Synergic effect of diabetes mellitus and *H. pylori* infection on proliferation of cells via downregulating the expression of PTEN by hypermethylating its promoter region

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Abstract

Background It has been reported that CagA of *H. pylori* reduced the expression of PTEN by enhancing its promoter methylation. Furthermore, DM may also promote the methylation status of PTEN, a tumor suppressor gene in Gastric cancer. It is intriguing to explore whether DM may strengthen the tumorigenic effect of HP by promoting the methylation of PTEN promoter and whether the administration of metformin may reduce the risk of GC by suppressing the methylation of PTEN promoter.

Methods Bisulfite sequencing PCR was performed to measure the DNA methylation of PTEN promoter in GC patients and HGC-27 cells treated under different conditions. Quantitative real-time PCR was carried out to measure the expression of PTEN mRNA. Immunohistochemistry and Western blot were used to evaluate the expression of PTEN protein. Immunofluorescence and flow cytometry were performed to analyze the apoptosis of GC tissue samples and HGC-27 cells treated under different conditions. MTT assay was carried out to examine the proliferation of HGC-27 cells.

Results DNA methylation of PTEN promoter was synergetic enhanced by HP infection and Diabetes mellitus in patients with Gastric cancer. Accordingly, the expression of PTEN was suppressed in GC patients with HP infection and DM. Furthermore, cell apoptosis was decreased in GC patients with HP infection and DM. Metformin showed an apparent effect on maintaining CagA induced elevation of PTEN promoter methylation, thus attenuating the PTEN expression, increasing the proliferation and suppressing the apoptosis of HGC-27 cells.

Conclusion In this study, we collected GC tumor tissues from GC patients with or without DM/HP to compare their PTEN methylation and expression while testing the effect of Metformin on the methylation of PTEN promoter. Our study provided evidence for the mechanism underlying the therapeutic role of Metformin in GC treatment.

Background

Gastric cancer (GC) is one of highly frequently diagnosed cancers in the world. GC causes the 2nd highest death rate among all types of cancers. The majority of GC cases are actually gastric carcinoma and gastric antrum cancer, although the occurrence of carcinoma at gastroesophageal junction has actually been rising steadily [1–3]. Review of the onset of GC showed that the occurrence of GC is slowly increasing among youth adults as well as in children. Also, the occurrence, death rate, as well as metastasis of GC are pretty high, along with low rate of early diagnosis, good prognosis, as well as 5 year survival [4].

The gene of phosphatase and Tensin homologue on chromosome 10 (PTEN) is deemed as a possible tumor suppressor in numerous forms of cancers in human [5]. PTEN is likely to suppress phosphatidylinositol-3-kinase (PI3K) expression through catalyzing the elimination of D3 phosphate from phosphatidylinositol (3,4,5)- triphosphate (PIP3) to dysregulate the production of phosphatidylinositol 4,5-bisphosphate (PIP2) as well as PIP3 [6–8]. PTEN is actually downregulated in GC, and the expression

of PTEN is also negatively correlated to metastasis in lymph nodes, depth of invasion, as well as the age of GC patients [9–11]. PTEN was shown to suppress the cell cycle arrest, apoptosis, as well as metastasis of GC cells [12, 13]. Formerly, Tet1 has actually been shown to prevent the metastasis as well as development of GC through PTEN demethylation as well as its expression. Tet1 reduces the migration, development, as well as invasion of GC through the demethylation of CpG islands in the promoter region of PTEN, thus downregulating focal adhesion kinase as well as AKT activity [14].

The role of DM in the risk of cancer has been analyzed in various meta analyses [15]. Epidemiologic research reviewing the relationship between DM and the risk of GC has generated contradictory results [16, 17]. It was shown that PTEN promoter hypomethylation is actually quite common among Uyghur patients carrying wild type T2DM, which might contribute to T2DM pathogenesis. The abnormal methylation of CpG sites in the promoter of PTEN might function as a biomarker for T2DM diagnosis.

Helicobacter pylori are a global threat that has infected about 4 billion individuals [18]. *Helicobacter pylori* are actually the primary reason for peptic ulcer disease, gastritis, as well as GC. Previous research has actually revealed that the infection by *helicobacter pylori* caused an epithelial-to-mesenchymal transition of epithelial cells in the stomach [19]. It was revealed that CagA considerably lowered the PTEN as well as Tet1 expression. Furthermore, it was uncovered that CagA lowered PTEN expression through enhancing its own methylation, which was actually dramatically decreased by Tet1 [20]. Metformin is a compound extracted from *Galega officinalis* and has actually been utilized for years in medical treatment to type 2 diabetes mellitus (T2DM) [21]. It was shown that metformin dramatically reduces AKT-dependent phosphorylation [22].

