lncRNA ABHD11-AS1 drives gastric cancer development by targeting miR-1301-3p/PDPK1 signaling

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Abstract

Background Long noncoding RNAs (lncRNAs) has long been reported to associate with multitudinous kinds of cancer. The ABHD11-AS1, a newly identified lncRNAs, has been demonstrated as a new marker of gastric cancer (GC). In this study, a significant upregulation of ABHD11-AS1 was observed in GC, compared to adjacent normal tissues. Method Immunohistochemical analysis for ABHD11-AS1, 3-phosphoinositide dependent protein kinase 1 (PDPK1), miR-1301-3p was performed on gastric tumor and adjacent normal tissues. The gene expression was evaluated by qRT-PCR. The proliferation was assayed by CCK8 and Annexin V/PI assay. Results ABHD11-AS1 is highly expressed in GC tissues compared with adjacent normal tissues(*p<0.01) Knock-down of ABHD11-AS1 could suppress the aberrant proliferation of GC cells compared with si-Scramble(*p<0.01). LncRNA-ABHD11-AS1 served as a sponge for miR-1301-3p in GC cell. miR-1301-3p/ABHD11-AS1 regulated the expression of PDPK1 in GC cells compared with control *p<0.01). miR-1301-3p/ ABHD11-AS1 co-mediated proliferation and apoptosis of GC cell compared with si-Scramble/Mock and si-ABHD11-AS1/Mock (*p<0.01) Conclusion PDPK1 was a downstream target of miR-133a-3p and ABHD11-AS1 subsequently exerted its biological effects via modulating the expression of PDPK1in gastric cancer cells. Key lncRNA,ABHD11-AS1, gastric cancer, miR-1301-3p, PDPK1

Background

Gastric cancer(GC) is one kind of common tumors with high morbidity around the world[1]. Although great progress has been made in diagnostic methods as well as treatments for GC, there are still a growing number of GC patients with poor prognosis[2-4]. Even worse, a large proportion of patients with GC remain undiagnosed until late stage which is incurable. The pathogenic mechanism mediating the fast proliferation of GC is still not fully understood yet. Long noncoding RNAs (lncRNAs), comprising more than 200 nucleotides and belonging to noncoding RNAs, play a vital role in physical progress and in many human diseases[5, 6]. Aberrant expression of lncRNAs has been reported to mediate the initiation and metastasis of tumor, which can also serve as a prognostic marker of many kinds of disease.[7-9].

LncRNA ABHD11-AS1, known as long noncoding RNA ABHD11 Antisense RNA 1, has been reported to associate with the oncogenesis and metastasis of gastric tumor[6, 10]. The expression of ABHD11-AS1 was significantly increased in tumor tissues compared with adjacent normal tissues. miRNAs are a group of automatically occurring single-stranded short 21-23 nt non-coding RNAs, which exist in eukaryotic organisms[11, 12]. miRNA can prevent the transcription of targeted mRNAs and by which the expression of target gene is inhibited[13, 14]. LncRNAs can serve as miRNA sponges, diminishing the regulatory effects of miRNAs on mRNAs[15, 16]. 3-phosphoinositide dependent protein kinase 1 (PDPK1) is a serine/threonine kinase with many substrates including PKA, AKT, PKC-zeta, and p90S6K, functioning as a major control point by activating a rang of signaling pathways involved in cellular proliferation and apoptosis[17, 18]. The downstream molecules of PDPK1 are usually hyper-activated in many kinds of cancer cells and the overexpression of PDPK1 has been observed in a many kinds of human cancer cell lines, including GC[19-21]. Therefore, PDPK1 is expected to function as an oncogenic protein.

In this study, we demonstrated that the overexpression of ABHD11-AS1 could serve as a clini-copathological factor for GC patients. We pioneeringly identified that miR-1301-3p acts as a key mediator for the regulation of ABHD11-AS1 on the expression of PDPK1.

Methods

2.1 Patients and tissue samples

GC tissues and adjacent normal tissues were collected from 120 volunteers at jiangshu Province People's Hospital from January to December,2017. Each specimen was snap-frozed in liquid nitrogen and stored temporarily in a −80℃ freezer before RNA isolation and qRT-PCR analysis. All specimens were collected before patients received either chemotherapy or radiotherapy. Written informed consents were obtained from all patients for the agreement regarding the use of tissue samples in our research. Selection bias of GC samples collection in our study was avoided.

