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HDAC3-mediated lncRNA-LOC101928316 contributes to cisplatin resistance in gastric cancer via activating the PI3K-Akt-mTOR pathway

Hengjie REN, Liyun TANG*

Nursing Department, First Affiliated Hospital of Jinzhou Medical University, Jinzhou, Liaoning, China

**Correspondence: tcns312@163.com*

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The present study aimed to explore how histone deacetylases 3 (HDAC3) regulated the resistance to cisplatin by inhibiting the transcription of lncRNA-LOC101928316 (LOC101928316) in gastric cancer (GC). We revealed that HDAC3 expression in cisplatin-resistant cell lines was significantly higher than that of GC parental cell lines. Besides, knockdown of HDAC3 inhibited the cell activity, cell invasion, and migration but promoted the apoptosis of GC cisplatin-resistant cell lines. To our surprise, silencing HDAC3 inhibited the transcription of LOC101928316 by promoting the level of acetylation of H3K4 on the LOC101928316 promoter, thus promoting the LOC101 expression in GC cisplatin-resistant cell lines. Together, the overexpression of HDAC3 mediated LOC101928316 to promote GC resistance to cisplatin by activating the PI3K-Akt-mTOR pathway. Therefore, HDAC3 may serve as a potential target of cisplatin resistance in GC.

Key words: cisplatin resistance, gastric cancer, histone deacetylases 3, lncRNA-LOC101928316

Gastric cancer is a gastrointestinal tumor that is sensitive to chemotherapy [1]. Platinum drugs including cisplatin, carboplatin, and oxaliplatin are the most commonly used cycle non-specific antitumor drugs in clinical practice [2]. Although drug combination therapy had improved the overall survival rate of gastric cancer, drug resistance is still one of the main obstacles to the treatment of gastric cancer [3]. Therefore, it is crucial to study the drug resistance mechanism of gastric cancer for the treatment of gastric cancer.

Histone acetylation is a typical epigenetic method [4]. HDACs are a class of histone deacetylases. Class I HDACs including HDAC1, 2, 3, and 8 are mainly located in the nucleus and are commonly expressed in human tissues [5]. Recently, HDAC and HDAC inhibitors have been reported to be widely involved in tumor drug resistance. For instance, Wang et al. [6] found that the expression level of HDAC1 was significantly upregulated in the NSCLC cisplatin-resistant cell line and mediated the expression of TRIB1 and p53 to promote drug resistance. Moreover, HDAC inhibitors were proven to reverse the resistance to cisplatin in NSCLC. Kim et al. [7] showed that HDAC3 and HDAC2 were significantly upregulated in ovarian cancer cell lines exposed to cisplatin. Moreover, HDAC inhibitor trichostatin A exerted an anti-tumor effect through the expression profiles of miRNA in breast cancer resistant cell lines [8]. However, the effect of HDAC3 on cisplatin resistance in gastric cancer has not been studied.

lncRNA is a long-chain non-coding RNA longer than 200 nucleotides [9]. lncRNAs are widely involved in the resistance of gastric cancer to cisplatin and regulate various phenotypes of gastric cancer cells such as proliferation, migration, invasion, and apoptosis [9]. Silencing lncRNA UCA1 was proven to inhibit GC cell proliferation and promoted cisplatin-induced apoptosis by recruiting EZH2 [10]. lncRNA PCAT-1 acted as a sponge for miR-128 to contribute to GC resistance to cisplatin by regulating ZEB1 [11]. On the other hand, lncRNA ADAMTS9-AS2 was demonstrated to suppress gastric cancer resistance to cisplatin by mediating the miR-223-3p/NLRP3 axis [12]. Mechanically, most of the research in the past focused on the regulation of downstream genes by lncRNA, but its upstream regulation mechanism is needed to be further explored. Recent studies have shown that HDAC3 was involved in regulating drug resistance in a variety of tumors [7, 8, 13]. Multiple lncRNAs were proven to affect the process of gastric cancer resistance to cisplatin [7, 8, 13]. Moreover, HDAC3 epigenetically regulated lncRNA-LET expression and participated in the invasion and migration of gastric cancer cells [14]. HDAC3 mediated the process of HCC regulated by lncRNA RUNX1-IT1 [15]. Recently, lncRNA-

LOC101928316 served as a novel lncRNA to promote the process of gastric cancer resistance to cisplatin [16].

Based on the above research, the present study focused on the role of HDAC3 in the cisplatin-resistant cell lines of GC and the regulatory mechanism of HDAC3 on lncRNA. The results showed that HDAC3 inhibited the lncRNA-LOC101928316 promoter H3K4ac level to suppress the lncRNA-LOC101928316 transcription, and activated the PI3K-Akt-mTOR signaling pathway to promote cell activity, invasion, migration, and apoptosis of the cisplatin-resistant cell lines. Our study will provide a theoretical basis for HDAC3 in the treatment of gastric cancer chemotherapy resistance.

Materials and methods

Cell cultures. Human gastric cancer cell lines (AGS and BGC-823) and cisplatin-resistant cell lines (AGS/DDP and BGC-823/DDP) were all purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. All cells were cultivated in DMEM/RPMI-1640 medium (Boguang Biotechnology, Shanghai, China) supplemented with 10% fetal bovine serum (Sanger Biotechnology, Shanghai, China) and the culture environment was set at 37 °C with 5% CO_2.