CagA of *H. pylori* reduces the expression of PTEN by enhancing its promoter methylation [20]. Furthermore, DM may also promote PTEN methylation [23]. Giving the fact that DM and HP infection may synergistically elevate the risk of GC as well as the ability of metformin to suppress the methylation of PTEN, we hypothesized that DM may strengthen the tumorigenic effect of HP by promoting PTEN promoter methylation, and the administration of metformin may reduce the risk of GC by suppressing PTEN promoter methylation [23–25]. In this study, we collected GC tumor tissues from GC patients with or without DM/HP to compare their PTEN methylation and expression while testing the effect of Metformin on the methylation of PTEN promoter.

Materials And Methods

Human subjects sample collection

In this study, we enrolled 107 patients with gastric cancer and divided them into three groups according to their status of HP (*H. Pylori*) infection and Diabetes mellitus, i.e., 1. HP(-) DM(-) group (N = 39), 2. HP(+) DM(-) group, (N = 32), and 3. HP(+) DM(+) group, (N = 36). The information of participants, including their age, gender, smoking history, drinking history, staging of gastric cancer, location of gastric cancer, was

collected and compared among different groups. Institutional ethical committee has approved the protocol of this study.

Bisulfite Sequencing

We used bisulfite sequencing to determine the level of DNA methylation in the promoter of the PTEN gene. In brief, genomic DNA in the samples was isolated by making use of a QIAamp DNA Mini assay kit (Qiagen, Hilden, Germany) in accordance with the routine assay protocol provided by the kit manufacturer. Then, Bisulfite conversion was carried out via adding 5 M of salt sodium bisulfite to each 1.8 µg of the DNA sample. A universal primer free of CpG was made use of for the amplification of both demethylated as well as methylated promoter of the PTEN gene at 55.0 °C annealing temperature. The product of PCR was then quantitatively evaluated through using DHPLC on a WAVE DNA Fragment Analysis System in accordance with the routine assay protocol provided by the manufacturer.

Rna Isolation And Real-time Pcr

Real time PCR was done to measure the expression of PTEN and CagA mRNA in collected samples. In brief, total RNA in each sample was isolated by utilizing a PureLink RNA Mini assay kit (Thermo Fisher Scientific, Waltham, MA) in accordance with the routine assay protocol provided by the kit manufacturer. Then, cDNA was generated from isolated total RNA by making use of a PrimeScript RT Reagent assay kit (Thermo Fisher Scientific, Waltham, MA) in accordance with the routine assay protocol provided by the kit manufacturer. In the next step, the real time PCR was carried out on an iCycler real time PCR instrument (Bio-Rad Laboratories, Hercules, CA) by utilizing an iQSYBR Green master kit (Bio-Rad Laboratories, Hercules, CA) in accordance with the routine assay protocol provided by the kit manufacturer. The $\Delta\Delta C_t$ method was used to determine the relative expression of PTEN and CagA mRNA in each sample, with housekeeping gene GAPDH serving as the internal reference.

Cell Culture And Transfection

HGC-27 cells were bought from American Type Culture Collection (ATCC, Manassas, VA) and maintained in an RPMI 1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA) added with 10% of fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA) as well as a suitable amount of penicillin and streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA). The culture was carried out at 37 ° C in an incubator containing 5% CO₂. Prior to the experiments, the cells were randomly divided into 4 groups, i.e., 1. Untreated group (HGC-27 cells treated with PBS only); 2. Metformin group (HGC-27 cells treated with 100 µM of Metformin); 3. CagA group (HGC-27 cells treated with CagA); 4. CagA + Metformin group (HGC-27 cells treated with CagA and 100 µM of Metformin). After 48 h of treatment, the cells were collected for subsequent assays.