2.2 Cell culture and cell transfection

All of the GC cell lines (RGM-1, MGC-803, BGC-823, SGC-7901, MKN-28, AGS) and normal human gastric epithelial cell (GES-1) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM (Gibco BRL) medium, supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). All cells were cultured in a humidified chamber at 37℃ with 5% CO2.

SiRNA were obtained from Sigma-Aldrich (Sigma). miR-1301-3p mimics, miR-133a-3p inhibitor and the negative control were all purchased from GenePharma (Shanghai, China). Cells were transfected with Lipofectamine 3000 (Life Technologies) according to the manufacturer's guide. The ABHD11-AS1 sequences were as follows:

CCCAGAGTACCAGGCGGCTGGAGCACTGGGGGACACCCGGACAGAACCCTCCCTGGACCAAGTCCTCCAGGAACGGGATGAAGCCATTGCCAAGAAGCAGGCGGTGGA

2.3 Quantitative real-time PCR (qRT-PCR) assay

Total RNA from tissues and cells was extracted by TRIzol reagent (InvitrogenCarlsbad,CA) and cDNA was reverse-transcripted using SuperScript First-StrandSynthesis System (Invitrogen) according to the manufacturer's direction. Quantitative RT-PCR was conducted with ABI 7500 system (Applied

Biosystems, CA, USA) to measure the relative expression of target genes. GAPDH or U6 was used as the internal normalizer for target genes or miRNA, respectively. All the primers were designed and synthesized by Shanghai Sheng gong Biotechnology (Shanghai). The primer sequences were as follows:

GAPDH,F:TCGGAGTCAACGGATTTGGT;R:TTCCCGTTCTCAGCCTTGAC

U6, F: CTCGCTTCGGCAGCACA; R: AACGCTTCACGAATTTGCGT

2.5 Cell proliferation assay

CCK8 assay was used to measure the cell proliferation activity by a cell counting kit (CCK8, MedChem Express) kit according to manufacturer's recommend protocol.

2.6 Colony formation assay

For clonogenic assay, 200 cells per well of CRC cells were seeded in six-well plates and incubated for 2 weeks until visible colonies formed (cell number more than 50). Colonies were washed, fixed with 4% formaldehyde, stained with 0.1% crystal violet, and counted.

2.7 Cell apoptosis detection assay

For cell apoptosis, active caspase 3 human ELISA kit (Invitrogen) was used to evaluate the cell apoptosis according to the manufacture's recommended protocol. Annexin V/PI assay was performed to measure the apoptosis percent of GC. The procedure was the same as the previous description

2.8 Reporter vectors and dual-luciferase reporter assay

The putative miR-133a-3p target binding sequence in ABHD11-AS1 and its mutant of the binding sites were amplified by PCR and the PCR products were cloned into pMir Reporter plasmid (shanghaishenggong, shanghai, china) to generate the ABHD11-AS1-wild-type(WT-ABHD11-AS1) vector and ABHD11-AS1-mutated-type (MUT-ABHD11-AS1) vector. Subsequently, mutated or wild-type pMir Reporter luciferase vectors and miR-1301-3p mimic or NC-mimic were co-transfected into MGC803 and BGC823 cell lines in 96-well plates for 24 h with Lipofectamine 3000 (Invitrogen,Carlsbad,CA,). The luciferase activity was detected by the luciferase reporter assay system (Promega) according to the manufacture's protocol.

2.9 RNA immunoprecipitation (RIP) assay

We utilized the Magna RIP RNA-binding protein immunoprecipitation kit (Sigma) to perform RIP assay according to the manufacturer's recommended procedure. Cells were lysed by RIP lysis buffer. Then, whole cell lysate was co-incubated with RIP immunoprecipitation buffer containing magnetic beads conjugated with human anti-Argonaute2 (Ago2) antibody (Sigma), and negative control normal mouse IgG (Sigma). Immunoprecipitated RNA was acquired and then the expression levels of miR-133a-3p and ABHD11-AS1, which emerged in the sediments, was analyzed by qRT-PCR analysis.

