

# Aberrant histone methylation and the effect of Suv39H1 siRNA on gastric carcinoma

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**Abstract.** The present study aimed to investigate the involvement of the Suv39H1 histone methyltransferase in the epigenetic changes in the euchromatic promoter in gastric carcinoma. We retrospectively analyzed the protein of Suv39H1 and tri-methylated histone H3 lysine 9 (H3K9) and histone H3 lysine 4 (H3K4) in 175 cases of gastric carcinoma by immunohistochemistry. Suv39H1 was depleted by siRNA, and cell apoptosis and cell proliferation were assessed by TUNEL and MTT assays, respectively. Histone methylated H3K9 and histone acetylated H3 and H4 were evaluated by western blotting. We found that the expression of Suv39H1 and tri-methylated H3K9 in gastric carcinoma was higher than that in benign gastric diseases ( $p < 0.05$ ). Tri-methylated H3K4 was similar in both tissue types ( $p > 0.05$ ). Both Suv39H1 and tri-methylated H3K9 were positively correlated with the degree of differentiation, depth of infiltration and lymphatic invasion ( $p < 0.05$ ) in gastric carcinoma. In addition, tri-methylated H3K9 was positively correlated with tumor stage, and node and metastatic statuses ( $p < 0.05$ ). Activation of Suv39H1 and overexpression of H3K9 tri-methylation may play an important role in tumorigenesis. They may be useful as a predictor for poor prognosis in gastric carcinoma. Silencing of the Suv39H1 gene decreased tri-methylated H3K9 and increased histone H3 acetylation, which caused activation of gene transcription, while there was no change in histone H4 acetylation. Depletion of Suv39H1 induced apoptosis and inhibited cell proliferation in the gastric cancer MGC803 cell line, while decreasing

BCL-2, pro-caspase-9, pro-caspase-3 and C-myc. Suv39H1 may be a potential gene target for anti-gastric carcinoma therapy.

## Introduction

Histone modifications have been reported to include acetylation, phosphorylation, methylation, ADP ribosylation and ubiquitination (1). Histone methylation indicates the methylation on lysine (K) and arginine residues on the N-terminal of histones H3 and H4. Histone tail modifications can be markers for both active and repressed chromatin and are also interdependent. For histone lysine methylation, there are currently five described positions in the N-terminal of histone H3 (K4, K9, K27 and K36) and histone H4K20. Methylation of histone H3 lysine 4 (H3K4), H3K36 and H3K79 are mainly correlated with transcriptional stimulation, whereas methylation of H3 lysine 9 (H3K9), H3K27 and H4K20 comprise imprints for transcriptionally silent chromatin (2,3). Histone methylation, especially tri-methyl modification, was once believed to be irreversible, and to have an impact on long-term epigenetic memory. Yet, recent studies have discovered several demethylases, such as LSD-1, LSD-2 and JHDM1, which can demethylate histone and cause epigenetic changes (4-6).

Histone methylation on lysines is important for transcriptional silence. Suv39H1 is the first described histone methyltransferase (HMT) which is the mammalian homologues of *S. pombe* Clr4 and *Drosophila* Su (Var) 39, two proteins that are involved in silencing by pericentric heterochromatin (7-10). The Suv39H1 can be classified as the major H3K9 tri-methylating enzyme which appears to use an H3K9 0 monomethylated residue as the *in vivo* substrate. This interpretation is supported by *in vitro* HMT assays indicating a much higher preference for H3K9 monomethylated substrates (11).

Suv39H1 may be involved in tumorigenesis. The PML-RAR $\alpha$  fusion protein causes acute promyelocytic leukemia (PML), presumably via redistribution of the Suv39H1 to PML bodies and perturbed histone lysine methylation at RAR $\alpha$  target promoters (12). Other examples include association of heterochromatic HMTs with the tumor suppressor retinoblastoma protein (RB) (13). Furthermore, the SET domain genes EZH2 and MLL1 are involved in the progression of prostate cancer and mixed-lineage leukemia (14,15).

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**Abbreviations:** H3K9, histone H3 lysine 9; H3K4, histone H3 lysine 4; K, lysine; HMT, histone methyltransferase; PML, promyelocytic leukemia; RB, retinoblastoma protein; TNM, tumor-node-metastasis; PCR, polymerase chain reaction; HDAC, histone deacetylase

**Key words:** gastric carcinoma, histone methylation, Suv39H1, siRNA interference

Table I. Clinical significance of Suv39H1 expression in the gastric carcinoma cases (n=175).

Group	Suv39H1 protein expression		$\chi^2/t$	P-value
	Positive	Negative		
Gender (Male/Female)	97/30	36/12	0.036	>0.05
Body mass index (BMI)	20.1±3.4	21.4±3.6	2.09	0.37
Age (years)	60.5±12.3	57.1±9.8	2.04	0.34
Pathological type			0.493	>0.05
Papillary adenocarcinoma	14	6		
Tubular adenocarcinoma	67	25		
Poorly differentiated adenocarcinoma	20	8		
Signet-ring cell carcinoma	6	3		
Mucinous adenocarcinoma	20	6		
Level of differentiation			11.27	<0.05
Intermediate-highly differentiated	65	38		
Poorly differentiated	62	10		
Depth of infiltration			28.43	<0.05
T1+T2	14	23		
T3+T4	113	25		
Lymphatic metastasis			10.71	<0.05
No	12	14		
Yes	115	34		
Tumor-node-metastasis stage			0.071	>0.05
I+II	58	23		
III+IV	69	25		

Chakraborty *et al* reported that Suv39H1 methylated H3K9 and affected DNA transcriptional activity through interaction with the nut region of AML protein, leading to the development of acute myeloid leukemia (16).