RT-qPCR assays and cell transfection. We first used TRIzol reagent (Grammar Biotechnology, Shanghai, China) to extract total RNA from GC cell lines and GC cisplatin-resistant cell lines. Subsequently, 200 ng of total RNA was extracted from total RNA by using a first-strand cDNA synthesis kit. Besides, Nanodrop 2000 (Aolu Biotechnology, Shanghai, China) was applied to measure the concentration and quality of RNA. Power SYBR Green PCR master mix (Unicom Biotechnology, Shanghai, China) was used to determine real-time quantitative PCR (RT-qPCR). When analyzing the data, we applied the $2^{-\Delta\Delta Ct}$ method to calculate the experimental data. GAPDH served as an internal control. All interfering RNAs (siRNAs) targeting HDAC3 and LOC101928316 (si-HDAC3, si-LOC101928316, and LV-LOC101928316) or negative control (si-NC and LV-NC) were designed and constructed by Jima Pharmaceutical Technology, Shanghai, China. All primers were described as follows: HDAC3 forward (F) 5'-CCTGGCATTGACCCATAGCC-3' and reverse (R) 5'-CTCTTGGTGAAGCCTTGCATA-3'; LOC101928316 F 5'-AACAACGGGGACATTAGG-3' and R 5'-AACTGGAAACATCACATAGCA-3'; PI3K F 5'-GAAGTTGCTCTACCAGTGTCC-3' and R 5'-GATAGCCGTTCTTCATTTGG-3'; AKT F 5'-ACTCATTCCAGACCCACGAC-3' and R 5'-AGCCCGAAGTCCGTTATCTT-3'; mTOR F 5'-ACTGCTTTGAGGTCGCTATGA-3' and R 5'-TTGCCTTTGGTATTTGTGTCC-3'; GAPDH F 5'-ACCACAGTCCATGCCATCAC-3' and R 5'-TCCACCACCCTGTTGCTGTA-3'.

MTT. We utilized Counting Kit-8 (CCK-8, Kulaibo Technology, Beijing, China) for cell viability analysis. Briefly, we first seeded 5,000 cells/well in 96-well plates and then treated them with cisplatin at different concentration gradients (0, 1.25, 2.5, 5, 10, 20 μg/ml) for 24 h. Finally, the cell viability of GC cell lines and GC cisplatin-resistant cell lines were measured by the absorbance at 450 nm.

Transwell assay. We firstly inoculated GC cells and cisplatin-resistant cells into the upper layer of the Transwell chambers (Xinshengyuan Biomedical Technology, Beijing, China) at a density of 5×10^4 cells. Medium containing 20% fetal calf serum (FBS) was added to the lower compartment. After incubating all cells for 24 h under standard conditions, the invading cells were then fixed in methanol and stained with 0.1% crystal violet to visualize the cells. Finally, we observed and took pictures under an optical microscope.

ChIP. We utilized the Upstate Biotechnology ChIP kit (Yubo Biology, Shanghai, China) to perform chromatin immunoprecipitation (ChIP). Briefly, we first fixed the tissue in 1% formaldehyde and sheared the cell lysate by sonication in 1% sodium dodecyl sulfate lysate in order to obtain stains with an average length of 200 to 1,000 bp Quality fragment. HDAC3 (Abcam), H3K14ac (Haoran Biology, Shanghai, China), and equivalent amounts of isotype control IgG (Bohan Biological Technology, Shanghai, China) specific antibodies were then used to immunoprecipitate the chromatin overnight at 4 °C. Finally, the input DNA and DNA levels in the complex were analyzed by RT-PCR to quantify the enrichment.

Western blot assays. Western blot experiments were used to measure protein levels in different cell lines. Briefly, RIPA lysis buffer (Pulilai Gene Technology, Beijing, China) was first applied to obtain the total protein from the cells, and we used 10% SDS-PAGE gel (Biolab Technology, Beijing, China) and transfer the same amount of protein to the PVDF membrane. The membrane was then blocked with 5% skimmed milk for 2 h, the antibody protein (BSA) was incubated with 5% bovine serum at 4 °C for 12 h, the peroxidase-labeled secondary antibody (1:2500, cat. no. ab150077, Abcam, MA, USA) was used for 1 h. The antibodies used in this study were as follows: anti-HDAC3 (1:5000, cat. no. ab32369, Abcam, MA, USA), anti-β-actin (1:15000, cat. no. ab6276, Abcam, MA, USA), anti-PI3K (1:100, cat. no. ab32089, Abcam, MA, USA), anti-phospho-PI3K (1:500, cat. no. ab278545, Abcam, MA, USA), anti-Akt (1:500, cat. no. ab8805, Abcam, MA, USA), anti-phospho-Akt (1:1000, cat. no. ab38449, Abcam, MA, USA), anti-mTOR (1:10000, cat. no. ab134903, Abcam, MA, USA), anti-phospho-mTOR (1:5000, cat. no. ab109268, Abcam, MA, USA). Finally, ECL reagent (Xinshengyuan Biomedical Technology, Beijing, China) was applied to observe the immunoblot, and ImageJ software (NIH, Bethesda, MD, USA) was used to check the gray value of the band.

Cell apoptosis assay. Briefly, the apoptosis rate was measured by using Annexin V-FITC/Propidium Iodide (PI) double-stain kit (BD Bioscience, USA) based on the manufacturer's instructions. Finally, flow cytometry (FCM) (Thermo Fisher Scientific, USA) was applied to examine the apoptosis rate.

