of the NAP-related protein *in vitro*. The down-regulation of the *TSPY* gene in melanoma progression is caused by promoter hypermethylation.[32] A recent study showed that sudden infant death with dysgenesis of the testes syndrome (SIDDT, OMIM no. #608800) was due to a frameshift mutation in the *TSPYL1* gene, and this resulted in truncation at codon 169 leading to the loss of a region with high homology to the NAP domain.[18]

The purpose of this study was to investigate gene silencing of the *TSPYL5* mediated by promoter hypermethylation in gastric cancers, and the correlation between the inactivation of *TSPYL5* and gastric cancers. We evaluated the methylation status of a CpG island in the promoter and first exon of *TSPYL5* and also used a demethylating agent to reactivate gene expression. Our data indicate that the *TSPYL5* is frequently inactivated by hypermethylation and could be a candidate gene for epigenetic targeting in gastric cancers.

MATERIALS AND METHODS

Cell Culture and Tissue Samples

Human gastric adenocarcinoma SNU-1, SNU-16, SNU-484, SNU-601, SNU-620, SNU-638, SNU-668, and SNU-719 cell lines were obtained from the Korean cell line bank.[33] AGS and MDA-MB-231 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (WelGene, Korea). Gastric carcinoma tumor tissue samples and matching non-cancerous tissues were obtained from the Seoul National University Hospital (Seoul, Korea), and the frozen tissue samples were handled as described earlier.[15]

Treatment of 5-Aza-2′-deoxycytidine and Trichostatin A

One day after seeding, cells were treated with 5-aza-2′-deoxycytidine (5-aza-dC; Sigma, St Louis, MO, USA) at 5 μM or at different concentrations (0.1, 0.5 and 1 μM) for 4 days. The medium including 5-aza-dC was replaced every other day. Cells were exposed on trichostatin A (TSA; Wako, Japan) at 0.1 μM for 24 h. For cotreatment with 5-aza-dC and TSA, cells were exposed on both agents for an additional 24 h after a single treatment with 5-aza-dC for 96 h.

RT-PCR Analysis

Total RNA was isolated using TRI Reagent® (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. cDNA was synthesized from 2 μg of total RNA using ImProm-II™ reverse transcriptase (Promega, Madison, WI, USA). The cDNA was amplified by using r*Taq* DNA polymerase (Takara, Japan) under the following conditions: 94°C for 5 min, 29–32 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and the final extension of 72°C for 10 min. Amplification of β-actin from cDNA samples was used as an internal control for the reverse transcription-PCR (RT-PCR) reaction. The primer sequences are listed in Table 1. The amplified PCR products were separated on 2% agarose gels.

Table 1 Primer sequences for RT-PCR analysis

Gene		Primer sequence (5′→3′)	NCBI no.	Length (bp)
TSPYL1	F	AGTGGCGGAGGAGGATAGAT	NM_003309	274
	R	CCTAATCATGGCGGACAACT		
TSPYL2	F	CAATGAGGGCAGTGATGATG	NM_022117	262
	R	ATCTTCCCCTTCTTCCCAGA		
TSPYL3	F	TCAGCTGGTTTTCACAGCAC	BC101558	173
	R	GAACCCGTAGGGCCTAGAAG		
TSPYL4	F	CTACGGCTTCTATGCCAAGC	NM_021648	281
	R	GGAATGTCCAATTTGGGTTG		
TSPYL5	F	ATCCCCAGCTAGCATCCTTT	NM_033512	219
	R	GGAGCCACTGGATTGGAGTA		
TSPYL6	F	GCTTTCCTGTCAGCACACAA	NM_001003937	235
	R	GGAAGGGAGGCAAATAAAGG		
β-Actin	F	TCATCACCATTGGCAATGAG	BC013835	155
	R	CACTGTGTTGGCGTACAGGT		

F, forward primers; R, reverse primers; all primer sequences were designed by using Primer3 program.