Aberrant histone methylation has not been well characterized in human disease, especially in gastric carcinoma. The function of Suv39H1 is still not well known. In the present study, we investigated the expression of Suv39H1 and histone methylation in gastric carcinoma, and evaluated the correlation between Suv39H1, histone methylation and clinicopathological features, and prognosis in gastric carcinoma. Furthermore, we applied the siRNA technique to deplete Suv39H1 and measure consequent histone modification, gene transcription, cell proliferation and apoptosis in MGC-803 cells.

## Materials and methods

### Collection of patient samples and data

**Ethics statement.** The present study consisted of a total of 175 gastric carcinoma patients who underwent curative surgical resection at the Department of Surgery of Zhangzhou Affiliated Hospital of Fujian Medical University between January 2001 and December 2011. The surgically removed tissue samples were collected from all participants who had signed an informed consent form indicating their willingness to participate. Experimental procedures were approved by the

Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University.

Pathological stage was determined by the tumor-node-metastasis (TNM) classification. Gastric carcinoma was classified according to the degree of differentiation, depth of infiltration, lymphatic invasion and TNM stage as described in Table I. Patient samples from chronic superficial gastritis (n=30), chronic atrophic gastritis (n=30) and moderate-severe atypical hyperplasia (n=30) were used as control.

### Tissue microarray construction and immunohistochemistry.

A representative tumor section paraffin block (donor block) was collected from each case, and two tissue cores (2 mm in diameter) were obtained using a trephine apparatus. Since gastric carcinoma frequently shows histological heterogeneity, we sampled duplicate tissue cores from separate areas in individual paraffin-embedded gastric tumors for better representation of the tumor. Trephinated paraffin tissue cores were then arranged in a new recipient paraffin block (tissue array block). Cores containing tumor in >50% of the area were considered adequate. Immunohistochemical staining was performed using commercially polyclonal rabbit anti-histone antibodies to histone methyltransferase Suv39H1, tri-methyl-histone H3K9 and H3K4 (Upstate Biotechnology, Lake Placid, NY, USA). Tissue array blocks were sectioned at a thickness of 4  $\mu$ m and mounted on pre-coated glass slides. The sections

were deparaffinized and hydrated prior to immunohistochemistry. The immunohistochemical S-P method was performed according to the manufacturer's protocol. Tissues positive for all of the purchased antibodies were used as positive controls; sections prepared with phosphate-buffered saline instead of the primary antibody were used as negative controls. Positive controls exhibited a brown color in the nuclei. Chevallier's semi-quantity system analysis was used for the calculation of the immunohistochemistry results. Results are presented as the sum of scores presenting color density and the percentage of stained cells. According to color density, non-stained cells were scored as 0; slightly stained as 1; intermediate-stained as 2 and strongly stained as 3. When the number of positive cells was <25, 25-50, >50-75, or >75%, the immunoreactivity was scored as 1+, 2+, 3+ and 4+, respectively. The two scores for color density and the number of positive cells were added for each slide. A sum of 0 was considered negative; 1-2, +; 3-4, ++; 5-6, +++ and 7 as +++. If the sum of the two scores was  $\leq 2$ , it was considered negative staining;  $> 2$  was considered positive staining. The scores for each patient group were averaged.

**Cell line and culture.** Human gastric carcinoma MGC-803 cells were purchased from the American Type Culture Collection. Cells were cultured in 10% fetal bovine serum and RPMI-1640 containing L-glutamine under 37°C, saturated humidity and 5% CO<sub>2</sub>. Cells were subcultured every 3-5 days. The subculture of cells was performed using 0.25% trypsin to digest the attached cells for 2-3 min.

**RNA interference.** The approach by transient transfection using Lipofectamine™ 2000 was employed to deplete the Suv39H1 gene in the MGC-803 cell line. Suv39H1 siRNA sense: 5'-CGUGGAUUGUCUCAGGGAATT-3' and antisense 5'-UUCCCUGAGACAAUCCACGTG-3' were synthesized by Shanghai GenePharma Co., Ltd. (China). Transfection with siRNA was performed according to the Lipofectamine™ 2000 manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Inducible MGC-803 cells (1x10<sup>5</sup> cells/well) were seeded onto 24-well plates, (Costar Life Science, Acton, MA, USA) and transiently transfected with 0, or 60, 120 or 240 nmol/l Suv39H1 siRNA. All experiments were conducted in triplicate using independent cultures. Both total RNA and protein were extracted after a 24-h transfection.