Statistical analysis. All experimental data were expressed as mean ± standard deviation. Methodologically, we used a t-test to compare between two groups and applied analysis of variance to compare between multiple groups. Statistical analysis was performed by using SPSS 15.0 statistical software (SPSS IBM, Armonk, NY USA). Besides, $p<0.05$ was considered statistically significant.

Results

HDAC3 was significantly overexpressed in cisplatin-resistant GC cell lines. To explore the role of HDAC3 in gastric cancer cisplatin resistance, we used qRT-PCR to detect the expression level of HDAC3 in cisplatin-resistant cell lines and parental cells. As a result, HDAC3 was significantly upregulated in AGS/DDP and BGC-823/DDP cells, the mRNA level was close to 3-fold compared to AGS and BGC parental cells. Moreover, the expression level of HDAC3 in GC cells was also significantly higher than that in gastric mucosal epithelial cells (GES-1) (Figure 1A). The results of western blot also confirmed that HDAC3 protein level was elevated in drug-resistant GC cell lines (Figure 1B). Subsequently, the expression of HDAC3 in AGS/DDP and BGC-823/DDP cells decreased significantly after transfection of si-HDAC3 (Figures 1C, 1D). Meanwhile, we compared the cell viability of AGS/DDP and BGC-823/DDP cells transfected with si-NC and si-HDAC3 at different concentrations (0, 1.25, 2.5, 5, 10, 20 μg/ml). As a result, high concentration inhibited cell viability of GC cisplatin-resistant cell line. More importantly, knocking down HDAC3 suppressed cell viability of cisplatin-resistant cell lines compared with transfection of the si-NC group (Figures 1E, 1F). The above results suggested that HDAC3 was significantly upregulated in drug-resistant GC cell lines and may enhance the resistance of GC to cisplatin.

Low expression of HDAC3 significantly suppressed the invasion, migration but promoted apoptosis of GC cisplatin-resistant cell lines. To further investigate the biological function of HDAC3 on gastric cancer cisplatin-resistant cell lines, Transwell assay and flow cytometry were applied to examine the effects of si-HDAC3 transfection on the invasion, migration, and apoptosis of cisplatin-resistant cells. The results showed that the cell migration of AGS/DDP and BGC-823/DDP cells was significantly lower than that of the control group after si-HDAC3 transfection (Figures 2A, 2B). Moreover, after knocking down HDAC3, the cell invasion of AGS/DDP and BGC-823/DDP cells was significantly reduced (Figures 2C, 2D). However, the silencing of HDAC3 obviously promoted the apoptosis of AGS/DDP and BGC-823/DDP cells (Figures 2E, 2F).

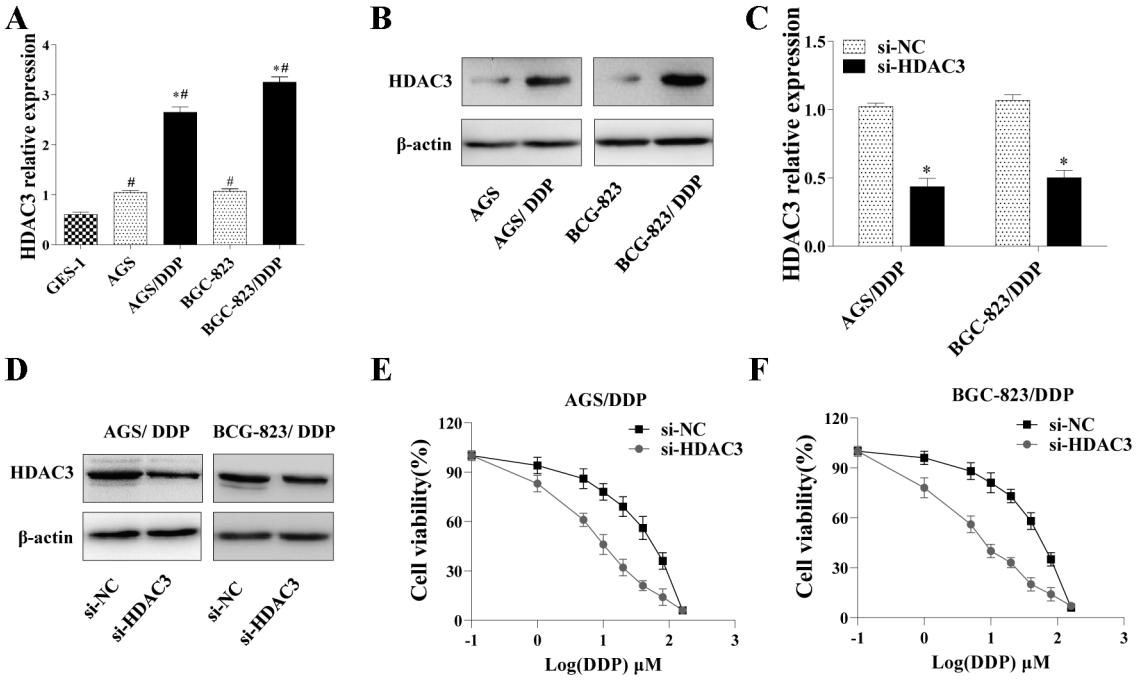

Figure 1. HDAC3 was significantly overexpressed in cisplatin-resistant GC cell lines. A, B) The mRNA and protein levels of HDAC3 in GC parental cell lines (AGS and BGC-823) and GC cisplatin-resistant cell lines (AGS/DDP and BGC-823/DDP) were detected by RT-qPCR and western blot. C, D) Transfection of si-HDAC3 significantly inhibited the mRNA and protein level of HDAC3 in GC cisplatin-resistant cell lines. E, F) Knockdown of HDAC3 suppressed the cell viability of GC cisplatin-resistant cell lines. *p<0.05. Abbreviations: HDAC3-Histone deacetylase 3; GC-Gastric cancer; DDP-Cisplatin; RT-qPCR-Real-time quantitative PCR