Methylation-Specific PCR and Bisulfite Sequencing

We isolated genomic DNA from cells was isolated using the DNA extraction kit (Intron Biotechnology, Korea). Genomic DNA from frozen tumor samples and paired normal tissue samples was isolated by standard phenol–chloroform extraction and ethanol precipitation methods. Based on the previous methods,[34] 1 μg of genomic DNA was treated with sodium bisulfite by using a kit from In2Gen (Korea). To determine the methylation status of the promoter region of *TSPYL5*, a CpG island region was searched and the specific primers for methylation-specific PCR (MSP) were designed by using MethPrimer program.[35] Primer sequences for MSP of the *TSPYL5* are as follows: 5′-GAGCGTATATTAGA GAAATTCGTCGA-3′ (F) and 5′-GCTATAACCCTACGACTC CTAACG-3′ (R) for methylated DNA, and 5′-GAGTGTATAT TAGAGAAATTTGTTGA-3′ (F) and 5′-ACTATAACCCTA CAACTCCTAACACC-3′ (R) for unmethylated DNA. PCR amplification was performed with Ampli*Taq*® Gold DNA polymerase (Applied Biosystems, Foster, CA, USA) under the following conditions: 95°C for 10 min, 30–33 cycles of 94°C

for 30 s, 60°C for 30 s, and 72°C for 30 s, and the final extension of 72°C for 10 min. For a statistical analysis of the MSP results of primary gastric tissues, Fisher's exact test, the *t*-test and χ^2-test were used to determine the significance of associations between different variables by using SPSS 12.0K software. The level of statistical significance was $P<0.05$. For bisulfite sequencing analysis, the promoter region of *TSPYL5* (from −182 to +94) was sequenced after PCR amplification with specific primers, 5′-GAAGAGATGAAATGGTAGTAT-3′ (F) and 5′-AAAACAACTTCAAAAACAC-3′ (R) using an Ampli*Taq*® Gold DNA polymerase. The amplified PCR products were subcloned into pCR®2.1 TOPO (Invitrogen, Carlsbad, CA, USA) and the DNA insert was sequenced using an ABI PRISM 3730 DNA analyzer (Applied Biosystems).

Construction of Expression Plasmids

pcDNA3-TSPYL5 was constructed by subcloning the PCR product generated from the full-length clone for the *TSPYL5* sequence (NCBI no. BC045630) obtained from Open Biosystems (Huntsville, AL, USA) with Takara Ex *Taq* DNA polymerase. PCR primers were designed to produce the full length of a coding region of the TSPYL5 protein, with recognition sites of the restriction enzymes (*Eco*RI and *Xba*I) at the ends. The sequence of construct and proper orientations were confirmed by sequencing. An expression plasmid for the *CDKN2A* gene has been used as described previously.[16]

Transfection and Colony Formation

For colony formation assay using monolayer culture, cells were seeded at a density of 2.5×10^5 cells per well in six-well plates. Cells were transfected with TSPYL5 expression plasmids or the empty vector (2 μg each) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Forty-eight hours after transfection, the transfectants were selected using G418 (Cellgro, Herndon, VA, USA) at 600 μg/ml. After 10–14 days, the colonies were washed with PBS, fixed with absolute methanol, and stained with a crystal violet solution (0.5%) for 20 min. At least 150 of surviving colonies for the control (empty vector) were counted using a Bio-Rad gel documentation system. Total RNA from the transfected cells was isolated and analyzed the overexpression of TSPYL5 by RT-PCR. All assays were repeated three times, and data are presented as the mean ± s.d.

RESULTS

The Loss of *TSPYL5* Expression and Restoration by a Demethylating Agent in Gastric Cancer Cells

The mRNA expression of the *TSPYL* genes was analyzed in human gastric cancer cell lines by RT-PCR analysis (Figure 1a). Unlike other members of the *TSPYL* family (*TSPYL1*, *TSPYL2*, *TSPYL3*, *TSPYL4*, and *TSPYL6*), the mRNA expression of the *TSPYL5* reflected gene silencing in some cancer cell lines. Among the nine gastric carcinoma cell lines, two cell lines (SNU-484 and SNU-668) expressed *TSPYL5* mRNA, while seven cell lines (SNU-1, SNU-601, SNU-620, SNU-638, SNU-719, AGS, and MKN28) did not express *TSPYL5* mRNA. We also examined the mRNA expression of the *TSPYL*s in normal gastric tissue and observed that all of the *TSPYL* mRNAs were expressed (Figure 1a). To investigate whether demethylation could restore the loss of the *TSPYL5* expression in nonexpressing cells, we used the demethylating agent 5-aza-dC. Seven *TSPYL5*-nonexpressing cells (SNU-1, SNU-601, SNU-620, SNU-638, SNU-719, AGS, and MKN28) were treated with 5 μM of 5-aza-dC for 4 days, and the mRNA expression levels of *TSPYL5* were analyzed (Figure 1b). The addition of 5-aza-dC resulted in the restoration of *TSPYL5* mRNA in all seven *TSPYL5*-nonexpressing cells. Moreover, *TSPYL5* mRNA was induced dose dependently in SNU-601 cells after exposure to increasing concentrations of 5-aza-dC for 4 days (Figure 1c). These results suggest that the loss of the *TSPYL5* expression is caused by CpG methylation of the promoter sites and that the expression of the *TSPYL5* is activated by the demethylating agent. To confirm whether the activities of both DNA methyltransferase and histone deacetylase play a role in the loss of *TSPYL5* expression, two epigenetic inhibitors were used in combination. SNU-719 cells were exposed on 5-aza-dC at 5 μM and TSA at 0.1 μM to for 4 days and an additional 12 h. We observed that the combined treatment showed additive induction of *TSPYL5* mRNA compared with cells treated with 5-aza-dC alone. The treatment with TSA alone for 24 h showed no effect on re-expression of *TSPYL5* mRNA (Figure 1d).