**Real-time polymerase chain reaction (PCR).** Total RNA was extracted from samples of 1x10<sup>6</sup> cells using TRIzol (Invitrogen). The quantity and quality of RNA samples were measured by absorbance at 260 and 280 nm. RNA samples with an A260:A280 ratio 1.8-2.0 were stored at -80°C until use. cDNA synthesis was performed using an Avian Myeloblastosis Virus Reverse Transcriptase kit, according to the manufacturer's protocol (Promega, Madison, WI, USA).  $\beta$ -actin was used as the internal control. Primers used in the PCR amplifications were: Suv39H1 forward, 5'-TGCGTATCCTCAAGCAGTTCC-3' and Suv39H1 reverse, 5'-CCGTCCAGGTCCACCTCATTC-3';  $\beta$ -actin forward, 5'-CTCCTTAATGTCAC GCACGATTTTC-3' and  $\beta$ -actin reverse, 5'-CTACAATGAGCTGCGTGTGGC-3'. Amplicon size was 217 base pairs (bp) and 517 bp for Suv39H1 and  $\beta$ -actin, respectively. PCR reaction conditions were: 94°C for 45 sec, 56°C for 1 min, 72°C for 80 sec which was repeated

for 30 cycles. The PCR-amplified products were electrophoresed on 1.0% (w/v) agarose gels with 1  $\mu$ g/ml ethidium bromide. Experiments were repeated twice.

**Cell proliferation as measured by MTT assay.** The MGC-803 cells were seeded at a density of 1x10<sup>5</sup>/well in 96-well culture dishes (Costar Life Science). After 0, 24, 48, 72 and 96 h transfection (n=5) with 0, 30, 60, 120, 240 or 480 nmol/l Suv39H1 siRNA, cell proliferation was measured using MTT assays (0.5 mg/ml; Sigma). The spectrophotometric absorbance of the samples was determined by using an Ultra Multifunctional Microplate Reader (Tecan, Durham, NC, USA) at 492 and 630 nm. Suppression ratio was also calculated. The experiment was repeated in triplicate.

**Apoptosis as detected by TUNEL assay.** Cells at a logarithmic phase of growth were transfected with negative control siRNA and 60, 120 and 240 nmol/l Suv39H1 siRNA, and were seeded in 6-well plates (1x10<sup>6</sup> cells/well; Costar Life Science) with a sterile cover glass placed at the bottom of each well. Cells were used for the detection of apoptosis 24 h after transfection by TUNEL, according to the manufacturer's protocol (DeadEnd™ Fluorometric TUNEL System; Promega).

**Western blot analysis.** Total protein lysate and western blot analysis were performed as previously described (17). Briefly, MGC-803 cells were plated on culture dishes and transfected with Suv39H1 siRNA at 60, 120 and 240 nmol/l for 24 h. Control cells were incubated in the medium with Na<sub>2</sub>CO<sub>3</sub> using the same time points. After incubation, total proteins were prepared from each culture condition with a lysis buffer containing freshly prepared protease inhibitors. The protein concentration was then measured using the BCA protein assay (Pierce, Rockford, IL, USA). Cell extracts were subjected to 12% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with monoclonal anti-acetyl-L-HistoneH3, anti-acetyl-L-Histone H4, tri-methyl-Histone H3K9, Suv39H1 (Upstate, USA), BCL-2, pro-caspase-9, pro-caspase-3 and C-myc antibodies (Santa Cruz, Santa Cruz, CA, USA). After being washed with TBS, the membranes were incubated with the secondary antibody conjugated with peroxidase. The signal was then detected using the chemiluminescence detection system (Pierce) and analyzed by a color image analysis system (AlphaDigiDoc; Alpha Innotech).

**Statistical analysis.** The Student's t-test for mean and standard deviation or the Mann-Whitney test for median and range were performed for comparisons between the groups in regards to continuous data. Comparisons among groups regarding categorical data were analyzed by performing the Chi-square test. All data were analyzed using the SPSS, version 16.0 computer program (SPSS, Inc., Chicago, IL, USA), and the significance of these differences was defined as  $p < 0.05$ .

## Results

**Overexpression of Suv39H1 and histone tri-methylation of H3K9 in gastric carcinoma.** We assessed the staining score for Suv39H1, tri-methylation of H3K9 and H3K4 in 175 cases