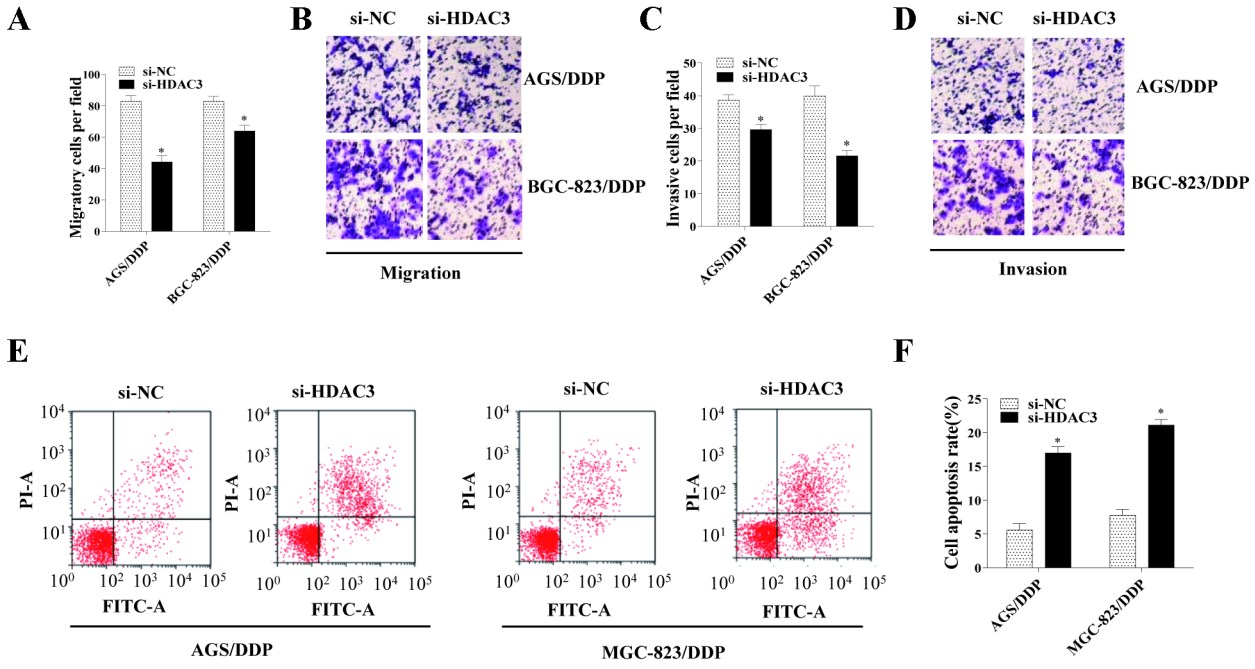

Figure 2. Low expression of HDAC3 significantly suppressed the invasion, migration but promoted apoptosis of GC cisplatin-resistant cell lines. A, B) Histogram and representative images characterized the level of migratory cells in GC cisplatin-resistant cell lines. C, D) Knocking down HDAC3 significantly suppressed the level of cell migration in GC cisplatin-resistant cell lines. E, F) Silencing HDAC3 promoted apoptosis of GC cisplatin-resistant cell lines. *p<0.05. Abbreviations: HDAC3-Histone deacetylase 3; GC-Gastric cancer; DDP-Cisplatin; RT-qPCR-Real-time quantitative PCR

HDAC3 inhibited the transcription of LOC101928316 by inhibiting the H3K14 acetylation of the LOC101928316 promoter in GC cisplatin-resistant cell lines. To explore the effect of knockdown of HDAC3 on the expression of lncRNA in GC cisplatin-resistant cells, we detected the RNA-Seq of lncRNA expression profile under transfection of si-HDAC3 and si-NC. As a result, lncRNA LOC101928316 was identified to increase the most after knocking down HDAC3 (Figure 3A). The high acetylation level of gene promoter was one of the essential signs of high gene transcription. Besides, HDAC3 has been reported to suppress lncRNA transcription by inhibiting lncRNA promoter acetylation. To our surprise, the result of the ChIP experiment confirmed that transfection of HDAC3 significantly reduced the enrichment of HDAC3 in the LOC101928316 promoter. We found that after silencing HDAC3, the expression level of HDAC3 recruited into the promoter region of LOC101928316 in AGS/DDP and BGC-823/DDP cells decreased significantly (Figure 3B). More importantly, transfection of si-HDAC3 significantly downregulated the H3K14ac enrichment of the LOC101928316 promoter compared with transfection of si-NC in AGS/DDP and BGC-823/DDP cells. Knocking down HDAC3 increased the expression level of H3K14ac in the LOC101928316 promoter region (Figure 3C). This suggested that knocking down HDAC3 promoted the transcription of LOC101928316 by increasing the acetylation level of H3K14 in the LOC101928316 promoter. The further experiment also proved that the overexpression of HDAC3 significantly downregulates the expression level of LOC101928316 in GC drug-resistant cell lines (Figures 3D, 3E). We also found that the expression level of LOC101928316 in GC drug-resistant cell lines was also downregulated in comparison with parental cells (Figure 3F). All these evidences indicated that HDAC3 could suppress LOC101928316 expression by decreasing LOC101928316 promoter acetylation level in GC cisplatin-resistant cells.