Promoter Methylation of *TSPYL5* in Gastric Cancer Cells

CpG promoter methylation is one of the causes of the gene silencing of tumor suppressor genes. For methylation analysis, the sequence from 1 kb upstream of the translation start site to first exon of *TSPYL5* was analyzed using the MethPrimer program.[35] The criteria for a CpG island were a high GC percentage (>60%) and more over 0.7 of observed/expected CpG ratio. As a result, the 925-bp fragment around the transcriptional start site (TSS) of the *TSPYL5* was revealed as a CpG island satisfying the criteria (Figure 2a). To determine the potential for CpG methylation of *TSPYL5*, the methylation status was analyzed by MSP in the gastric cancer cell lines. As shown in Figure 2b, no band for CpG methylation was observed in SNU-484 and SNU-668 cells that congenitally express *TSPYL5* mRNA, whereas the *TSPYL5*-nonexpressing cell lines (SNU-1, SNU-601, SNU-620, SNU-638, SNU-719, AGS, and MKN28) showed a pattern of complete methylation. To test whether the restoration of *TSPYL5* mRNA expression is associated with a change in the methylation status of *TSPYL5*, MSP analysis was carried out on genomic DNA of 5-aza-dC-treated cells (Figure 2c). SNU-1 cells treated with 5-aza-dC showed the appearance of an unmethylated band, which was absent in untreated cells, and a similar result was seen in AGS cells. We believe that this result can be attributed to the demethylation effect at a CpG island of *TSPYL5*. For a delicate methylation analysis, we