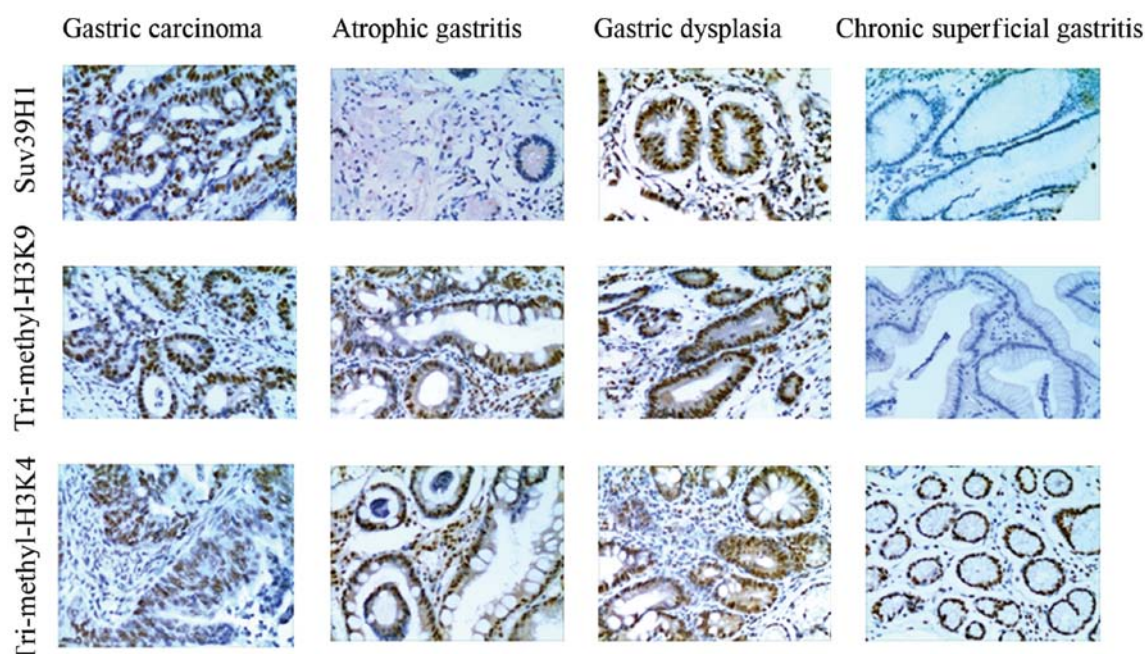


Figure 1. Expression of Suv39H1, tri-methyl-H3K9 and tri-methyl-H3K4 in gastric carcinoma and benign gastric diseases. The expression of Suv39H1 and H3K9 in gastric carcinoma was higher than that in chronic superficial gastritis, chronic atrophic gastritis and moderate-severe dysplasia,  $p < 0.05$ . The histone tri-methylation of H3K4 in gastric carcinoma was similar to that in the other disease tissues,  $p > 0.05$ . H3K9, histone H3 lysine 9; H3K4, histone H3 lysine 4.

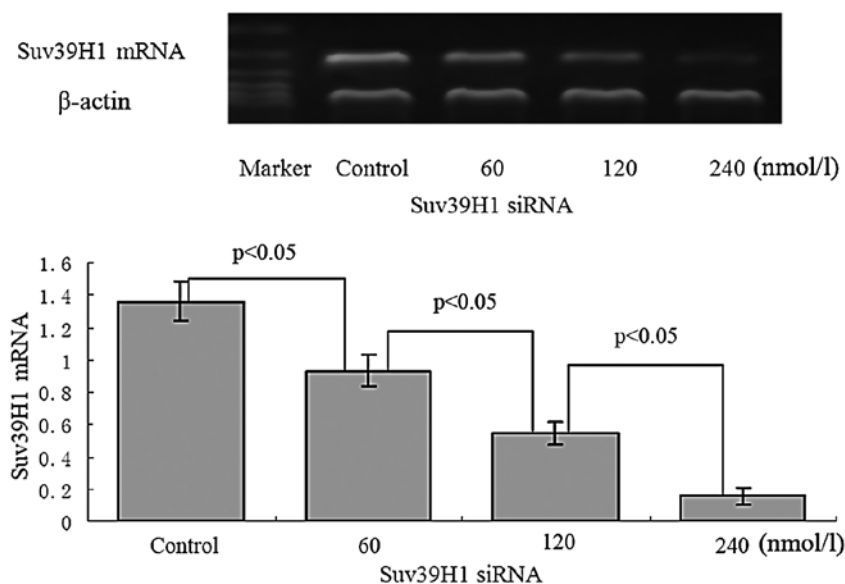


Figure 2. Silencing efficiency of Suv39H1 siRNA on expression of the Suv39H1 gene. Amplification of Suv39H1 mRNA was attenuated in a concentration-dependent manner.

of gastric carcinoma. The expression of Suv39H1 in gastric carcinoma was 72.57%, higher than that in the chronic superficial gastritis (33.33%,  $p < 0.05$ ), chronic atrophic gastritis (30.00%,  $p < 0.05$ ) and moderate-severe dysplasia (46.67%,  $p < 0.05$ ) (Fig. 1). The expression of histone tri-methylation of H3K9 in gastric carcinoma was 85.47%, higher than that in the chronic superficial gastritis (36.67%,  $p < 0.05$ ), chronic atrophic gastritis (33.33%,  $p < 0.05$ ) and moderate-severe dysplasia (53.33%,  $p < 0.05$ ) (Fig. 1). The histone tri-methylation of H3K4 in gastric carcinoma was 59.43%, which was similar to that of chronic superficial gastritis (46.67%,  $p > 0.05$ ), chronic atro-

phic gastritis (60.00%,  $p > 0.05$ ) and moderate-severe dysplasia (50.00%,  $p > 0.05$ ) (Fig. 1).

**Correlation of Suv39H1 and histone tri-methylated H3K9 with clinicopathological variables in the gastric carcinoma cases.** To evaluate the correlation between the histone methylation status and various clinicopathological data, we divided the level of staining scores into two groups: negative (score  $\leq 2$ ) and positive (score  $> 2$ ). The data showed that the Suv39H1 status was positively correlated with the degree of differentiation ( $\chi^2 = 11.27$ ,  $p < 0.05$ ), depth of infiltration ( $\chi^2 = 28.43$ ,  $p < 0.05$ )

Table II. Clinical significance of histone H3K9 methylation in the gastric carcinoma cases (n=175).