Overexpression of LOC101928316 deactivated the PI3K-Akt-mTOR signaling pathway in GC cisplatin-resistant cell lines. LncRNAs play an important role in the process of gastric cancer resistance. It has been reported that low expression LOC101928316 was involved in the progression of GC by activating the PI3K-Akt-mTOR signaling pathway [16]. Therefore, we focused on the relationship between LOC101928316 and PI3K-Akt-mTOR signaling pathway in gastric cancer drug resistance. The expression of LOC101928316 in AGS/DDP and BGC-823/DDP cells was significantly upregulated after the transfection with LV-LOC101928316 (Figure 4A). To our surprise, western blot indicated that p-PI3K, p-Akt, and p-mTOR protein levels in AGS/DDP and BGC-823/DDP cells suppressed significantly after transfection of LV-LOC101928316, while PI3K, Akt, and mTOR did not change significantly (Figures 4B–4D).

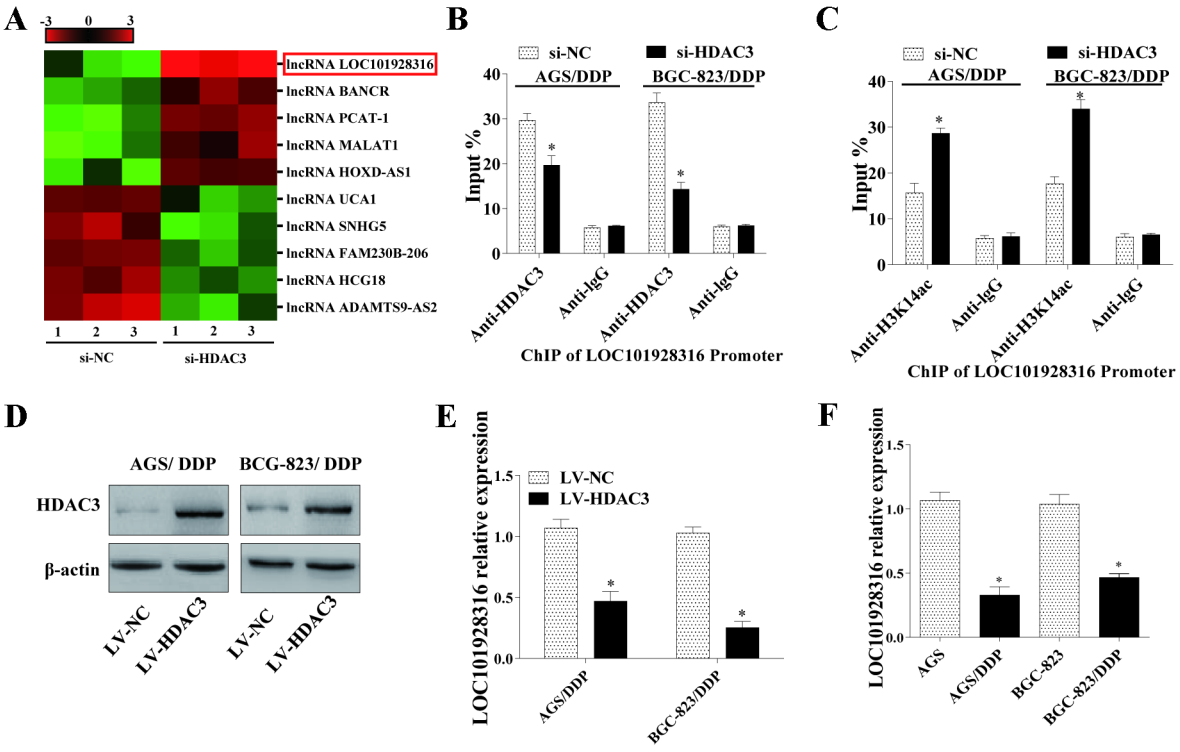

Figure 3. HDAC3 inhibited the transcription of LOC101928316 by inhibiting the H3K14 acetylation of the LOC101928316 promoter in GC cisplatin-resistant cell lines. A) RNA-Seq of lncRNA expression profile in three GC drug-resistant cell lines samples was detected after transfection of si-HDAC3 and si-NC. B) Knockdown of HDAC3 suppressed the recruitment of HDAC3 on LOC101928316 promoter in AGS/DDP and BGC-823/DDP cells. C) Silencing HDAC3 downregulated H3K14ac levels in the promoter region of LOC101928316. D, E) The effect of overexpressing HDAC3 on the expression level of LOC101928316 in AGS/DDP and BGC-823/DDP cells was measured by RT-qPCR. F) LV-LOC101928316 expression level in GC cisplatin-resistant cell lines was detected. *p<0.05. Abbreviations: HDAC3-Histone deacetylase 3; GC-Gastric cancer; DDP-Cisplatin; RT-qPCR-Real-time quantitative PCR

The above results indicated that the overexpression of LOC101928316 deactivated the PI3K-Akt-mTOR signaling pathway in GC cisplatin-resistant cell lines.

