Depletion of G9a gene induces cell apoptosis in human gastric carcinoma

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Abstract. G9a is a mammalian histone methyltransferase that contributes to the epigenetic silencing of tumor suppressor genes. Evidence suggests that G9a is required to maintain the malignant phenotype, but little documentation show the role of G9a function in mediating tumor growth. We retrospectively analyzed the protein of G9a and monomethylated histone H3 lysine 9 (H3K9 me1), and dimethylated histone H3 lysine 9 (H3K9 me2) in 175 cases of gastric carcinoma by immunohistochemistry. RNAi-based inhibition of G9a in MGC803 cancer cell line was studied. G9a depletion was done by transient transfection using Lipofectamine 2000. Depletion efficiency of G9a was tested using real-time PCR and western blot analysis. Cell apoptosis and proliferation were detected by TUNEL assay and MTT, respectively. The proteins of H3K9 me1, me2, trimethylation of H3K9 (H3K9 me3), monomethylated histone H3 lysine 27 (H3K27 me1), dimethylated histone H3 lysine 27 (H3K27 me2) and histone acetylated H3, apoptotic proteins were studied by western blot analysis. G9a and H3K9 me2 expression was higher in gastric cancer cells compared to the control ($P<0.05$). Both G9a and H3K9 me2 were positively correlated with the degree of differentiation, depth of infiltration, lymphatic invasions and tumor-node-metastasis stage in gastric carcinoma, ($P<0.05$). RNAi-mediated knockdown of G9a induced cell apoptosis and inhibited cell proliferation. Depletion of G9a reduced the levels of H3K9 me1 and me2, H3K27 me1 and me2. Nonetheless, it did not activate acetylation of H3 and H3K9 me3. These data suggest that G9a is required in tumorigenesis, and correlated with prognosis. Furthermore, G9a plays a critical role in regulating epigenetics. Depletion of G9a inhibits cell growth and induces cells apoptosis in gastric cancer. It might be of therapeutic benefit in gastric cancers.

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Abbreviations: H3K9 me1, monomethylated histone H3 lysine 9; H3K9 me2, dimethylated histone H3 lysine 9; H3K9 me3, trimethylation of H3K9; H3K27 me1, monomethylated histone H3 lysine 27; H3K27 me2, dimethylated histone H3 lysine 27; TNM, tumor-node-metastasis; HMT, histone methyltransferases; GLP, G9a-like protein; DNMT, DNA methyltransferase

Key words: gastric carcinoma, histone methylation, G9a, siRNA interference

Introduction

Methylation of several conserved lysine residues on histone H3 and H4 tails by histone methyltransferases (HMTs) plays a key role in chromatin structure and gene regulation. The role of HMTs in promoting tumorigenesis and the progression of human cancers has begun to emerge. Methylation of H3K9 is generally associated with heterochromatin and gene repression and is a docking site for HP1 (1-3). Histone lysine methylation is mediated by HMT, many of which contain a conserved SET [Su(var)3-9, Enhancer of zeste, Trithorax] domain, such as Suv39h1 (suppressor of variegation 39h1) and G9a (4,5). Suv39h1 belongs to a family of peri-centromeric proteins and is responsible for H3K9 trimethylation (6-9). G9a is the major methylase responsible for mono- and di-methylation of H3K9 and H3K27 euchromatic regions (10,11), but it may also be present in heterochromatic regions (12). G9a plays an important role in the silencing and subsequent *de novo* DNA methylation of embryonic and germ-line genes during normal development and is necessary for the maintenance of the DNA methylation profile of mammalian cells (13,14). G9a and G9a-like protein (GLP) are the primary HMTs responsible for histone H3 lysine 9 methylation in euchromatic DNA (11).

Aberrant histone methylation has not been well characterized in human disease, especially in gastric carcinoma. The function of G9a is still not well known. In the present study, we characterized the G9a, H3K9 me1 and H3K9 me2 expression in gastric carcinoma tissue, evaluated the correlation between G9a, H3K9 me2 and the clinicopathological features, in gastric carcinoma. Furthermore, we applied the siRNA technique to deplete G9a and measured consequent histone methylation in H3K9 me1, me2 me3 and H3K27 me1 and H3K27 me2 and histone acetylation of H3, cell proliferation and apoptosis in MGC-803 cells to characterize the function of G9a in gastric carcinoma.

Table I. Clinical significance of G9a and H3K9 me2 expression in gastric carcinoma (n=175).