Group	Histone H3K9 protein expression		$\chi^2/t$	P-value
	Positive	Negative		
Gender (Male/Female)	113/34	20/8	0.381	>0.05
Body mass index (BMI)	20.3±3.6	21.3±3.8	2.13	0.39
Age (years)	59.3±11.3	58.1±10.7	2.20	0.56
Pathological type			Fisher's precise probability	0.865
Papillary adenocarcinoma	16	4		
Tubular adenocarcinoma	78	14		
Poorly differentiated adenocarcinoma	23	5		
Signet-ring cell carcinoma	7	2		
Mucinous adenocarcinoma	23	3		
Level of differentiation			7.46	<0.05
Intermediate-highly differentiated	80	23		
Poorly differentiated	67	5		
Depth of infiltration			16.65	<0.05
T1+T2	23	14		
T3+T4	124	14		
Lymphatic metastasis			6.33	<0.05
No	17	9		
Yes	130	19		
Tumor-node-metastasis stage			20.84	<0.05
I+II	57	24		
III+IV	90	4		

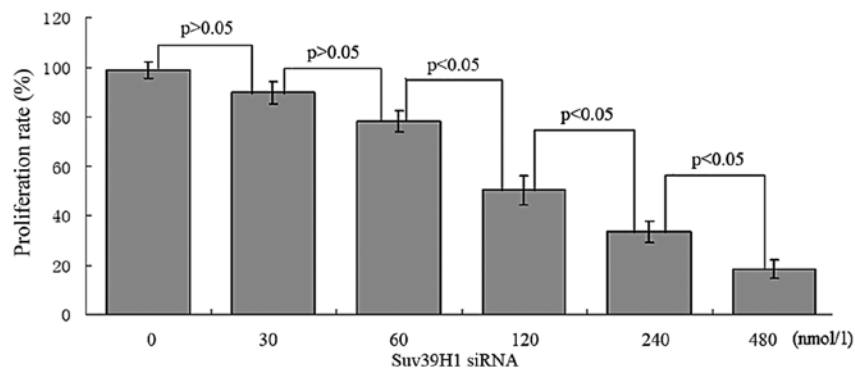


Figure 3. Assessment of cell proliferation after transfection of MGC-803 cells with Suv39H1 siRNA. Cell proliferation was inhibited in the MGC-803 cells after 24 h of transfection with the indicated concentrations of Suv39H1 siRNA.

and lymphatic invasion ( $\chi^2=10.71$ ,  $p<0.05$ ). The Suv39H1 status was not correlated with TNM stage ( $\chi^2=0.071$ ,  $p>0.05$ ), age, ( $t=2.04$ ,  $p>0.05$ ), gender ( $\chi^2=0.036$ ,  $p>0.05$ ), body mass index (BMI) ( $t=2.09$ ,  $p>0.05$ ) or pathological type ( $\chi^2=0.493$ ,  $p>0.05$ ) (Table I).

The tri-methylated H3K9 status was positively correlated with the degree of differentiation ( $\chi^2=7.46$ ,  $p<0.05$ ), depth of infiltration ( $\chi^2=16.65$ ,  $p<0.05$ ), lymphatic invasion ( $\chi^2=6.33$ ,  $p<0.05$ ) or TNM stage ( $\chi^2=20.84$ ,  $p<0.05$ ). It was not correlated

with age ( $t=2.20$ ,  $p>0.05$ ), gender ( $\chi^2=0.381$ ,  $p>0.05$ ), BMI ( $t=2.13$ ,  $p>0.05$ ) or pathological type ( $p=0.865$ ) (Table II).

*Silencing efficiency of the Suv39H1 gene following transfection of Suv39H1 siRNA in MGC-803 cells.* After 24 h of transfection of MGC-803 cells with Suv39H1 siRNA at 60, 120 and 240 nmol/l, the amplification of Suv39H1 mRNA was attenuated in a concentration-dependent manner. Gray value (to  $\beta$ -actin) showed that the amplification of Suv39H1 was

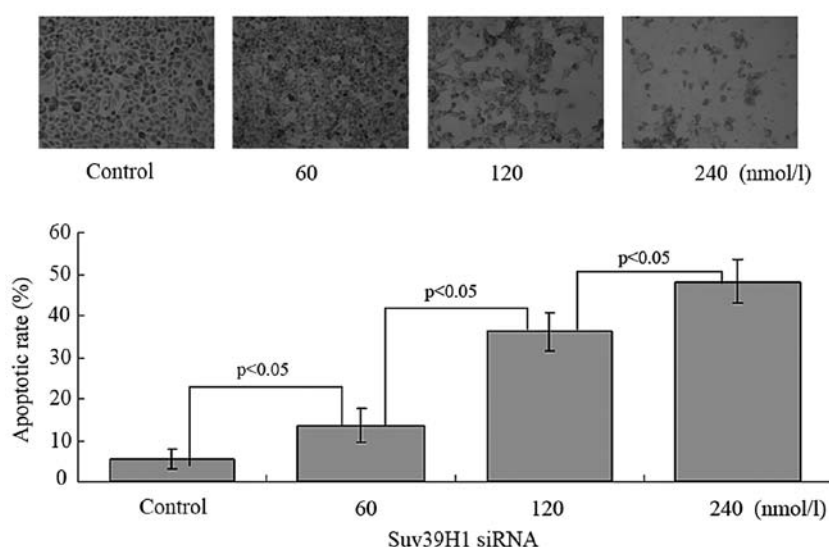


Figure 4. Assessment of cell apoptosis after transfection of MGC-803 cells with Suv39H1 siRNA. Cell apoptosis was induced after 24 h of transfection with the indicated concentrations of Suv39H1 siRNA in the MGC-803 cells.