Silencing LOC101928316 reversed the effect of knocking down HDAC3 on GC cisplatin-resistant cell lines. To further verify that HDAC3 was involved in the process of GC cisplatin resistance by mediating LOC101928316, we compared the phenotypic differences of three groups of GC resistant cell lines co-transfected with si-NC, si-HDAC3, si-HDAC3, and si-LOC101928316. The results showed that transfection with si-LOC101928316 significantly inhibited the expression of LOC101928316 in AGS/DDP and BGC-823/DDP cells (Figure 5A). Meanwhile, silencing LOC101928316 reversed the inhibitory effect of knocking down HDAC3 on the cell viability of AGS/DDP and BGC-823/DDP cells (Figures 5B, 5C). Moreover, the results of apoptosis, invasion, and migration experiments also showed similar patterns, transfection of si-LOC101928316 reversed the effect of knockdown of HDAC3 on cisplatin-resistant cell lines (Figures 5D–5F). The above results indicated that silencing HDAC3 negatively mediated LOC101928316 to suppress the process of GC cisplatin resistance.

Wortmannin (PI3K-Akt-mTOR pathway inhibitor) further enhanced the effect of overexpressing LOC101928316 on GC drug-resistant cell lines. We also verified that LOC101928316 regulates the development of cisplatin resistance in GC by inhibiting the PI3K-Akt-mTOR signaling pathway. The results showed that Wortmannin (PI3K-Akt-mTOR inhibitor) further enhanced the inhibitory effect of overexpression of LOC101928316 on cell viability of cisplatin-resistant cell lines (Figures 6A, 6B). Furthermore, Wortmannin further promoted the effect of overexpression of LOC101928316 on apoptosis, invasion, and migration of cisplatin-resistant cell lines (Figures 6C–6E). Together, overexpression of LOC101928316 inhibited the process of GC cisplatin resistance by inactivating the PI3K-Akt-mTOR pathway.

Overexpression of HDAC3 inhibited the transcription of LOC101928316 to promote gastric cancer resistance to cisplatin by activating the PI3K-Akt-mTOR pathway. In summary, we explored the biological function and potential mechanism of HDAC3 in GC resistance to cisplatin. HDAC3 was proven to be significantly upregulated in cisplatin-resistant cell lines. Besides, highly expressed HDAC3 suppressed

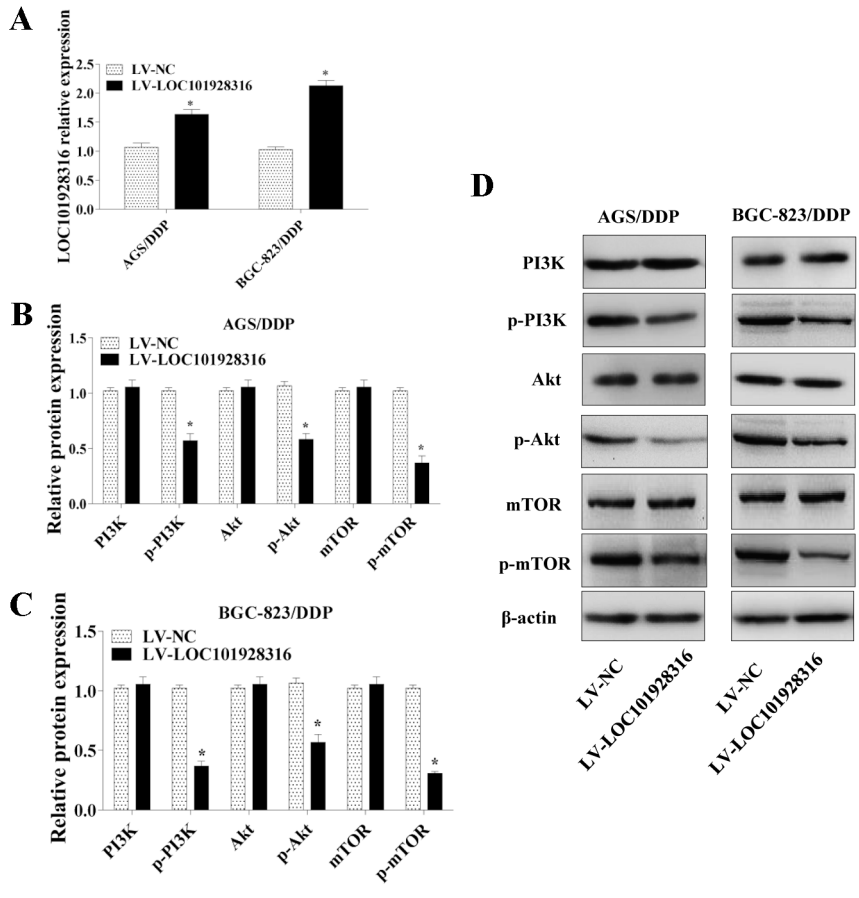

Figure 4. Overexpression of LOC101928316 deactivated the PI3K-Akt-mTOR signaling pathway in GC cisplatin-resistant cell lines. A) Transfection of LV-LOC101928316 inhibited LOC101928316 expression in AGS/DDP and BGC-823/DDP cells. B–D) The histogram and western blot images suggested that the overexpression of LOC101928316 significantly inhibited the protein levels of p-PI3K, p-Akt, and p-mTOR in AGS/DDP and BGC-823/DDP cells. *p<0.05. Abbreviations: LOC101928316-lncRNA LOC101928316; GC-Gastric cancer; DDP-Cisplatin; RT-qPCR-Real-time quantitative PCR