Construction Of Recombinant Plasmids And Packaging Of Lentiviral Vectors

HEK 293T cells and HGC 27 cells, a human GC cell line, were bought from American Type Culture Collection (ATCC, Manassas, VA) and maintained in a Dulbecco Modified Eagle's Medium (DMEM, Gibco, Thermo Fisher Scientific, Waltham, MA) added with 10% of fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA) as well as a suitable amount of penicillin and streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA). The culture was carried out at 37 ° C in an incubator containing 5% CO₂. Then, the catalytic domain of Tet1 was utilized to create 3 shRNAs. After annealing, the shRNAs were incubated with dATP and T4 DNA polymerase, and then inserted into a pds019-pl6.3- SHRNA-BSD vector (EMD Millipore, Billerica, MA). In the next step, the vector was transformed into *E. coli* and the resulting plasmids were called CL946-3, CL946-2 as well as also CL946-1. Moreover, the catalytic domain of Tet1 was inserted into a PDS087_pL6-TO-V5-GIM vector (EMD Millipore, Billerica, MA) and the resulting plasmid was called CL981_pL6-TO-V5-tet1-CD. In a similar way, a vector overexpressing CagA was produced. Ultimately, an Opti-Mum Essential Medium (MEM, Thermo Fisher Scientific, Waltham, MA) and a packaging reagent (Thermo Fisher Scientific, Waltham, MA) were used with 3 µg of the lentiviral plasmid and Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in accordance with the routine assay protocol provided by the kit manufacturer to transfect HEK 293T cells. After 48 h of transfection, the supernatant of cells was centrifuged for 10 minutes at 1,500 g and ambient temperature to collect the viral vectors.

MTT cell proliferation assay

The proliferation status of treated cells was measured by utilizing a CellTiter-Glo MTT assay (Promega, Madison, WI) in accordance with the routine assay protocol provided by the kit manufacturer.

Western Blot Analysis

Collected tissue and cell samples were lysed by using a Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the routine assay protocol provided by the reagent manufacturer to obtain protein lysate, which was then resolved via electrophoresis by making use of a 10% SDS-PAGE gel. After transferring the resolved protein samples onto PVDF membranes, the membranes were blocked by making use of 5% skim milk and then incubated overnight at 4 ° C with anti-PTEN primary antibody (R&D systems, Minneapolis, MN). In the next step, the membranes were washed and further incubated at room temperature for 1 h with horseradish peroxidase-labeled secondary antibody. Finally, the relative expression of PTEN protein in each sample was analyzed after the protein blot was developed by making use of an Immobilon Western Chemiluminescent HRP Substrate assay kit (EMD Millipore, Billerica, MA) in accordance with the routine assay protocol provided by the kit manufacturer.

Apoptosis Analysis

The status of apoptosis in each collected cell and tissue sample was analyzed by using a propidium iodide/annexin V-FITC apoptosis assay kit (Sigma Aldrich, St. Louis, MO) in accordance with the routine assay protocol provided by the kit manufacturer. The detection of apoptosis was carried out on a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA) at a 488 nm wavelength.

Immunofluorescence And Immunohistochemistry

Immunofluorescence and immunohistochemistry assays were used to detect the expression of PTEN and the status of apoptosis of collected tissue samples, respectively. In brief, for immunohistochemistry assays, the tissue samples were fixed by using 4% of formaldehyde (Sigma Aldrich, St. Louis, MO), deparaffinized, and rehydrated using gradient alcohol. In the next step, the sections were stained with anti-PTEN primary antibody (Abcam, Cambridge, MA) and suitable biotin-labeled secondary antibody (Thermo Fisher Scientific, Waltham, MA) before the relative PTEN expression in each sample was evaluated under a Zeiss LSM700 confocal laser-scanning microscope in accordance with the instruction provided by the manufacturer. For immunofluorescence assays, the status of apoptosis of collected tissue samples was evaluated by using a Vybran Apoptosis Assay Kit (Thermo Fisher Scientific, Waltham, MA) in accordance with the routine assay protocol provided by the kit manufacturer.

Statistical analysis

All statistical evaluations were conducted by making use of GraphPad Prism 8.0 for Microsoft Windows (GraphPad, La Jolla, CA). All data were shown as mean $\pm$ SEM. The difference between two different groups was evaluated by using a two-tailed Student's t test. A p value of < 0.05 was deemed significant statistically.

Results

The characteristics of patients

We enrolled 107 gastric cancer patients and divided them into three groups according to their status of HP (H. Pylori) infection and Diabetes mellitus: 1. HP(-)DM(-)(N = 39), 2. HP(+)DM(-)(N = 32), 3. HP(+)DM(+)(N = 36). The information of participants was collected and listed in Table 1. Student's t test was utilized to perform the statistical comparison, and revealed that there was no obvious difference in all above characteristics among the three groups.