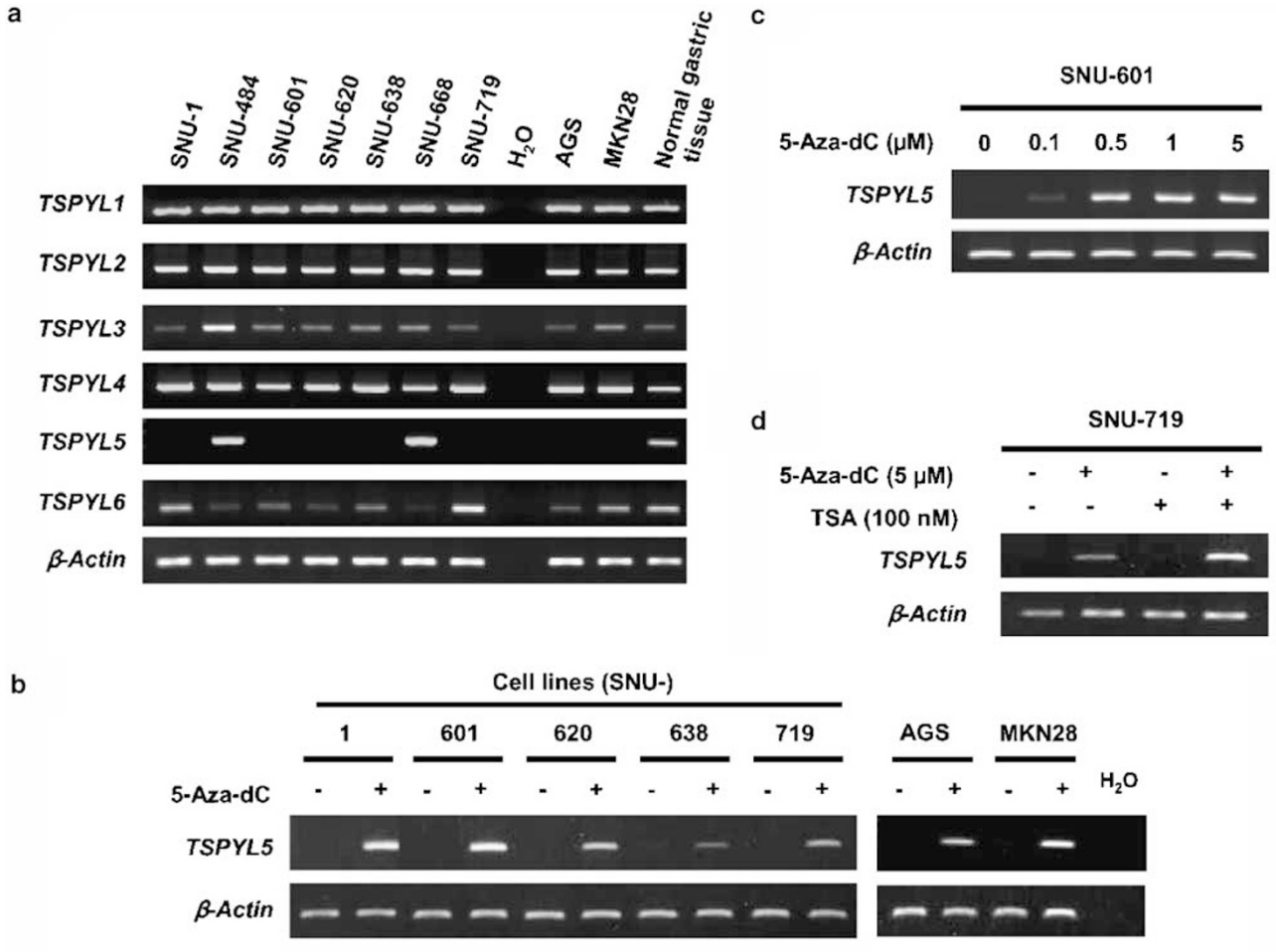

Figure 1 The mRNA expression of the *TSPYL* genes, and the epigenetic loss of *TSPYL5*. (**a**) The mRNA expression of the *TSPYL*s. Gene silencing is apparent only in the *TSPYL5* mRNA. β-Actin was used as a loading control for RT-PCR. (**b**) RT-PCR analysis of *TSPYL5* after treating with 5-aza-dC for 4 days. (**c**) SNU-601 cells were exposed to 0.1, 0.5, 1, or 5 μM of 5-aza-dC for 4 days. (**d**) The combinatorial treatment of SNU-719 cells with 5-aza-dC at 5 μM and TSA at 0.1 μM.

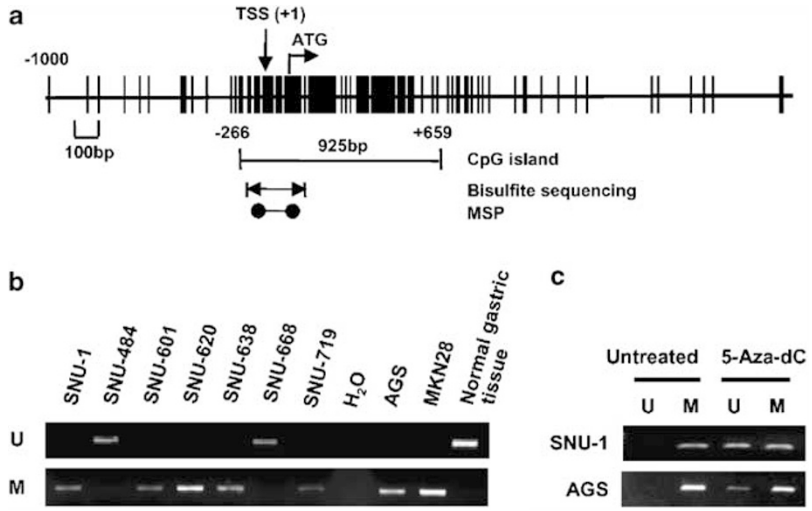

Figure 2 Methylation status at a CpG island of the *TSPYL5* in gastric cancer cell lines. (**a**) Schematic structure of the *TSPYL5* and location of a CpG island. Vertical bars represent the locations of CpG sites and locations investigated by MSP and bisulfite sequencing in this study are indicated. TSS means transcriptional start site and ATG indicates the start point for translation. (**b**) MSP analysis from bisulfite-modified DNA in gastric cancer cells with primers specific for unmethylated (U) or methylated (M) DNA. (**c**) Demethylation was analyzed by MSP in SNU-1 and AGS cells before and after the exposure to 5-aza-dC at 5 μM for 4 days.