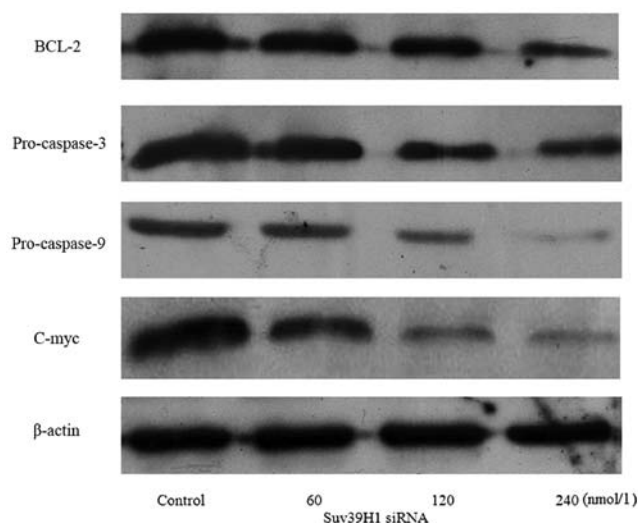


Figure 5. Expression of apoptosis-related proteins after transfection with Suv39H1 siRNA in MGC-803 cells. The apoptosis-related proteins were inhibited after 24 h of transfection with Suv39H1 siRNA at the indicated concentrations. The protein levels of BCL-2, pro-caspase-3, pro-caspase-9 and C-myc were suppressed after transfection with Suv39H1 siRNA.

$0.93 \pm 0.10$  at 60 nmol/l,  $0.55 \pm 0.07$  at 120 nmol/l and  $0.16 \pm 0.05$  at 240 nmol/l respectively, compared to the control ( $1.36 \pm 0.12$ ) ( $p < 0.05$ ) (Fig. 2).

*Suv39H1 siRNA inhibits cell proliferation and promotes apoptosis in MGC-803 cells.* Suv39H1 siRNA significantly suppressed cell proliferation. Twenty-four hours after transfection with Suv39H1 siRNA,  $\sim 50.12 \pm 5.98\%$  reduction in cell density was noted at 120 nmol/l,  $33.37 \pm 4.13\%$  at 240 nmol/l and  $18.35 \pm 3.96\%$  at 480 nmol/l (Fig. 3).

The apoptotic cells exhibited brown staining in the nucleus. The apoptotic rate was  $5.4 \pm 2.3$ ,  $13.5 \pm 4.1$ ,  $36.3 \pm 4.6$  and  $48.3 \pm 5.4\%$  after 24 h of transfection with Suv39H1 siRNA at 0, 60, 120 and 240 nmol/l, respectively. The apoptotic rate was increased in a concentration-dependent

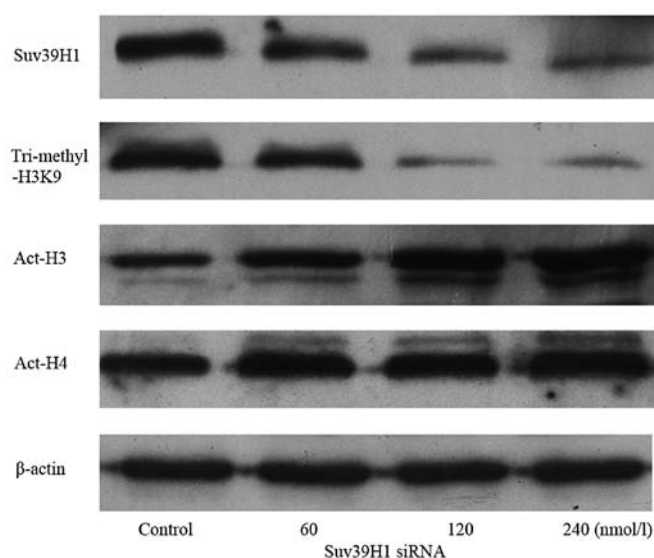


Figure 6. Expression of Suv39H1, tri-methylated H3K9, acetylation of H3 (Act-H3) and H4 (Act-H4) after transfection with Suv39H1 siRNA. The modulation of Suv39H1, histone methylation and acetylation after transfection with Suv39H1 siRNA for 24 h in MGC-803 cells. Suv39H1 siRNA downregulated Suv39H1 and histone tri-methylated H3K9, and upregulated histone acetylation of H3. Histone H4 acetylation was not affected.

manner ( $p < 0.05$ ) (Fig. 4). We further assessed expression of the apoptosis-associated proteins: BCL-2, pro-caspase-3, pro-caspase-9 and C-myc. Suv39H1 siRNA significantly inhibited BCL-2, pro-caspase-3, pro-caspase-9 and C-myc (Fig. 5).

*Suv39H1 siRNA modulates histone tri-methylation of H3K9 and acetylation of H3 and H4.* Protein of Suv39H1, and histone tri-methylated H3K9 were downregulated, while acetylated H3 was upregulated after a 24 h transfection with Suv39H1 siRNA, when compared with the control. The relative (to  $\beta$ -actin) gray value showed that Suv39H1 was  $1.18 \pm 0.1$ ,  $0.82 \pm 0.08$ ,  $0.43 \pm 0.12$ ,  $0.4 \pm 0.11$  and tri-methylated H3K9 was

1.21±0.11, 0.89±0.09, 0.26±0.12 and 0.25±0.10; Act-H3 was 0.6±0.05, 0.91±0.04, 1.22±0.11 and 1.61±0.10 in the control, and at 60, 120, 240 nmol/l Suv39H1 siRNA, respectively ( $p<0.05$ ). There was no change in histone H4 acetylation. The expression of Act-H4 was 1.22±0.05, 1.31±0.10, 1.25±0.06, 1.33±0.11 in the control and at 60, 120, 240 nmol/l Suv39H1 siRNA, respectively ( $p>0.05$ ) (Fig. 6).