2.10 Western blot analysis

Cells were collected and lysed with RIPA lysis buffer supplemented with proteinase inhibitors on ice (Roche). And then, the lysates were collected and treated with ultrasonication and centrifugation at 15000 rpm for 8 min. The supernatants of cell specimen were the kept as purified target protein. And target proteins were seperated by electrophoresis in a 10% sodium-dodecyl sulfate polyacrylamide gel (SDS-PAGE) and blotted onto PVDF membrane. After blocking with 5% BSA buffer for 1h, the blots were incubated with primary antibodies for 2 h at room temperature. The primary antibodies used in this study included β-actin antibody (Rabbit, Sigma) and PDPK1 antibody (Rabbit, Cell Signaling Technology). After subsequent incubation with secondary antibody, the bands were scanned by using LI-COR-Odyssey scanner (LI-COR). Signal intensity of bands were further analyzed by Image J software.

2.11 Statistical methods

All the statistical analyses were performed with GraphPad Prism 5.0. The data are presented as the means ± standard deviation (S.D.). Student's t-test or one-way analysis of variance (ANOVA) were used to analyze the difference between groups for expression of apoptosis percent, colony formation, cell proliferation, target gene. The log-rank test and Kaplan–Meier method was conducted to evaluate PFS or OS. two-tailed P-values less than 0.05 were considered statistically significant. (*P <0.05, **P <0.01, ***P <0.01).

Results

3.1 ABHD11-AS1 is highly expressed in GC tissues

To prove oncogenic role of lncRNA ABHD11-AS1 in GC, we firstly analyzed the relationship between the expression level of ABHD11-AS1 and survival duration of GC patients in open databases (Figure1A,B). We found a worse prognosis of gastric cancer patients was correlated with a higher level of ABHD11-AS1.

Based on the analysis, we further explore whether the level of ABHD11-AS1 was significantly increased in GC specimens and cell lines. Firstly, we determined the expression level of ABHD11-AS1 in GC tissues and adjacent normal tissues with RT-PCR analysis, and the gene expression was normalized to GAPDH. As expected, the expression level of ABHD11-AS1 was significantly upregulated in GC tissues compared with normal counterparts (*$p<0.01$*, Firgure 1C). Moreover, to further validate whether the expression level of ABHD11-AS1 was significantly increased in GC, we measured the expression of ABHD11-AS1 in various GC cell lines by RT-PCR analysis and normalized the gene expression to β-actin. Compared to normal gastric epithelium cell line (GES-1), two cell lines (BGC-823, MGC-803) exhibited significant higher levels of ABHD11-AS1(*$p<0.01$*,Figure1 D). Therefore, we focused on these two cell lines to explore the biological functions of ABHD11-AS1 in GC. In short, the aberrant overexpression of ABHD11-AS1 may be related to GC.

3.2 Knock-down of ABHD11-AS1 could suppress the aberrant proliferation of GC cells.

Yang et al has proved that the overexpression of ABHD11-AS1 could promote the proliferation of GC cells[22]. To explore the biological effects of ABHD11-AS1 knock-down on GC cells, we knocked-down ABHD11-AS1 with siRNA in two GC cell lines (BGC-823, MGC-803) and evaluated the effects on cell proliferation as well as apoptosis. The knock-down efficiency of ABHD11-AS1 was confirmed with RT-PCR compared with Scramble (*$p<0.01$*, Firgure2 A). CCK8 assay revealed that the reduced expression of ABHD11-AS1 significantly suppressed the cell proliferation in GC cell lines compared to the si-Scramble (*$p<0.01$*, Figure 2B,C). Likewise, colony formation assay showed that the reduced expression of ABHD11-AS1 markedly decreased the clonogenic survival percent of GC cell lines (BGC-823, MGC-803) compared to si-Scramble (*$p<0.01$*, Figure2F,G).

Meanwhile, the results of Annexin V-FITC staining assay proved that the knock-down of ABHD11-AS1 promoted the apoptosis percent of GC cell lines compared to si-Scramble (*$p<0.01$*, Figure2H,I). Since apoptosis-promoting gene Bax and enzyme caspase-3 could mediate the process of cell apoptosis[23-25], their protein levels were measured by RT-PCR and Elisa assay in our study. The results of Elisa assay suggested that inhibition of ABHD11-AS1 enhanced the level of apoptosis-promoting enzyme caspase-3 compared to si-Scramble (*$p<0.01$* ,Figure2J) and the RT-PCR analysis showed that Bax was remarkably higher in ABHD11-AS1 knock-down group comapred to si-Scramble (*$p<0.01$*, Figure2k).