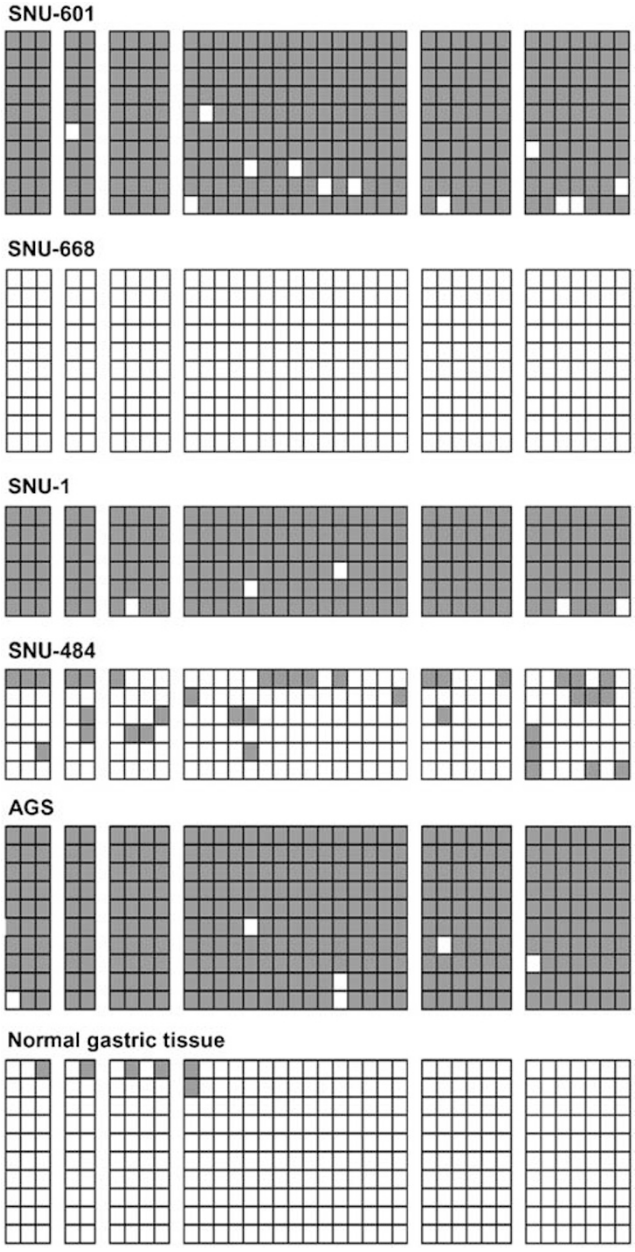

Figure 3 Bisulfite sequencing results at a CpG island of *TSPYL5* in gastric cancer cell lines. The methylation status of the 37 CpG sites at the CpG island of *TSPYL5* was analyzed by sequencing. Each square indicates a CpG site, and each line of squares represents a single cloned allele. Open and closed squares represent unmethylated and methylated CpG sites, respectively.

used bisulfite genomic sequencing about the 276-bp amplified PCR product of the bisulfite-modified genomic DNA containing 37 CpG sites on the promoter at the *TSPYL5* (Figure 3). SNU-484 and SNU-668 cells showed a low frequency of methylation at the amplified region of the *TSPYL5* (17% in SNU-484 and 0% in SNU-668 cells). However, in consistence with the MSP results (Figure 2b), CpG islands in SNU-1, SNU-601 and AGS cells were nearly completely

Table 2 Clinicopathological features of 36 gastric cancer patients and methylation status of *TSPYL5* in cancer tissues and matching non-cancerous tissues

	Case no.	Methylation (%)	Unmethylation (%)	*P*-value
Non-cancer	36	5 (14)	31 (86)	
Cancer	36	23 (64)	13 (36)	
Gender				0.72[a]
Male	24	16 (67)	8 (33)	
Female	12	7 (58)	5 (42)	
Age (mean, years)		57.4 ± 11.8	51.2 ± 12.0	0.14[b]
Differentiation				1.00[a]
Poor	21	13 (62)	8 (38)	
Moderate/well	15	10 (67)	5 (33)	
Tumor stage				0.21[c]
T1	0	0	0	
T2	22	16 (44.4)	6 (16.7)	
T3	13	6 (16.7)	7 (19.4)	
T4	1	1 (2.8)	0	
Lymph nodes				0.27[a]
Negative	10	8 (80)	2 (20)	
Positive	26	15 (58)	11 (42)	

[a]Fisher's exact test; [b]*t*-test; [c]χ^2-test.

methylated (98, 97, and 98%, respectively) at the amplified region of the *TSPYL5*. Taken together, these results indicate that the methylation status at a CpG island of the *TSPYL5* is closely correlated with its transcriptional silencing in gastric cancer cells.