## Discussion

H3K9 methylation is a major component of gene regulation and chromatin organization. Suv39H1 methylates H3K9 at the pericentric heterochromatin region and participates in the maintenance of genome stability. In the present study, we described the overexpression of Suv39H1 and histone tri-methylated H3K9 in gastric carcinoma. Suv39H1 and histone tri-methylated H3K9 were positively correlated with the degree of differentiation, depth of infiltration and lymphatic invasion in gastric carcinoma. The expression of histone methylated H3K9 was correlated with TNM stage. This indicates that overexpression of Suv39H1 and histone tri-methylated H3K9 may be involved in the initiation and development of gastric carcinoma. This is an initial study of Suv39H1 in gastric carcinoma and its clinicopathological characteristics.

The role of Suv39H1 in pericentric heterochromatin has been extensively investigated, but only a few studies have suggested the involvement of Suv39H1 in tumorigenesis. Overexpression of Suv39H1 is associated with colon cancer (18). We previously found that aberrant histone methylation occurs in acute leukemia and lymphoma (19). Park *et al* studied 261 gastric adenocarcinoma samples and concluded that tri-methylation of H3K9 was positively correlated with tumor stage, lymphovascular invasion, cancer recurrence, and a higher level of H3K9 tri-methylation was correlated with a poor survival rate (20). Multivariate survival analysis showed that H3K9 tri-methylation status is an independent prognostic factor. Differential expression of Suv39H1 was reported in different types of cancer, and a prominent increase in the expression of Suv39H1 was observed in preneoplastic nodules and liver tumors induced by methyl deficiency in rats (21,22). The mechanism of Suv39H1's tumorigenesis involves Suv39H1-associating partner molecule HP1 which is able to interact with RB (23), raising the possibility that Suv39H1 could be directly linked to specific tumor-suppressor proteins (24,25). This may be the reason why overexpression of Suv39H1 and H3K9 is involved in carcinogenesis.

We further silenced the Suv39H1 gene by transfection of Suv39H1 siRNA into gastric carcinoma MGC-803 cells to study the effect on cell growth and apoptosis. The results demonstrated that silencing of the Suv39H1 gene inhibited cell proliferation and induced cell apoptosis in the MGC-803 cells, along with downregulation of pro-caspase-3 and -9, BCL-2 and C-myc, which are related to the apoptosis pathway. The escape from replicative senescence in Suv39H1 transgenic erythroblasts was previously found to be correlated with reduced amounts of the 'antiproliferative' cell cycle regulator p21, deregulated abundance of RB, aberrant cytoplasmic levels of p53 and elevated expression of the p53-destabilizing protein mdm (26,27). Suv39H1 is targeted to promoters of

cell-cycle control genes by RB and also induces the silencing of S-phase genes through H3K9 methylation in differentiating cells (13,28). H3K9 methylation is a prerequisite for DNA methylation to occur (29,30). Loss of Suv39H1/2 in knockout mouse cells also altered the DNA methylation pattern of their pericentric heterochromatin (31).

The present study revealed that suppression of the expression of Suv39H1 not only downregulates H3K9 methylation, but also upregulates histone H3 acetylation, which is consistent with previous results (32). We previously found that silencing of Suv39H1 upregulated histone acetylated H3K9, H3 and P15 in HL60 cells. It did not affect H3K27, H3K14 and H4. Vaute *et al* found that interaction with histone deacetylase could be mediated by the N-terminus of Suv39H1, which contains a chromodomain (33). Histone deacetylases (HDACs) associated with Suv39H1 may include HDAC1, HDAC2 and HDAC3. Multiple residues on each of the four core histones have been identified as potential modification sites, and some lysine residues, such as H3K9, can be either methylated or acetylated. Recent studies have shown that switching acetylation to methylation on H3K9 contributes to euchromatin gene silencing in many organisms (34).

In summary, utilizing a large number of specimens from patients with gastric carcinoma, we demonstrated for the first time that Suv39H1 is positively correlated with initiation, development and migration of gastric carcinoma. Suv39H1 is also positively correlated with the level of H3K9 methylation in these tissues. In addition, we confirmed that Suv39H1 affects H3K9 methylation and H3 acetylation, and inhibits cell growth and induces cell apoptosis *in vitro*. The results from this study highlight the potential of H3K9 methyltransferases as therapeutic targets for gastric carcinoma.

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## References

1. Jenuwein T and Allis CD: Translating the histone code. *Science* 293: 1074-1080, 2001.
2. Fischle W, Wang Y and Allis CD: Histone and chromatin cross-talk. *Curr Opin Cell Biol* 15: 172-183, 2003.
3. Lachner M, O'Sullivan RJ and Jenuwein T: An epigenetic road map for histone lysine methylation. *J Cell Sci* 116: 2117-2124, 2003.
4. Klose RJ, Kallin EM and Zhang Y: JmJc-domain-containing proteins and histone demethylation. *Nat Rev Genet* 7: 715-727, 2006.
5. Cang S, Ma Y and Liu D: New clinical developments in histone deacetylase inhibitors for epigenetic therapy of cancer. *J Hematol Oncol* 2: 22, 2009.
6. Tan J, Cang S, Ma Y, Petrillo RL and Liu D: Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. *J Hematol Oncol* 3: 5, 2010.
7. Rea S, Eisenhaber F, O'Carroll D, *et al*: Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406: 593-599, 2000.