In conclusion, ABHD11-AS1 could be a potential therapeutic target to suppress the growth of GC.

3.3 LncRNA-ABHD11-AS1 served as a sponge for miR-1301-3p in GC cell

Growing number of evidences have demonstrated that ABHD11-AS1 could regulate the expression of miR-133a in colorectal cancer cell (CRC). We hypothesized that another miRNA interacting with ABHD11-AS1 might exist in GC cell lines. Indeed, we noticed that the expression of miR-1301-3p was significantly upregualted after the knockdown of ABHD11-AS1 in GC cell lines (BGC-823, MGC-803) compared to si-Scramble (*$p<0.01$*,Figure3A,B). Therefore, we suspected miR-1301-3p as a potential target molecule interacting with ABHD11-AS1 in this research. To further explore whether ABHD11-AS1 functioned by sponging miRNA or acting as competing endogenous RNAs (ceRNAs), bioinformatics tools (microRNA.org and miRcode) were utilized to identify the microRNA binding sites of ABHD11-AS1(Figure 3C). qPCR assay showed miR-1301-3p expression level was significantly decreased in GC tissues compared with adjacent normal tissues (*$p<0.01$* , Figure 3D).

To determine whether ABHD11-AS directly interacted with miR-1301-3p, dual-luciferase reporter assay demonstrated that miR-1301-3p could significantly weaken the luciferase activity of the WT-ABHD11-AS1 compared with mock, while similar observation was not found for MUT-ABHD11-AS1 (*$p<0.01$,* Figure 3E,F). To further investigate whether RNA-induced silencing complex (RISC) was involved in the mutual suppression between ABHD11-AS1 and miR-1301-3p, RNA immunoprecipitation assay (RIP) were conducted with antibody against Ago2, a major component of RISC complex. ABHD11-AS1 and miR-1301-3p were remarkably higher in pellet pulled-down by Ago2 antibody in GC cell lines (BGC-823, MGC-803) compared with IgG (*$p<0.01$,* Figure 3G,H). Eventually, biotin-labeled pull-down assay further demonstrated miR-1301-3p could directly interact with ABHD11-AS1 compared with Bio-control (*$p<0.01$*,Figure3I).

3.4 miR-1301-3p/ABHD11-AS1 regulated the expression of PDPK1 in GC cells

By searching public database (starbase), Pearson correlation analysis suggested that there was a strong negative correlation between miR-1301-3p and PDPK1 expression in GC tissues (Firgure 4A). Bioinformatics analysis by TargetScan and miRBase predicted that PDPK1 had matched binding bases with PDPK1 (Figure 4B). Luciferase reporter assay revealed that the over-expression of miR-133a remarkably suppressed PDPK1 3'-UTR activity while the activity of the mutant PDPK1 3'-UTR was unaffected compared with mock (*$p<0.01$* , Figure 4C). Moreover, the up-regulation of miR-133a had a significant negative effect on the expression of PDPK1 at the levels of both mRNA and protein, compared to mock treatment (*$p<0.01$* , Figure4D,E). To determine whether ABHD11-AS1 competitively inhibited the binding of miR-1301-3p with PDPK1, luciferase reported assays were conducted. The results suggested that ABHD11-AS1 overexpression could offset the suppressing effect of miR-1301-3p on endogenous ABHD11-AS1 in GC cell lines (*$p<0.01$*,Figure H,I). In addition, q-PCR

analysis and western blot assays demonstrated that there was a mutual antagonism between miR-1301-3p and ABHD11-AS1 on binding to PDPK1 (Figure 4J,K,L). In short, miR-1301-3p/ABHD11-AS1 behave as counteract regulators on the expression of PDPK1 in GC lines.