Aberrant Methylation of *TSPYL5* in Primary Gastric Cancer Tissues

We next examined whether a CpG island of the *TSPYL5* is also methylated in primary gastric cancer tissues as seen *in vitro*. Table 2 shows the results of the MSP analysis for the specific region of the *TSPYL5* obtained from 36 primary tumor samples. The results indicate that the CpG island of *TSPYL5* was hypermethylated in 23 cases of 36 primary gastric cancer tissues (64%). Figure 4a shows a representative subset of the MSP results from the 36 primary tumor tissues and corresponding non-cancerous gastric tissues. The frequency of hypermethylation in non-cancerous gastric tissues was significantly lower than that in primary gastric cancer tissues regarding the analyzed region. Since most cancer tissues showed the coexistence of both unmethylation and methylation, we could not rule out a possibility of contamination of non-cancerous cells in gastric cancer tissue, which appeared to be methylated and also unmethylated. By bisulfite sequencing analysis, we examined the methylation

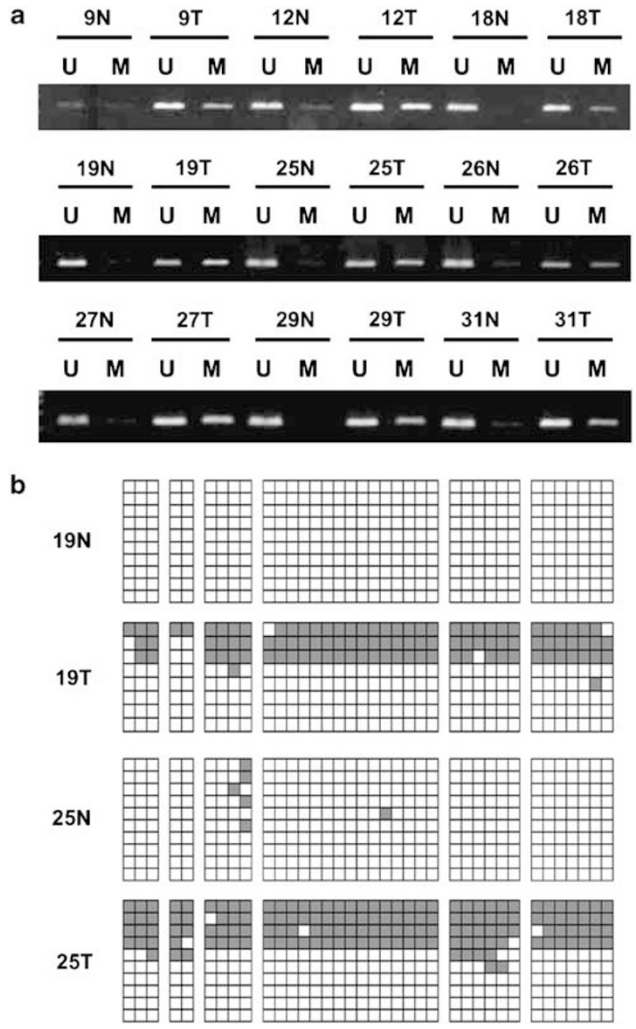

Figure 4 Methylation analysis at a CpG island of *TSPYL5* in primary gastric cancers. (**a**) The representative MSP results at a CpG island of *TSPYL5* in primary gastric tissues. Parallel amplification reactions in cancer tissue samples and matching non-cancerous tissue samples were performed using primers specific for unmethylated (U) or methylated (M) DNA. (**b**) The methylation status of *TSPYL5* was determined by bisulfite sequencing in cancer tissue samples and corresponding non-cancerous tissue samples. Each line of squares represents a single cloned allele. T, tumor tissue DNA; N, non-cancerous tissue DNA.