		G9a expression				H3K9 me2			
Group	n	Positive	Negative	χ^2/t	P-value	Positive	Negative	χ^2/t	P-value
Gender									
Male	133	113	20	2.2	>0.05	120	13	0.06	>0.05
Female	42	40	2			38	4		
Age (years)									
<60	67	54	13	2.97	>0.05	62	5	1.01	>0.05
≥60	109	99	10			96	13		
Pathological type									
Papillary	20	17	3	2.46	>0.05	18	2	2.86	>0.05
Tubular	92	81	11			85	7		
Poor-differention	28	24	4			25	3		
Signet-ring	9	8	1			7	2		
Mucinous	26	23	3			23	3		
Differentiation									
Intermediate-high	103	84	19	6.62	<0.05	88	15	5.43	<0.05
Poor	72	69	3			70	2		
Depth infiltration									
T1+T2	47	33	14	15.25	<0.05	36	11	11.67	<0.05
T3+T4	128	120	8			122	6		
Lymph metastasis									
No	26	18	8	7.35	<0.05	20	6	4.56	<0.05
Yes	149	135	14			138	11		
TNM stage									
I+II	81	66	15	3.89	<0.05	68	13	4.33	<0.05
III+IV	94	87	7			89	5		

Papillary, papillary adenocarcinoma; Tubular, tubular adenocarcinoma; Poor-differention, poorly-differentiated adenocarcinoma; Signet-ring, Signet-ring cell carcinoma; mucinous, mucinous adenocarcinoma.

Materials and methods

Collection of patient samples and data. There were 175 gastric carcinoma patients who underwent curative surgical resection at the Surgical Department of Zhangzhou Affiliated Hospital of Fujian Medical University between January 2001 and December 2011. None of these patients received chemotherapy or radiotherapy before the operation. Tumor specimens were collected in ten percent formalin and then embedded in paraffin for histopathological analysis. All cases were independently classified by two experienced pathologists as gastric carcinoma according to classification of the World Health Organization. The criteria of the TNM staging system were used to classify clinical pathological factors and clinical stages of gastric cancer (defined by the International Union Against Cancer TNM classification of malignant tumors, seventh Edition, 2009). All the patients gave signed informed consent. The gastric carcinoma cases consisted of 133 men and 42 women with a mean age of 63 (range, 41-84) at the time of operation. A summary of patient characteristics and pathological characteristics is presented 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. The research was approved by the Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University.

Tissue microarray construction and immunohistochemistry. Immunohistochemical studies on G9a, H3K9 me1, and H3K9 me2 were performed on formalin-fixed, paraffin-embedded tissue sections obtained from the aforementioned patients with gastric carcinoma and tissue microarrays. The primary antibodies were used rabbit monoclonal G9a antibody (1:500 dilutions; Upstate Biotechnology, Inc., Lake Placid, NY, USA), H3H9me1, H3H9me2 antibodies (1:500 dilutions; Upstate Biotechnology). 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. Because 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 commercial polyclonal rabbit anti-histone antibodies to HMT G9a, H3K9 me1 and H3K9 me2. Tissue array blocks were section at a thickness of 4 μm and mounted on precoated 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 the purchased antibodies were used as positive controls, sections prepared with phosphate-buffer saline instead of the primary antibody were used as negative controls. Positive control exhibited brown color in the nuclei. The staging was scored by two independent investigators without knowledge of patient outcomes. The sections were evaluated at low magnification (x100) to identify areas where G9a, H3K9 me1, H3K9 me2 were evenly stained. Chevallier's semi-quantity system analysis was used in the calculation of 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 1, intermediate-stained 2, strongly stained as 3. When the number of positive cells was <25%, 25-50%, or 50-75%, or >75%, the immunoreactivity was scored as 1+, 2+, 3+, 4+, respectively. The two scores presenting color density and the number of positive cells were added for each slide. The score of 0 was negative, 1-2 was presented as +, 3-4 as ++, 5-6 as +++, and 7 as ++++. If the sum of the two scores was ≤2, it was considered negative staining; >2 was considered positive staining. The scores of each patient group were averaged.

Cell culture analyses. Cell culture studies were done using human gastric carcinoma MGC-803 cells, which were purchased from the American Type Culture Collection. Cells were maintained in 10% fetal bovine serum, and RPMI-1640 containing L-glutamine under 37˚C, saturated humidity and 5% CO_2. 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 transfecting using Lipofectamine™ 2000 was employed to deplete G9a gene in MGC-803 cell lines. G9a siRNA sense: 5'-UUCAGU CUCUACUAUGAUUTT-3', antisense 5'-AAUCAUAGUAG AGACUGAATT-3' were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). Transfection with siRNA was performed according to the Lipofectamine 2000 manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Inducible MGC-803 cells ($1x10^5$ cells/well) were seeded onto 24-well plates (Corning Life Sciences, Acton, MA, USA) and transiently transfected with 0, or 40, 80 or 120 nmol/l G9a siRNA. The efficiency of siG9a and non-specific scrambled siRNA was tested using real-time PCR and western blot analysis. All experiments were conducted in triplicate using independent cultures. Both total RNA and protein were extracted after 24-h transfection.