Table 1
Demographic and clinical characteristics of recruited subjects

Characteristics	HP(-)DM(-) (N = 39)	HP(+)DM(-) (N = 32)	HP(+)DM(+) (N = 36)	P value
Age, years				0.279
<60	18 (46.2)	14 (43.8)	18 (50.0)	
≥60	21 (53.8)	18 (56.2)	18 (50.0)	
Gender				0.538
Male	30 (76.9)	24 (75.0)	31 (86.1)	
Female	9 (23.1)	8 (25.0)	5 (13.9)	
Smoking				0.608
Ever or current	20 (51.3)	18 (56.3)	20 (55.6)	
Never	17 (43.6)	13 (40.6)	15 (41.6)	
Missing	2 (5.1)	1 (3.1)	1 (2.8)	
Drinking				0.178
Ever or current	18 (46.2)	17 (53.1)	20 (55.6)	
Never	20 (51.2)	14 (43.8)	14 (38.8)	
Missing	1 (2.6)	1 (3.1)	2 (5.6)	
Stage				0.376
Early	15 (38.5)	13 (40.6)	18 (50.0)	
Advanced	13 (33.3)	8 (25.0)	8 (22.2)	
Missing	11 (28.2)	11 (34.4)	10 (27.8)	
Location				0.882
Cardia	11 (28.2)	8 (25.0)	10 (27.8)	
Non-cardia	25 (64.1)	23 (71.9)	25 (69.4)	
Missing	3 (7.7))	1 (3.1)	1 (2.8)	

HP infection and Diabetes mellitus synergistically increased the DNA methylation level of PTEN promoter in gastric cancer patients.

As PTEN expression was reported to be closely related to the pathogenesis of gastric cancer, and DNA methylation level of PTEN promoter is reversely correlated with PTEN expression, we performed bisulfite sequencing PCR to evaluate the DNA methylation status of PTEN promoter in the three groups of gastric cancer patients. As shown in Fig.1, DNA methylation of PTEN promoter was slightly increased in HP(+)DM(-) patients when compared with HP(-)DM(-) patients. However, DNA methylation of PTEN promoter was remarkably elevated in HP(+)DM(+) patients, indicating that HP infection and Diabetes mellitus showed a synergistic effect on promoting the DNA methylation of PTEN promoter in patients with gastric cancer.

HP infection and Diabetes mellitus suppressed the expression of PTEN mRNA and protein in gastric cancer patients in a synergistic manner.

It is well known that DNA methylation in the promoter region can repress gene expression. Therefore, we further performed quantitative real time PCR and immunohistochemistry assay to examine the PTEN expression in gastric cancer patients in the above three groups. As expected, the expression of PTEN mRNA (Fig.2) and protein (Fig.3) was obviously repressed in HP(+)DM(-) patients in comparison to that in HP(-)DM(-) gastric patients. Moreover, the expression of PTEN mRNA and protein in HP(+)DM(+) gastric cancer patients was more apparently suppressed.

The apoptosis of gastric cancerous tissues was inhibited by HP infection and Diabetes mellitus.

Furthermore, immunofluorescence was carried out to evaluate the apoptotic status of gastric tissues collected from patients in 1. HP(-)DM(-)(N=39), 2. HP(+)DM(-)(N=32), 3. HP(+)DM(+) (N=36) groups. The proportion of apoptotic gastric tissues was progressively decreased from HP(-)DM(-) group to HP(+)DM(-) and HP(+)DM(+) (Fig.4). These results demonstrated that HP infection and Diabetes mellitus played an antagonistic role in the apoptosis of gastric cancer tissues.

Metformin treatment alleviated CagA induced up-regulation of CagA mRNA expression in HGC-27 cells.

CagA is a toxic component of HP and was shown to alter the expression of PTEN through regulating the DNA methylation of PTEN promoter. We treated HGC-27 cells with CagA and Metformin, and then performed qPCR to measure the expression of CagA mRNA under different conditions. No obvious change of CagA was observed in HGC-27 cells treated with 100uM Metformin when compared with the control. On the contrary, CagA treatment remarkably increased the expression of CagA in HGC-27 cells. The expression of CagA mRNA in HGC-27 cells was apparently decreased under combined treatment with

CagA and 100uM Metformin when compared with that in HGC-27 cells treated with CagA alone (Fig.5). These results indicated that Metformin treatment reduced CagA-induced up-regulation of CagA mRNA in HGC-27 cells.