3.5 miR-1301-3p/ ABHD11-AS1 co-mediated proliferation and apoptosis of GC cell

Finally, we showed that ABHD11-AS1 could promote GC through regulating the binding between miR-1301-3p and PDPK1 mRNA. The blockage of miR-1301-3p counteracted the inhibitor effects of ABHD11-AS1 knock down on PDPK1 expression in GC cells (*$p<0.01$,Firgure5A-C). Moreover, simultaneous suppression of miR-1301-3p and ABHD11-AS1 expression could significantly enhance the expression of PDPK1. Additionally, CCK8 assay and colony formation assay demonstrated that the negative effect of ABHD11-AS1 down-regulation on proliferation of GC cells could be neutralized by miR-1301-3p inhibitor (*$p<0.01$,Figure5D,E,F). Meanwhile, Annexin V-FITC staining assay and caspase-3 activity assay showed that miR-1301-3p knockdown could partially abolish the pro-apoptotic effect of ABHD11-AS1 down-regulation in GC cells (Figure G, H, $p<0.01$).

In conclusion, the check and balance relationship between miR-1301-3p and ABHD11-AS1 could mediate the expression of PDPK1, disorder of which may contribute to the development of GC.

Discussion

Great progress has been made in high-throughput gene sequencing analysis , which updated our comprehension that only less than 2% of human genome have been proved to be transcribed, and many non-coding RNAs with limited or no protein-coding capacity exist in human genome[26-28]. Long non-coding RNAs (lncRNAs) are known as a class of non-coding RNA tran-scripts and are associated with some key process, such as, cellar proliferation and apoptosis. Growing number of evidences have demonstrated that lncRNAs may contribute to the tumorigenesis of GC[29-31]. LncRNA ABHD11 Antisense RNA 1 (ABHD11-AS1) has been demonstrated to serve as diagnostic and prognostic bio-marker in ovarian cancer, bladder cancer, gastric cancer[6, 32, 33]. However, the underlying molecular mechanisms of ABHD11-AS1 was still elusive in GC.

In this study, we firstly showed that the expression of lncRNA ABHD11-AS1 was upregulated in GC and ABHD11-AS1 expression could be one prognostic marker for GC patients. Further functional experiments showed that the knockdown of ABHD11-AS1 suppressed the proliferation of GC cell lines (BGC-823, MGC-803). These results fully demonstrated that ABHD11-AS1 exerted oncogenic role in the development of GC, besides the overexpression of ABHD11-AS1 has been validated in other tumors. Accumulating researches have indicated that lncRNAs act as ceRNAs for miRNAs to mediate tumor occurrence[34-36]. In our study, bioinformatics analysis and RNA pull-down assay also proved that biotin-labeled ABHD11-AS1 remarkably pull-down miR-1301-3p. Moreover, a negative correlation between miR-133a and ABHD11-AS1 was identified in this study. Simultaneously, luciferase reporter assays also revealed that miR-1301-3p and ABHD11-AS1 shared same binding site. Lastly, we found an endogenous interaction between miR-1301-3p and ABHD11-AS1 by using RIP with anti-Ago2 antibody. Although the antagonism relationship between ABHD11-AS1 and miR-1301-3p has not been reported in GC cells, nevertheless, it has been reported that miR-1301-3p contributed to the expansion of prostate cancer stem cell by targeting SFRP1 and MiR-1301-3p reduced the viability of human breast cancer cell through regulating cell cycle progression and apoptosis by targeting ICT1, indicating that miR-1301-3p could serve as a tumor suppressor[37, 38]. However, increased expression of ABHD11-AS1 has been found in GC[33]. Therefore, ABHD11-AS1 may exert oncogenic effect by negatively regulating miR-1301-3p expression in GC. Then bioinformatics analysis was performed to predict the latent downstream targets of miR-1301-3p. The analysis revealed that PDPK1 may be a downstream target of miR-1301-3p. In previous studies, PDPK1 has been identified as a marker of tumor[19]. Meanwhile, we observed that ABHD11-AS1 could positively regulate the expression of PDPK1 by targeting miR-1301-3p in GC cells. Interestingly, miR-1301-3p antagonized the carcinogenic effects of ABHD11-AS1 in GC cells. In all, these results suggest that lncRNA ABHD11-AS1 promotes gastric cancer development by regulating miR-1301-3p/PDPK1 signaling.