Real-time PCR for gene expression. Real-time PCR was done using RNA isolated from MGC-803 cells, using TRIzol reagent (Invitrogen) and purified using an RNeasy kit (Qiagen). 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). β-actin was used as internal control. Primers used in PCR amplifications were: G9a forward, 5'-UUCAGUCUCUACUAUGAUUTT-3' and reverse, 5'-AUCAUAGUAGAGACUGAATT-3'; β-actin forward, 5'-CTCCTTAATGTCACGCACGATTTC-3' and reverse: 5'-CTACAATGAGCTGCGTGTGGC-3'. Amplicon size was 292 base pairs and 517 bp for G9a and β-actin, respectively. PCR reaction conditions were: 94˚C 45 sec, 56˚C 1 min, 72˚C 80 sec, and was repeated 30 cycles. PCR-amplified products were electrophoresed in 1.0% (w/v) agarose gels with 1 μg/ml ethidium bromide. Experiments were repeated twice.

Proliferation assay. After cells were transfected with G9a siRNA, the proliferative activity was determined by the MTT method (0.5 mg/ml; Sigma). According to the manufacturer's instructions, cells were seeded into 96-well tissue culture plates at the density of $1x10^5$/well in triplicate for 0, 24, 48, 72 and 96 h with different concentrations (0, 40, 60, 80, 100 or 120 nmol/l) of G9a siRNA. The spectrophotometric absorbance of the samples was determined by using Ultra Multifunctional Microplate Reader (Tecan US, Inc., Durham, NC, USA) at 492 and 630 nm for absorbance. Suppression ratio was also calculated. The experiment was repeated in triplicate.

Apoptosis detected by TUNEL assay. Cells at logarithmic phase were transfected with negative control siRNA, and 40, 80 and 120 nmol/l G9a siRNA, and were seeded in 6-well plates ($1x10^6$ cells/well; Costar Life Sciences) with sterile coverglass placed at the bottom of each well. After 24-h transfection, apoptotic cells were detected by TUNEL, according to the manufacturer's protocol (DeadEnd™ Fluorometric TUNEL system; Promega).

Western blot analysis. Total protein lysates and western blot analysis were performed as previously described (15). Briefly, MGC-803 cells were plated on culture dishes and transfected by G9a siRNA with 40, 80 and 120 nmol/l for 24 h. Control cells were incubated in the medium with Na_2CO_3 using the same time-points. After incubation, total proteins were prepared from each culture condition with a lysis buffer containing freshly prepared protease inhibitors. Protein concentration was then measured using BCA protein assay (Pierce, Rockford, IL, USA). Cell extracts were subjected to 12% SDS-PAGE and electrophoretically transferred to nitrocellulose membrane. Membranes were incubated with monoclonal anti-mono-methyl-histone H3K9, anti-di-methyl-histone H3K9, anti-tri-methyl-histone H3K9, anti-mono-methyl-histone H3K27, anti-dimethyl-histone H3K27, anti-acetyl-histone H3, and G9a (Upstate Biotechnology), BCL-2, Bax, pro-caspase-3, (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After being washed with TBS, membrane was incubated with secondary