status of the 276-bp bisulfite-modified genomic DNA region containing 37 CpG sites of the *TSPYL5* from gastric cancer tissues and paired non-cancerous tissues (Figure 4b). The bisulfite sequencing results of two representative pairs from primary gastric tissues show that the methylation level was greatly increased in cancer regions (three of eight and four of 10) than corresponding non-cancer regions (zero of 10 and zero of 10, respectively). The findings demonstrate that methylation at *TSPYL5* promoter can be observed as a relatively common event in gastric cancers as seen *in vitro*. The clinical and pathological data in gastric cancer tissues and matching non-cancerous tissues are summarized in Table 2. Statistical analysis of the MSP results was performed to determine the significance of associations between different variables. However, methylation at *TSPYL5* promoter in primary gastric cancer tissue was not significantly correlated with gender, age, differentiation, tumor stage or lymph node stage of patients.

Growth Suppression by the Restoration of TSPYL5

Based on the correlation between the loss of expression and DNA methylation of the *TSPYL5* in gastric cancers, we examined whether the expression of TSPYL5 affects a growth-suppressive property in human gastric cancer cells. The effect of TSPYL5 expression on cell growth was assessed by using a colony formation assay (Figure 5). We cloned the full-length cDNA of *TSPYL5* into the pcDNA3 vectors, and the expression plasmids were transfected into the SNU-601 and SNU-719 cell lines. G418 was added for 2 weeks to select only the transfectants. The number of colonies of transfectants with empty vector as a negative control was considered to be 100%. The transfection of *CDKN2A* was used as a positive control and resulted in the significant suppression of growth in cancer cells. The expression of TSPYL5 after transfection was confirmed by real-time RT-PCR (data not shown). The growth inhibitory effect of TSPYL5 was further confirmed by stable transfection experiments and soft agar assay (Supplementary data). The results show that the overexpression of TSPYL5 yields a significant growth-suppressive effect (39.8% in SNU-601 and 62.4% in SNU-719 cells), suggesting that the *TSPYL5* may have the properties of a tumor suppressor gene in gastric cancers.

DISCUSSION

Silencing of tumor suppressor genes by aberrant methylation at CpG islands plays a crucial role in the development of various cancers. Many genes that are involved in the regulation of cell cycle, tissue invasion, DNA repair, and apoptosis have been shown to be inactivated by promoter hypermethylation in gastric cancer.[6,7]

In this study, we have shown that the mRNA expression of the *TSPYL5* was significantly reduced or absent in seven of nine gastric cancer cell lines compared with normal gastric tissue (Figure 1a). We found that the *TSPYL5* promoter was methylated in gastric cancer cells as demonstrated by methylation-detectable MSP analysis (Figure 2b). The assessment of DNA methylation by bisulfite sequencing also convincingly demonstrated that the promoter site on genomic DNA of *TSPYL5* was methylated with a high frequency in gastric cancer cells (Figure 3). These results also showed that the DNA methylation status of *TSPYL5* was inversely matched with the loss of expression. We found that the use of a demethylating agent (5-aza-dC) for 4 days could restore *TSPYL5* expression (Figure 1b). Furthermore, the CpG promoter of *TSPYL5* is methylated in the majority of gastric cancer cell lines (77.8%), and also in that of primary gastric tumors (63.9%) (Figure 2b; Table 2). These results