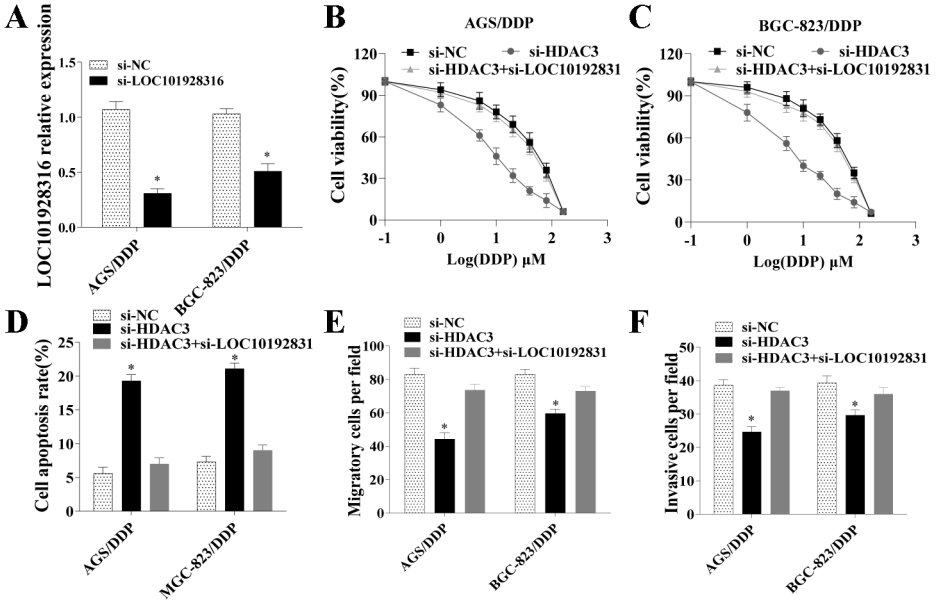

Figure 5. Silencing LOC101928316 reversed the effect of knocking down HDAC3 in GC cisplatin-resistant cell lines. A) Transfection with si-LOC101928316 decreased the expression level of LOC101928316 in GC cisplatin-resistant cell lines. B, C) Silencing LOC101928316 reversed the inhibitory effect of HDAC3 knockdown on cell viability. D–F) Knocking down LOC101928316 reversed the effect of silencing HDAC3 on apoptosis, migration, and invasion of GC cisplatin-resistant cell lines. *p<0.05, #p<0.05. Abbreviations: LOC101928316-lncRNA LOC101928316; GC-Gastric cancer

LOC101928316 transcription by inhibiting H3K14 acetylation of the LOC101928316 promoter, thereby activating the PI3K-Akt-mTOR pathway to promote cell viability, invasion, migration, and apoptosis inhibition of cisplatin-resistant cells (Figure 7).

Discussion

Gastric cancer is a common tumor with high morbidity and mortality [17]. The treatment of gastric cancer mainly includes surgery and chemotherapy [18]. Chemotherapy drugs mainly contain platinum-based drugs such as cisplatin or fluorouracil, and gastric cancer resistance to platinum-based drugs such as cisplatin had become a problem that hindered the treatment of patients [18]. Therefore, it was very urgent and necessary to study the resistance of gastric cancer to cisplatin.

HDAC, as a class of histone deacetylase, was reported to be abnormally expressed in different tumor-resistant cells [19–21]. HDAC3 was shown to be significantly upregulated in ovarian cancer cisplatin-resistant cell lines compared to parental cells [19–21]. The expression level of HDAC in NSCLC cells was significantly upregulated after transfection with cisplatin, and HDAC inhibitors showed anti-tumor drug resistance in NSCLC [6]. Our study suggested that HDAC3 was highly expressed in GC cisplatin-resistant cell lines (AGS/DDP and BGC-823/DDP) compared with parent cells. We also studied the effect of knocking down HDAC3 on the biological function of GC cisplatin-resistant cell lines. The results showed that silencing HDAC3 significantly inhibited the cell activity, invasion, migration, and promote apoptosis of cisplatin-resistant cells. This meant that HDAC3 was highly expressed in the GC cisplatin-resistant cell lines and promoted the resistance of GC to cisplatin.

LncRNA, as a star molecule for cancer suppression or anti-cancer, not only participated in the phenotypes of tumor metastasis and invasion but also profoundly affected the resistance of tumors to cisplatin [22–24]. High acetylation of gene promoters was one of the essential signs of transcriptional activation. As a deacetylation transferase, HDAC3 was proven to significantly downregulate the acetylation levels of gene promoters, thereby suppressing gene transcription [10, 11, 14–16]. RNA-Seq of lncRNA expression profile was measured in the GC cisplatin-resistant cell lines under transfection of si-NC and si-HDAC3, and LOC101928316 was demonstrated to increase the most by si-HDAC3 transfection. Mechanistically, the results of ChIP showed that after knocking down HDAC3, the level of acetylation of H3K14 on the LOC101928316 promoter decreased significantly. The above results indicated that HDAC3 negatively regulated the expression of LOC101928316 by inhibiting the acetylation level of the LOC101928316 promoter. This was consistent with the studies of Mao et al. and Sun et al. [14, 15]. The studies showed that HDAC3 could mediate the expression of lncRNA LET and lncRNA RUNX1-IT1 by inhibiting the deacetylation of the lncRNA promoter.