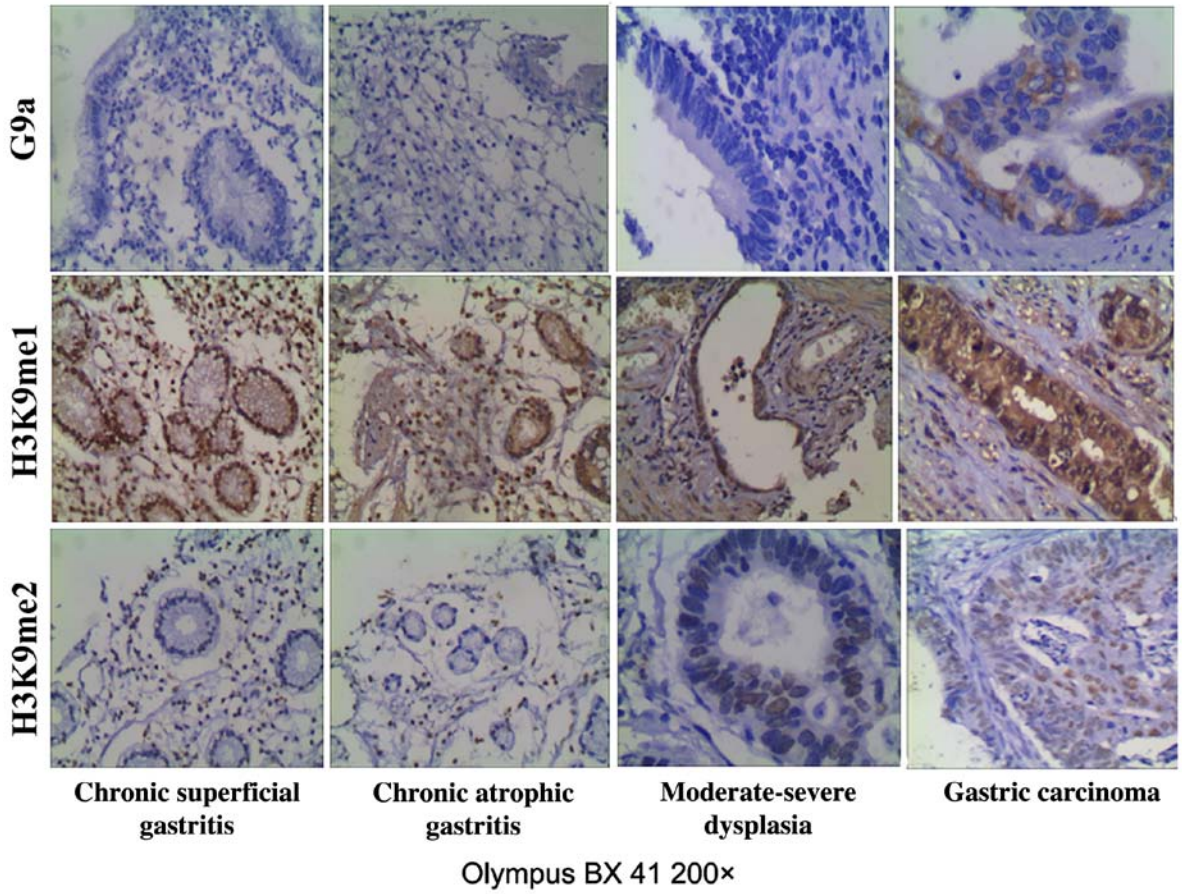

Figure 1. The expression of G9a, monomethylated H3K9 and dimethylated H3K9 in gastric carcinoma and benign gastric diseases. The expression of G9a and H3K9 me2 in gastric carcinoma was higher than that in chronic superficial gastritis, chronic atrophic gastritis and moderate-severe dysplasia, P<0.05. The histone H3K9 me1 in gastric carcinoma was also similar, P>0.05.

antibody conjugated with peroxidase. The signal was then detected using the chemiluminescent detection system (Pierce) and analysed by a color image analysis system (AlphaDigiDoc; Alpha Innotech, Corp., St. San Leandro, CA, USA).

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 G9a, H3K9 me2 in gastric carcinoma tissues. We found overexpression of G9a, and H3K9 me2 in gastric carcinoma tissues. The expression of G9a in gastric carcinoma was 87.43%, clearly higher than that in chronic superficial gastritis (33.33%, P<0.05), chronic atrophic gastritis (43.33%, P<0.05) and moderate-severe dysplasia (46.67%, P<0.05). The histone H3K9 me2 in gastric carcinoma was 90.28%, higher than that in chronic superficial gastritis (36.67%, P<0.05), chronic atrophic gastritis (40.00%, P<0.05), and moderate-severe dysplasia (56.67%, P<0.05). The expression of histone H3K9 me1 in gastric carcinoma was 50.86%, similar to chronic superficial gastritis (53.33%, P>0.05), chronic atrophic gastritis (50.00%, P>0.05) and moderate-severe dysplasia (56.67%, P>0.05) (Fig. 1).

G9a and H3K9 me2 expression in gastric carcinoma is associated with poor prognosis. The association of G9a, and H3K9 me2 expression with clinical outcomes was analyzed separately. We divided the level of staining scores into two groups: negative (score ≤2) and positive (score >2). Among these specimens, High G9a and H3K9 me2 expression positively correlated with differentiation (P<0.05), depth of infiltration (P<0.05), lymph node metastasis (P<0.05), and TNM stage (P<0.05). They were not correlated with age (P>0.05), gender (P>0.05) or pathological type (P>0.05), respectively. The relationships between the levels of G9a, and H3K9 me2 expression and the clinicopathplogical characteristics of gastric cancer are summarized in Table I.