8. Allshire RC, Nimmo ER, Ekwall K, Javerzat JP and Cranston G: Mutations derepressing silent centromeric domains in fission yeast disrupt chromosome segregation. *Genes Dev* 9: 218-233, 1995.
9. Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G and Reuter G: The protein encoded by the *Drosophila* position-effect variegation suppressor gene *Su(var)3-9* combines domains of antagonistic regulators of homeotic gene complexes. *EMBO J* 13: 3822-3831, 1994.
10. Aagaard L, Laible G, Selenko P, *et al*: Functional mammalian homologues of the *Drosophila* PEV-modifier *Su(var)3-9* encode centromere-associated proteins which complex with the heterochromatin component M31. *EMBO J* 18: 1923-1938, 1999.
11. Peters AH, Kubicek S, Mechtler K, *et al*: Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* 12: 1577-1589, 2003.
12. Di Croce L, Raker VA, Corsaro M, *et al*: Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 295: 1079-1082, 2002.
13. Nielsen SJ, Schneider R, Bauer UM, *et al*: Rb targets histone H3 methylation and HP1 to promoters. *Nature* 412: 561-565, 2001.
14. Varambally S, Dhanasekaran SM, Zhou M, *et al*: The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 419: 624-629, 2002.
15. Milne TA, Briggs SD, Brock HW, *et al*: MLL targets SET domain methyltransferase activity to *Hox* gene promoters. *Mol Cell* 10: 1107-1117, 2002.
16. Chakraborty S, Sinha KK, Senyuk V and Nucifora G: SUV39H1 interacts with AML1 and abrogates AML1 transactivity. AML1 is methylated in vivo. *Oncogene* 22: 1107-1117, 2003.
17. Ma X, Fang Y, Beklemisheva A, *et al*: Phenylhexyl isothiocyanate inhibits histone deacetylases and remodels chromatin to induce growth arrest in human leukemia cells. *Int J Oncol* 28: 1287-1293, 2006.
18. Kang MY, Lee BB, Kim YH, *et al*: Association of the SUV39H1 histone methyltransferase with the DNA methyltransferase 1 at mRNA expression level in primary colorectal cancer. *Int J Cancer* 121: 2192-2197, 2007.
19. Zou Y, Ma X, Huang Y, Hong L and Chiao JW: Effect of phenylhexyl isothiocyanate on aberrant histone H3 methylation in primary human acute leukemia. *J Hematol Oncol* 5: 36, 2012.
20. Park YS, Jin MY, Kim YJ, Yook JH, Kim BS and Jang SJ: The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 15: 1968-1976, 2008.
21. Ozdag H, Teschendorff AE, Ahmed AA, *et al*: Differential expression of selected histone modifier genes in human solid cancers. *BMC Genomics* 7: 90, 2006.
22. Pogribny IP, Ross SA, Tryndyak VP, Pogribna M, Poirier LA and Karpinets TV: Histone H3 lysine 9 and H4 lysine 20 trimethylation and the expression of Suv4-20h2 and Suv-39h1 histone methyltransferases in hepatocarcinogenesis induced by methyl deficiency in rats. *Carcinogenesis* 27: 1180-1186, 2006.
23. Williams L and Grafi G: The retinoblastoma protein - a bridge to heterochromatin. *Trends Plant Sci* 5: 239-240, 2000.
24. Dunaief JL, Strober BE, Guha S, *et al*: The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* 79: 119-130, 1994.
25. Trouche D, Le Chalony C, Muchardt C, Yaniv M and Kouzarides T: RB and hbrm cooperate to repress the activation functions of E2F1. *Proc Natl Acad Sci USA* 94: 11268-11273, 1997.
26. Cheng M, Olivier P, Diehl JA, *et al*: The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J* 18: 1571-1583, 1999.
27. Czvitkovich S, Sauer S, Peters AH, *et al*: Over-expression of the SUV39H1 histone methyltransferase induces altered proliferation and differentiation in transgenic mice. *Mech Dev* 107: 141-153, 2001.
28. Ait-Si-Ali S, Guasconi V, Fritsch L, *et al*: A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells. *EMBO J* 23: 605-615, 2004.
29. Tamaru H and Selker EU: A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* 414: 277-283, 2001.
30. Jackson JP, Lindroth AM, Cao X and Jacobsen SE: Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 416: 556-560, 2002.
31. Lehnertz B, Ueda Y, Derijck AA, *et al*: Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* 13: 1192-1200, 2003.
32. Czermin B, Schotta G, Hülsmann BB, *et al*: Physical and functional association of SU(VAR)3-9 and HDAC1 in *Drosophila*. *EMBO Rep* 2: 915-919, 2001.
33. Vaute O, Nicolas E, Vandel L and Trouche D: Functional and physical interaction between the histone methyl transferase Suv39H1 and histone deacetylases. *Nucleic Acids Res* 30: 475-481, 2002.
34. Lachner M and Jenuwein T: The many faces of histone lysine methylation. *Curr Opin Cell Biol* 14: 286-298, 2002.