Metformin treatment attenuated CagA-induced increase of DNA methylation of PTEN promoter in HGC-27 cells.

Bisulfite sequencing PCR was carried out to measure the DNA methylation of PTEN promoter in HGC-27 cells treated under different conditions. We found that DNA methylation of PTEN promoter was obviously elevated in HGC-27 cells treated with CagA when compared with that in the control cells, whereas no obvious difference was observed in Metformin-treated HGC-27 cells. However, the DNA methylation of PTEN promoter in HGC-27 cells enhanced by CagA treatment was effectively reduced by Metformin treatment (Fig.6).

Metformin treatment maintained CagA induced down-regulation of PTEN expression in HGC-27 cells.

Quantitative real time PCR and Western blot were used to analyze the expression of PTEN mRNA and protein in HGC-27 cells treated under different conditions. The expression of PTEN mRNA (Fig.7A) and protein (Fig.7B) in HGC-27 cells was significantly suppressed by CagA treatment, while Metformin treatment showed no obvious effect on the PTEN mRNA expression in untreated HGC-27 cells. However, Metformin treatment effectively restored CagA induced down-regulation of PTEN mRNA and protein in HGC-27 cells.

Metformin treatment restored CagA induced dysregulation of proliferation and apoptosis of HGC-27 cells.

Finally, we performed MTT assay and flow cytometry to evaluate the proliferation and apoptosis of HGC-27 cells treated under different conditions. CagA treatment notably increased the proliferation of HGC-27 cells. Further treatment with Metformin apparently decreased CagA-induced elevation of HGC-27 cell proliferation (Fig.8A). On the contrary, CagA treatment remarkably suppressed the apoptosis of HGC-27 cells. Metformin treatment effectively maintained CagA-induced decrease of HGC-27 cell apoptosis (Fig.8B).

Discussion

Metformin is well known for its anti-diabetic role [26]. It was also shown that metformin reduced the risk of cancer in patients with T2DM [27, 28]. The role of metformin in suppressing the proliferation of GC cells is related to its role in blocking cell cycles, which could explain why metformin decreased tumour

size in mice with xenograft GC tissues [29, 30]. It was shown that the use of metformin significantly decreased the risk of infection by helicobacter pylori dose dependently [31]. In this study, we recruited 107 gastric cancer patients and divided them into three groups according to their status of HP infection and DM to explore the molecular mechanism underlying gastric cancer pathogenesis. We found that HP infection and DM effectively increased the DNA methylation of PTEN promoter in GC patients.

PTEN acts as a crucial mediator in the carcinogenesis of a wide array of cancers [32]. PTEN can degrade the derivatives of PI3K through dephosphorylating position 30 of PIP3 as well as PIP2, subsequently boosting AKT phosphorylation and reducing apoptosis [33, 34]. It was shown that the methylation of PTEN promoter can be used as a prognostic marker of survival [35]. It was illustrated that GC cells transfected by PTEN-shRNA NC displayed considerably down-regulated expression of PTEN, thus substantially enhancing the expression of b-catenin. In addition, IFC as well as Western blotting results showed that PTEN down-regulation and b-catenin up-regulation in GC tissues triggered the phosphorylation of p-AKT as well as p-GSK-3b in GC tissues. In this study, we carried out qPCR and IHC to measure the expression of PTEN mRNA and protein in GC patients in the three groups. HP infection and DM evidently decreased the expression of PTEN mRNA and protein in GC patients. In addition, we used immunofluorescence assays to analyze the apoptosis of GC tissue samples collected from GC patients in the three groups. HP infection and DM notably attenuated the apoptosis of GC tissue samples.