G9a-specific siRNA inhibition of G9a in MGC-803 cells. After 24 h transfection with G9a siRNA of 40, 80 and 120 nmol/l to MGC-803 cells, the amplification of G9a mRNA attenuated concentration dependently. Gray value (to β-actin) showed the amplification of G9a was 0.21±0.10 with 40 nmol/l, 0.11±0.04 with 80 nmol/l, and 0.08±0.02 with 120 nmol/l, respectively, compared to the control (0.33±0.08), χ^2=17.43, P<0.05. The

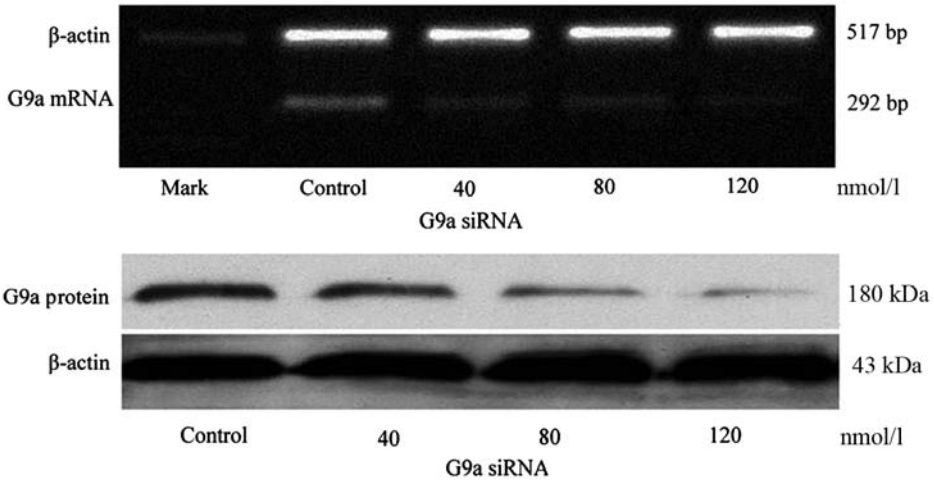

Figure 2. Silencing efficiency of G9a siRNA on G9a gene and G9a protein. Amplification of G9a mRNA and G9a protein was attenuated concentration-dependently.

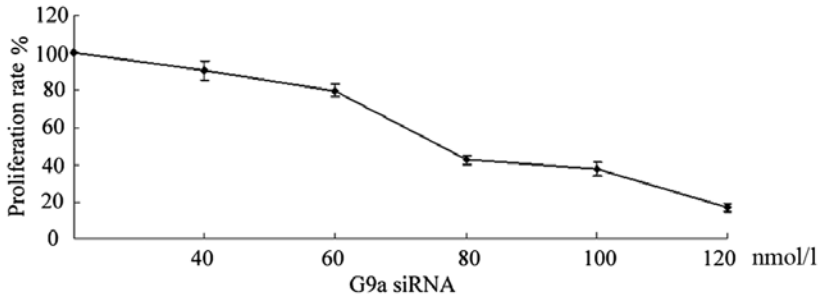

Figure 3. The observation of cell proliferation after transfected with G9a siRNA in MGC-803 cells. Cell proliferation was inhibited in MGC-803 cells after 24-h transfection of indicated concentrations of G9a siRNA.

protein of G9a also decreased concentration dependently (P<0.05; Fig. 2).

Depletion of G9a decreases cell proliferation and promotes apoptosis in MGC-803 cells. Depletion of G9a suppressed cell proliferation. To elucidate the effect of G9a on proliferation and apoptosis, G9a was knocked out by transfection of G9a siRNA in gastric cancer MGC-803 cells to exhibit cells biological changes. After 24-h transfection with G9a siRNA, 82.36±4.46% reduction in cell density was seen with 40 nmol/l, 76.24±3.24% with 60 nmol/l, 42.53±3.25% with 80 nmol/l, 17.45±1.83% with 120 nmol/l (Fig. 3). Apoptotic rate was 3.2±1.3, 17.4±3.2 and 39.7±9.2 and 68.5±5.4% after 24-h transfection of G9a siRNA with 0, 40, 80 and 120 nmol/l, respectively. The apoptotic rate was increased in a concentration-dependent manner (P<0.05; Fig. 4A). We further investigated the apoptosis-associated proteins BCL-2, pro-caspase-3 and Bax. It demonstrated that G9a siRNA inhibited BCL-2, pro-caspase-3, and promoted Bax significantly, thus promoting cell apoptosis (Fig. 4B).