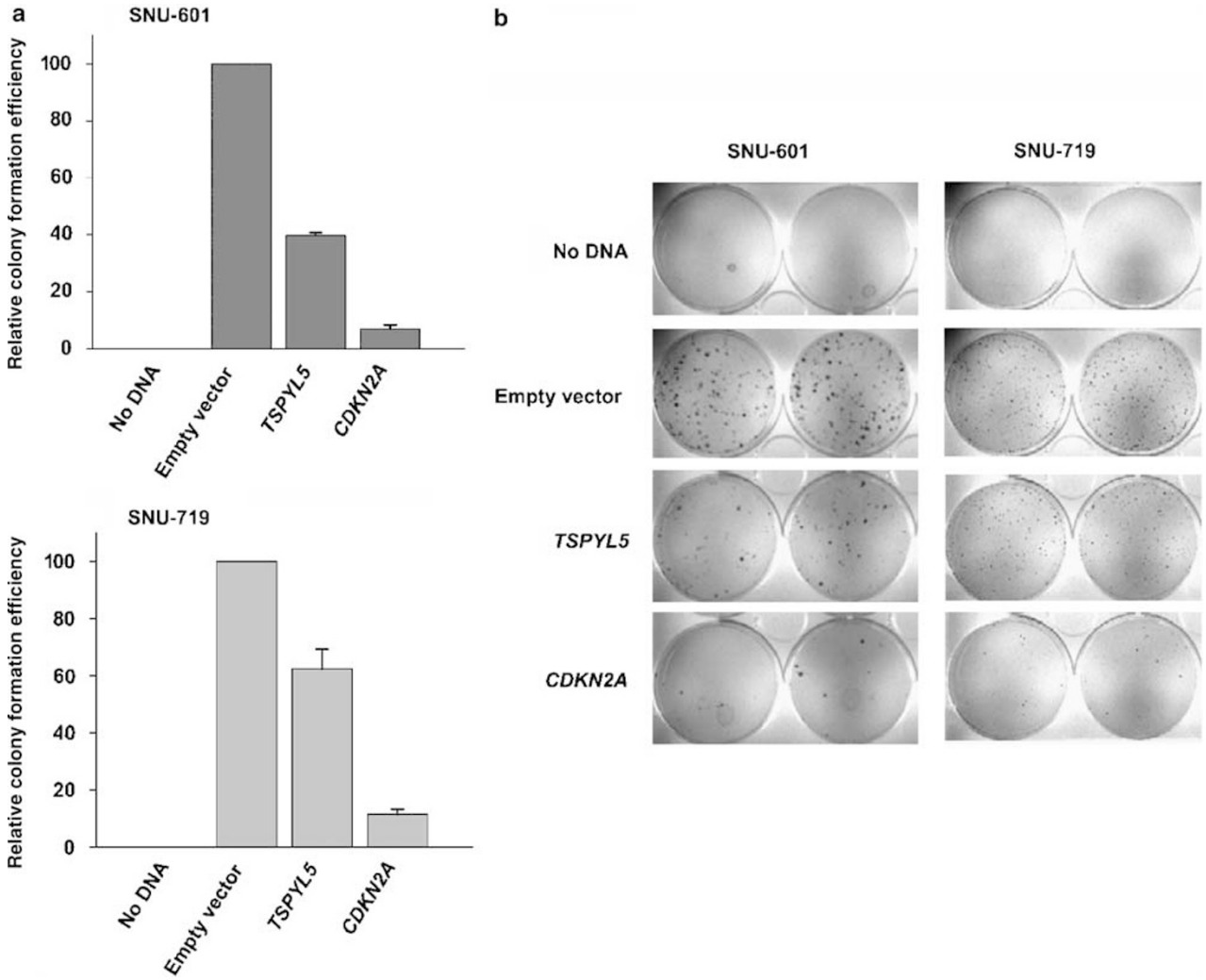

Figure 5 Growth-suppressive activity of *TSPYL5*. (**a**) SNU-601 and SNU-719 cells were transfected with 2 μg of a *TSPYL5* or a *CDKN2A* (a positive control) expression plasmids or an empty vector. Colonies were stained and counted 14–20 days after G418-addition at 600 μg/ml. Values are means ± s.d. of triplicate plates. In three separate experiments, a similar result was observed. (**b**) The representative results of colony formation assays in SNU-601 and SNU-719 cells.

demonstrate that DNA methylation at a CpG island of the *TSPYL5* may be involved in the mechanism of gene silencing.

We examined the cooperative effects of DNA methylation and histone deacetylation by using two inhibitors (5-aza-dC and TSA) on SNU-719 cells. We found that cotreatment with the two agents synergistically increased the level of *TSPYL5* mRNA compared with 5-aza-dC alone (Figure 1d). This result supports that DNMT and HDAC cooperatively could play an essential role in transcriptional silencing of several methylated genes.[36] However, the recovery of histone acetylation using TSA alone did not induce the mRNA expression of *TSPYL5*. This result suggests that DNA methylation might play a dominant role over histone deacetylation in the repression of *TSPYL5* and that histone deacetylation might have a modulating effect.

In summary, our findings demonstrated that CpG sites of the *TSPYL5* are frequently methylated in gastric cancers and that hypermethylation at the promoter is significantly associated with silencing of *TSPYL5*. Although further evidence is needed to fully understand the molecular mechanism of *TSPYL5* as a tumor suppressor gene, our data show that the expression of TSPYL5 suppresses the growth of gastric cancer cells, suggesting that it is a tumor suppressor candidate. Our results indicate that epigenetic mechanisms mediate the loss of *TSPYL5* expression and that DNA methylation may play an important role in human gastric carcinogenesis.

Supplementary Information accompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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