Helicobacter pylori infection is one of the most significant dangers for stomach cancers [36]. In a research on 114 GC patients, gastric adenocarcinoma was shown as accounting for 90% in all patients. In helicobacter pylori, the expression of a 120 kDa protein called cytotoxin associated gene A (CagA) was shown to be related to some of the essential features of helicobacter pylori to interact with kinases in host cells to trigger tyrosine phosphorylation [37–39]. So far, 4 distinguish EPIYA motifs have been found, i.e., EPIYA-A to EPIYA-D [40, 41]. It was likewise revealed that CagA dramatically lowered the expression levels of PTEN, APOBEC3A, Tet1, APOBEC3C as well as APOBEC3F in GC tissues. Furthermore, CagA lowered the expression levels of PTEN through boosting its levels of methylation, which was substantially blocked through upregulation of Tet1 [20]. It was additionally found that infection with helicobacter pylori strains positive for CagA expression triggers Akt signaling in the epithelial tissues in the stomach, thus attenuating cell apoptosis while promoting cell survival [42–44]. The activation of Akt is actually a common event in chemotherapy treatment of GC, indicating a significant role of Akt in causing apoptosis resistance [45, 46]. Previous results presented that etoposide boosted the phosphorylation of Akt while minimizing cell survival via the induction of GC apoptosis [47].

It was actually shown that the abnormal methylation in PTEN promoter was substantially reduced in Uyghur subjects suffering from mild cases of T2DM. It was further shown that the hypomethylation in PTEN promoter was actually pretty common in T2DM subjects, indicating that PTEN might contribute to T2DM pathogenesis among Uyghur people [23]. A previous research illustrated that the enhanced PTEN expression in adipose tissues as well as muscular tissues of T2DM rodents might exert a significant effect on insulin resistance in T2DM [48]. Zhu et al. proposed that PTEN controls the production of extracellular matrix in kidneys through activating Akt while enhancing CTGF in T2DM [49, 50]. In this

study, we tested the therapeutic effect of Metformin on HGC-27 cells stimulated by CagA. We found that Metformin treatment could effectively maintain CagA-induced dysregulation of PTEN promoter methylation, PTEN mRNA and protein expression, as well as the proliferation and apoptosis of HGC-27 cells.

Conclusion

In summary, these findings suggest that the hypermethylation of PTEN promoter is a common event in GC patients with DM and metformin treatment. DM may strengthen the tumorigenic effect of HP by promoting the methylation of PTEN promoter, while the administration of metformin may reduce the risk of GC by suppressing the methylation of PTEN promoter.

Declarations

Ethics approval and consent to participate

The Human Research Ethics Committees of The First Affiliated Hospital of Zhengzhou University has approved this research and all methods were performed in accordance with the last vision of the Declaration of Helsinki. Written informed consent was obtained from all patients before the study.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None

Authors' contributions

HL planned the study, HL, XH, JR and KR collected the data, ZL and QZ collected the literature, XH and JR analyzed the data, HL and QZ composed the manuscript, and all authors approved the final manuscript.

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Figures

Figure 1

Bisulfite sequencing PCR analysis showed increased DNA methylation of PTEN promoter in HP(-)DM(-) (N=39), HP(+)DM(-) (N=32) and HP(+)DM(+) (N=36) GC patients.

Figure 2

Decreased expression of PTEN mRNA in HP(-)DM(-) (N=39), HP(+)DM(-) (N=32) and HP(+)DM(+) (N=36) GC patients.

Figure 3

Decreased expression of PTEN protein in HP(-)DM(-)(N=39), HP(+)DM(-)(N=32) and HP(+)DM(+)(N=36) GC patients.

Figure 4

Immunofluorescence analysis showed that the apoptosis of gastric tissues was decreased in HP(-)DM(-) (N=39), HP(+)DM(-)(N=32) and HP(+)DM(+)(N=36) GC patients.

Figure 5

Metformin treatment decreased CagA induced up-regulation of CagA mRNA expression in HGC-27 cells (* P value < 0.05 vs. Untreated group; ** P value < 0.05, vs. CagA group).

Figure 6

Bisulfite sequencing PCR analysis showed that metformin treatment reversed CagA-induced hypermethylation of PTEN promoter.

Figure 7

Metformin maintained CagA-induced decrease of PTEN expression in HGC-27 cells. A: CagA-induced decrease of PTEN mRNA expression was effectively restored by Metformin treatment (* P value < 0.05 vs. Untreated group; ** P value < 0.05, vs. CagA group). B: CagA-induced decrease of PTEN protein expression was effectively restored by Metformin treatment.

Figure 8

Metformin restored CagA-induced dysregulation of proliferation and apoptosis of HGC-27 cells. A: CagA-induced increase of HGC-27 cell proliferation was effectively restored by Metformin treatment. B: CagA-induced attenuation of HGC-27 cell apoptosis was effectively restored by Metformin treatment (* P value < 0.05 vs. Untreated group; ** P value < 0.05, vs. CagA group).