G9a depletion associates with reduction of the H3K9 me1, H3K9 me2 and histone H3K27me and H3K27 me2 in MGC-803 cells, but not with H3K9 me3, histone acetyl H3. Lysine residues can be mono, di, or trimethylated, inducing different biological responses (16-18). The present study was performed to identify the histone modifications associated with the G9a, especially on histones methylation of H3K9 and H3K27. To this end, we transfect of G9a siRNA to gastric carcinoma MGC-803 cells and performed western blot analysis to identify the effect of depletion of G9a on histone H3K9 and H3K27 and histone acetylation of H3. We demonstrated that depletion of G9a for 24 h resulted in downregulation of histone H3K9 me1, H3K9 me2 and H3K27 me1, H3K27 me2, compared with the control. The relative (to β-actin), gray value showed H3K9 me1 was 1.21±0.19, 0.84±0.17, 0.42±0.14, 0.19±0.08, H3K9 me2 was 1.13±0.18, 0.86±0.15, 0.47±0.15, 0.16±0.04, H3K27 me was 1.01±0.12, 0.82±0.11, 0.51±0.14, 0.31±0.07; H3K27 me2 was 1.14±0.19, 0.91±0.14, 0.57±0.15, 0.33 ±0.05, in 0, 40, 80 and 120 nmol/l, respectively (P<0.05). There was no change in histone H3K9 me3 and histone H3 acetylation. The expression of H3K9 me3 was 1.08±0.17, 1.11±0.16, 1.09±0.17, 1.07±0.16, Act-H3 was 0.93±0.15, 0.87±0.13, 0.91±0.16, 0.89±0.15 in 0, 40, 80 and 120 nmol/l, respectively (P>0.05) (Fig. 5).

Discussion

Cancer cells are characterized by prominent epigenetic dysregulation, including altered chromatin modification. The histone methyltransferase G9a is overexpressed in a variety of cancer types, such as pancreatic adenocarcinoma, lung cancer, hepatocellular carcinoma (19-21), and promotes tumor