Subsequently, we analyzed the effect of overexpression of LOC101928316 on the biological function of GC cisplatin-resistant cells. Results revealed that the overexpression of LOC101928316 suppressed the migration, invasion,

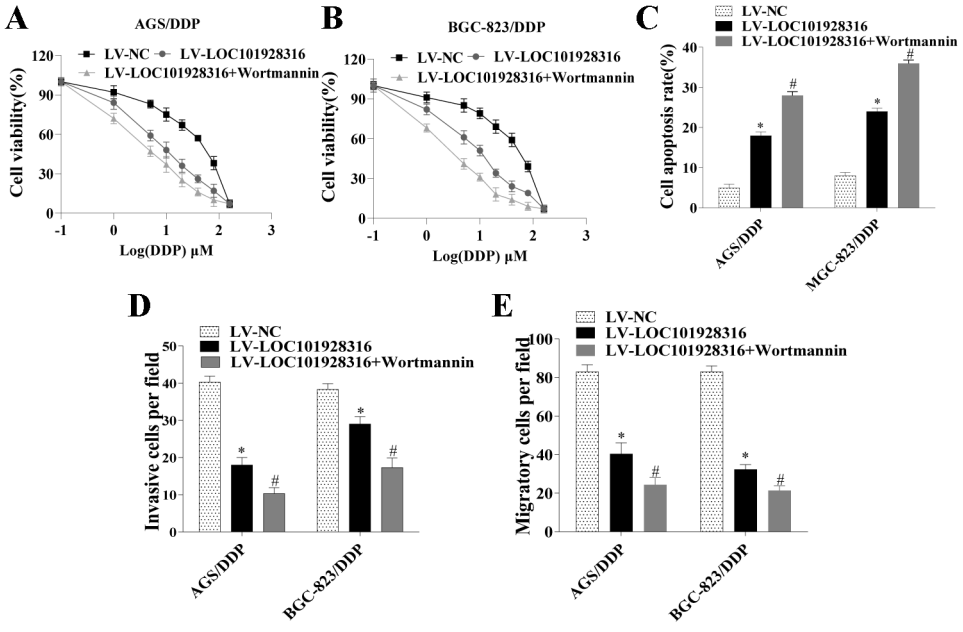

Figure 6. Wortmannin (PI3K-Akt-mTOR pathway inhibitor) further enhanced the effect of overexpressing LOC101928316 on GC drug-resistant cell lines. A–E) Wortmannin further promoted the effect of overexpression of LOC101928316 on the activity, apoptosis, invasion, and migration of GC drug-resistant cell lines. *p<0.05, #p<0.05. Abbreviations: LOC101928316-lncRNA LOC101928316; GC-Gastric cancer

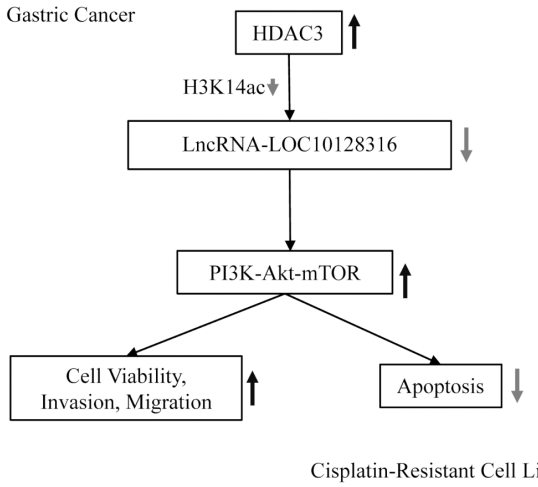

Figure 7. Overexpression of HDAC3 inhibited the transcription of LOC101928316 to promote gastric cancer resistance to cisplatin by activating the PI3K-Akt-mTOR pathway.

and promoted apoptosis of GC cisplatin-resistant cells by inhibiting the PI3K-Akt-mTOR activation. This was consistent with the study by Li et al. [16]. LOC101928316 served as a tumor suppressor gene to affect the progress of GC by activating the PI3K-Akt-mTOR pathway. Further experiments verified that silencing LOC101928316 reversed the effect of knocking down HDAC3 on GC cisplatin-resistant cell lines. Furthermore, Wortmannin (PI3K-Akt-mTOR inhibitor) further promoted the effect of overexpressing of LOC101928316 on GC cisplatin-resistant cell lines. The above results confirmed that HDAC3 mediated LOC101928316 to affect the process of GC resistance to cisplatin by activating the PI3K-Akt-mTOR pathway.

Taken together, we first discovered the role of HDAC3 in cisplatin resistance in gastric cancer. Mechanistically, HDAC3 reduced the recruitment of H3K14ac by inhibiting the acetylation of the LOC101928316 promoter, thereby inhibiting the transcription of LOC101928316. This will enrich epigenetic regulatory mechanisms, especially for histone acetylation-mediated abnormal expression of lncRNA in tumors. However, there were still some shortcomings in this study. First, the biological functions of HDAC3 need to be verified by *in vivo* animal experiments. Secondly, other histone acetylases such as HAT or HDAC family were not discussed. Overall, the downregulation of LOC101928316 by HDAC3 promoted gastric cancer resistance to cisplatin by activating the PI3K-Akt-mTOR pathway. We are convinced that HDAC3 will play an extremely important role in the treatment of gastric cancer resistance.

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