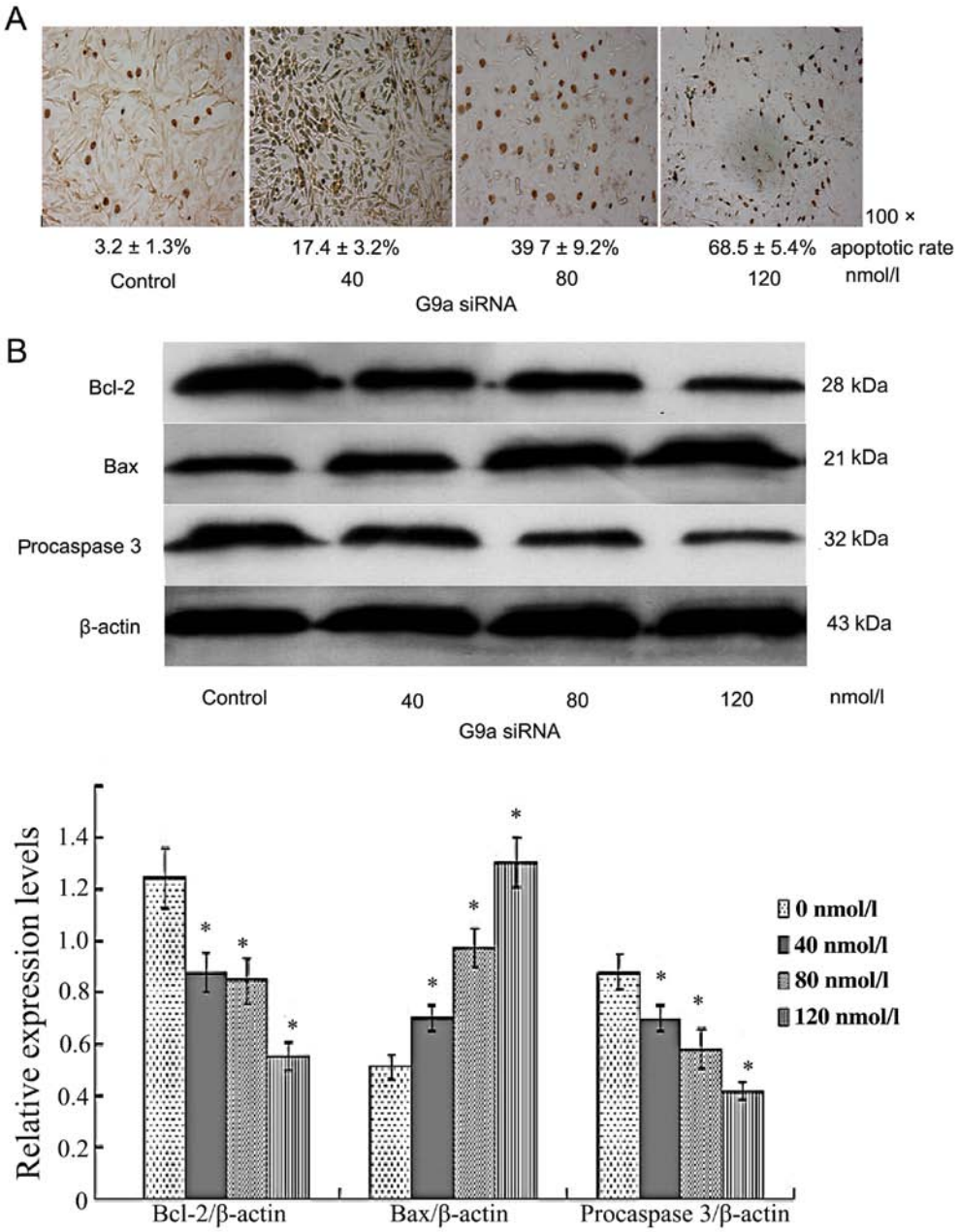

Figure 4. (A) The observation of cell apoptosis after transfected with G9a siRNA in MGC-803 cells. Cell apoptosis was induced after 24 h transfection with indicated concentrations of G9a siRNA in MGC-803 cells. (B) The expression of apoptosis related proteins after transfection with G9a siRNA in MGC-803 cells. The apoptosis related proteins were inhibited after 24 h transfection with G9a siRNA in indicated concentrations. The proteins of BCL-2, and procaspase-3 was degradated and Bax was upgradated after transfected with G9a siRNA.

invasiveness and metastasis, although the functional role of the HMTs overexpression in cancer remains unclear. Previously we found the level of SUV39H1, another HMT, was increased and associated with poor prognosis in gastric carcinoma (22). In the present study, we demonstrated that the expression of G9a, H3K9 me2 were aberrant in gastric carcinoma. The expression of G9a, H3K9 me2 in gastric carcinoma was 87.43 and 90.28%, respectively, which were higher than that in the controls ($P<0.05$). Downregulation of H3K9 me1 in gastric carcinoma need to be investigated. Tissue invasion and metastasis are the major causes of cancer-related death (23). The changes in global levels of individual histone modifications are independently predictive of the clinical outcome of prostate cancer, gastric adenocarcinomas, as well as breast, ovarian and pancreatic cancers (24-26). Some studies have found that HMTs specifically affect metastasis. EZH2 (the polycomb group protein enhancer of zeste homolog 2) is linked to cell proliferation and invasion in prostate and breast cancer (27,28) and significantly associated with distant metastases in gastric and prostate cancer (29,30). We found that G9a and H3K9 me2 were positively correlated with differentiation, depth of infiltration, lymph node metastasis and TNM stage. These results support the hypothesis that aberrant histone modification patterns are critically involved in the tumorigenetic process and indicate that G9a seems to be required for the maintenance of the malignant phenotype and could be valid therapeutic targets in human neoplasia. The elevated levels of G9a and H3K9 me2 predict poor prognosis. Chen *et al* (19)

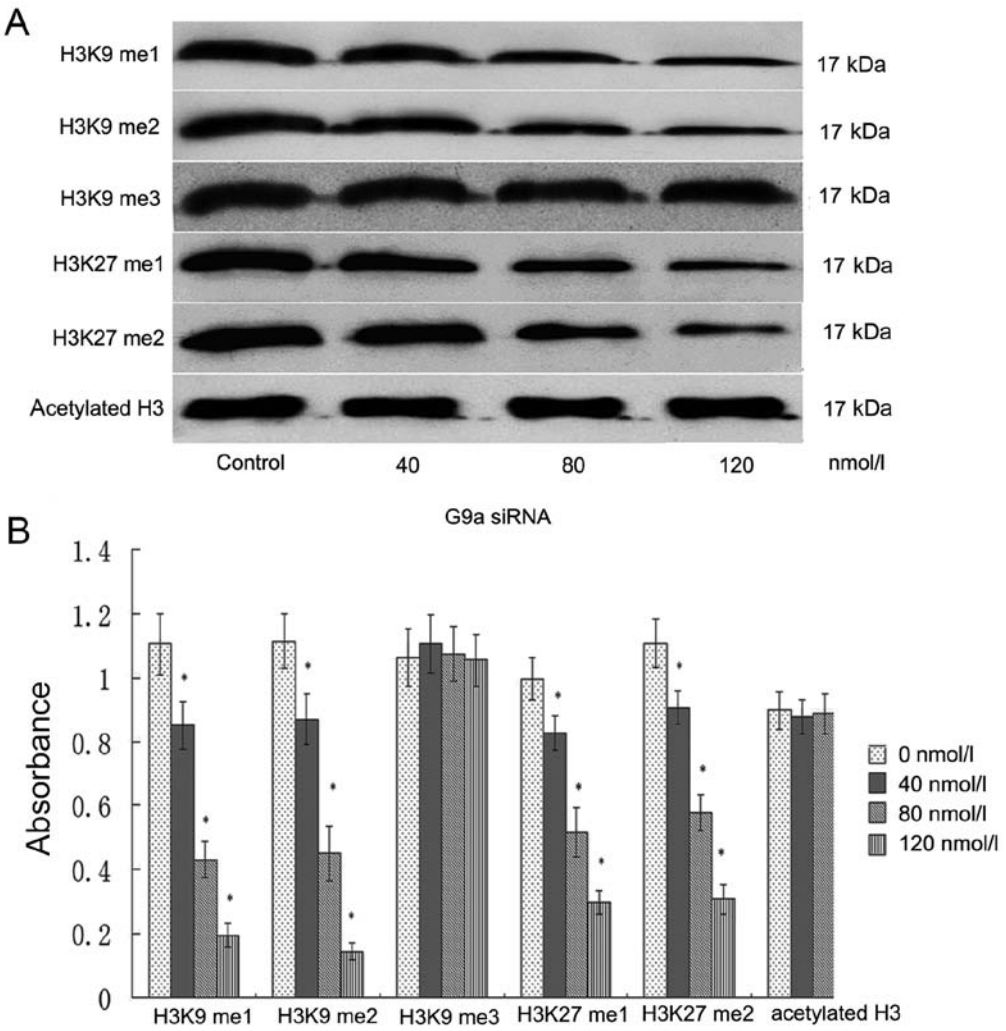

Figure 5. The expression of G9a, H3K9 me1, H3K9 me2, H3K9 me3, H3K27 me1, H3K27 me2, acetylation of H3 after 24 h transfected withG9a siRNA. The modulation of histone methylation H3K9 and H3K27 and acetylation H3 after transfected with G9a siRNA for 24 h in MGC-803 cells. G9a siRNA downregulated histone monomethylated H3K9, histone dimethylated H3K9, histone monomethylated H3K27, and histone dimethylated H3K27. Tri-methylated H3K9 and histone acetylation of H3 were not affected. $^{*}P<0.05$ vs. 0 nmol·l^{-1}

reported that G9a is endowed with methyltransferase activity to concomitantly repress the downstream effector Ep-CAM, thereby promoting the invasion step of the invasion-metastasis cascade. The possible mechanisms of G9a overexpression associated with cancer metastasis might be involved in post-translational regulation. It is possible that high G9a expressing cells may contain more cancer stem cell potential with higher capability for metastasis.

The aberrant overexpression of G9a in gastric carcinoma may make it a good candidate as a therapeutic molecular target. We transfered G9a siRNA to MGC-803 cells and observed their biological phenomenon. The evidence showed that depletion of G9a reduced cell proliferation, and induced apoptosis in human gastric cancer cell line MGC-803, along with downregulation of pro-caspase-3, BCL-2, and significant upregulation of Bax, which are related to apoptosis pathway. It was reported that G9a is linked to aberrant silencing of several tumor-suppressor genes through H3K9 methylation and DNA CpG methylation (31-33). Current evidence suggests that H3K9 methylation and silencing of the p16ink4a tumor suppressor gene can occur before CpG methylation (34). G9a has been characterized as transcriptional silencer by executing mono-, di- and tri-methylation of euchromatic H3K9 as well as by promoting DNA methylation (35). Reduction of G9a expression coincided with the decrease in DNA methyltransferase (DNMT) activity and indicated a functional impact of G9a on DNMT 1 operations. It was reported that RUNX3 was downregulated by hypoxia in several gastric cancer cells and this suppression was on account of histone deacetylation and methylation (36). This interaction enhanced the catalytic activity of recombinant DNMT 1 *in vitro*, in the absence of any other proteins (37). Regulation of DNA methylation by G9a is independent of its catalytic activity, implying that its physical presence, but not H3K9 methylation or methylation of DNMT1, is relevant (14).

Lysine residues can be mono, di, or trimethylated, inducing different biological responses (16,18,20). Thus, for example, highly condensed heterochromatic regions show a high degree of H3K9 me3, whereas euchromatic regions are preferentially enriched in mono- and dimethylated H3K9 (38). G9a and GLP are homologous HMT and mediate histones H3K9 me1 and H3K9 me2 and methylation at H3K27 (10). Studies in

knockout mice for Suv39h1/2 and G9a revealed that G9a is mainly responsible for monomethylation and dimethylation of H3K9, whereas Suv39h1 and Suv39h2 direct trimethylation of H3K9 (39). We found that depletion of G9a decreased H3K9 me1, me2, and H3K27 me1, me2, and could not change H3K9 me3 and histone acetylation of H3. Previously studied showed that silencing Suv39H1 downregulated histone acetylated H3, H3K9 me3, and enhanced P15 in MGC-803. It did not affect H3K27, H3K14 and H4 (22,40).

In conclusion, our data show that G9a and H3K9 me2 are highly expressed in gastric cancer, and associate with poor prognosis. Depletion of G9a reduces H3K9 me1, H3K9 me2, H3K27 me1, H3K27 me2, and producse tumor cell apoptosis and inhibits cell proliferation. The results from this study highlight the potential of G9a, a methyltransferase, that could be a therapeutic target for gastric carcinoma.

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