In conclusion, our results firstly demonstrated that the overexpression of ABHD11-AS1 was correlated with a poor prognosis of GC patients. ABHD11-AS1 could serve as a ceRNA to promote PDPK1 expression by neutralizing miR-1301-3p, consequently contributing to the proliferation and apoptosis of GC. These results demonstrated that ABHD11-AS1 could be a potential therapeutic target for GC treatment.

Conclusions

PDPK1 was a downstream target of miR-133a-3p and ABHD11-AS1 subsequently exerted its biological effects via modulating the expression of PDPK1in gastric cancer cells.

Abbreviations

CCK8: Cell Counting Kit-8; ABHD11-AS1: a subtype of Long noncoding RNAs; miR-1301-4p: a subtype of miRNA; PDPK1: 3-phosphoinositide dependent protein kinase 1; ceRNA: competing endogenous RNAs.

Declarations

Ethics approval and consent to participate

The study has been approved by the ethics committee ofThe Affiliated Cancer Hospital of Nanjing Medical University

All procedures performed in our study were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.Written informed consent was obtained from all individual participants included in the study.

Consent for publication

We have obtained written consent to publish from the participants.

Availability of data and materials

Not applicable

competing interest

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Author's contributions

Not applicable

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Figures

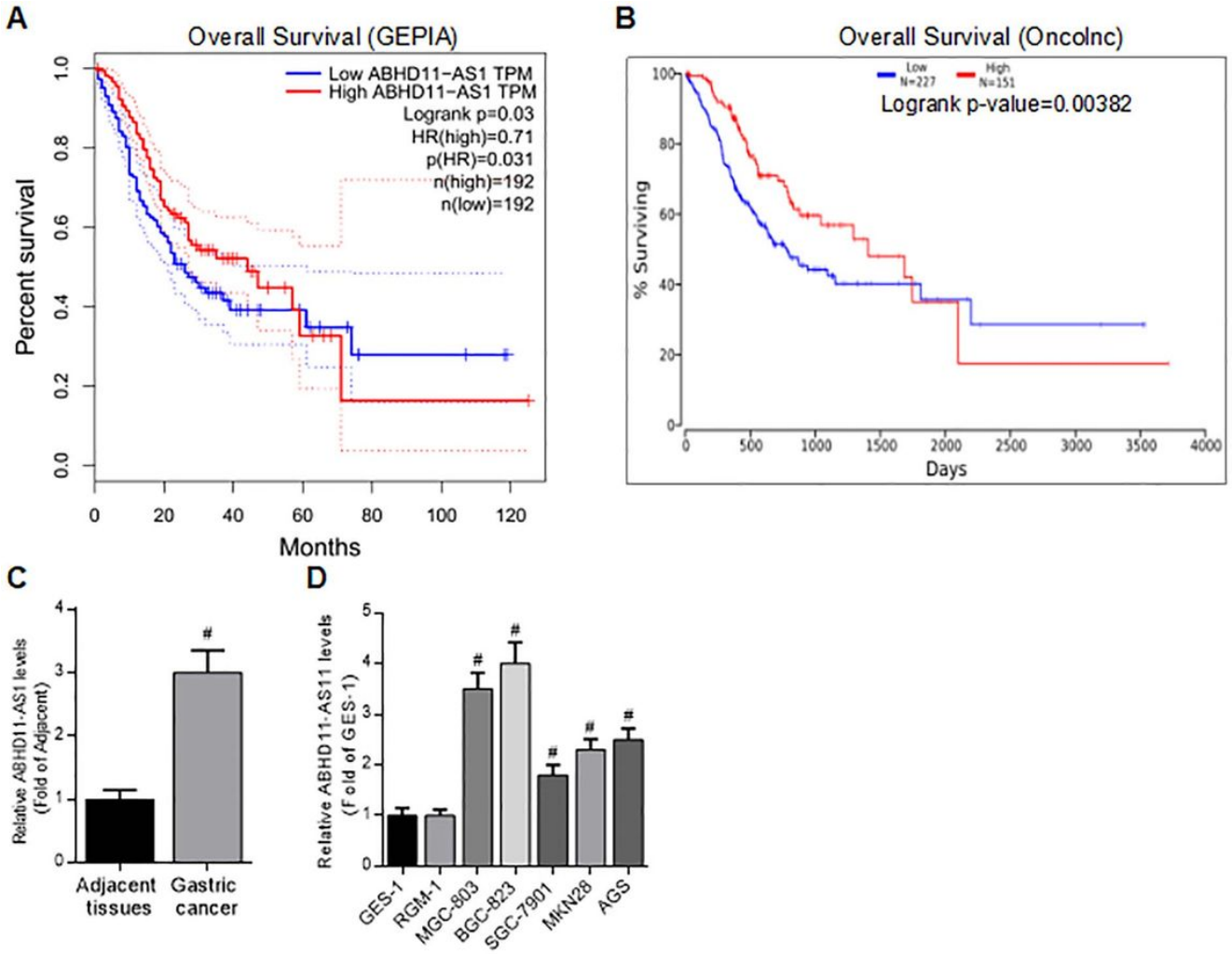

Figure 1

ABHD11-AS1 is overexpressed in GC cancer tissues and correlated with shorter survival time for GC patients. A. The relationship between the expression of ABHD11-AS1 and prognosis of gastric cancer by Kaplan–Meier analysis and log rank test. *p<0.01 (date from GEPIA and oncolnc date base). B. qRT-PCR analysis of ABHD11-AS1 expression levels in gastric cancer tissues (n=120), compared with the normal Adjacent tissues (n=120). C. qRT-PCR analysis of ABHD11-AS1 expression levels in gastric cancer cell lines (BGC-823, MGC-803, and SGC-7901) compared with the normal gastric epithelium cell line (GES-1). The statistical differences between samples were analyzed by paired samples t-test (n =63, *p<0.01).

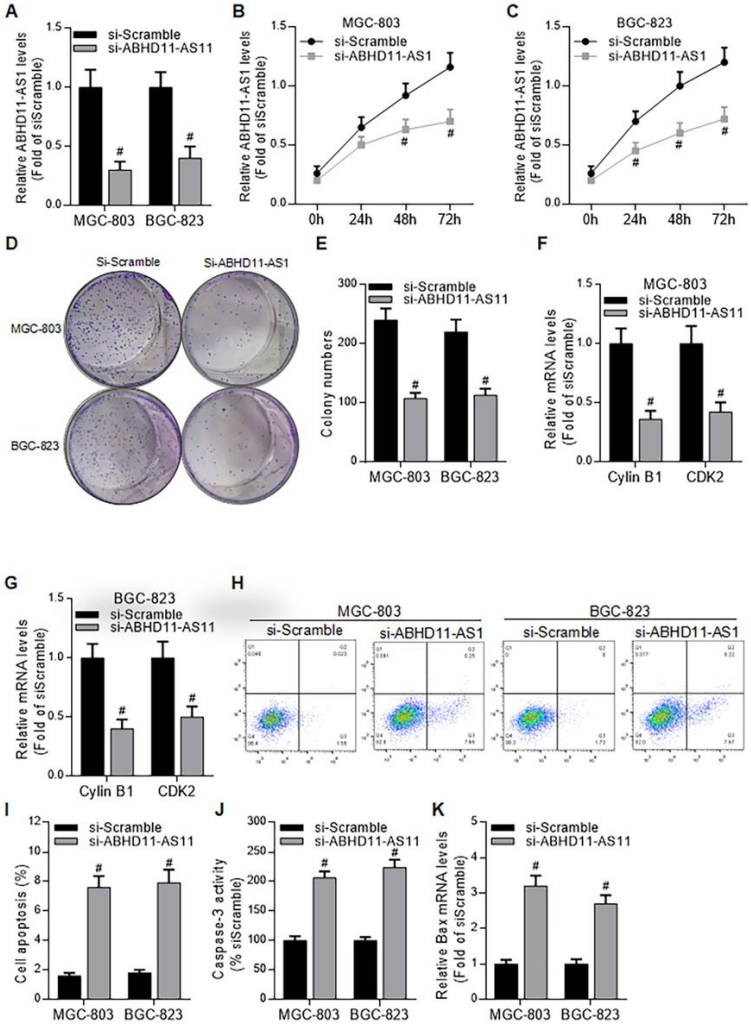

Figure 2

The effects of ABHD11-AS1 knocking-down on gastric cancer cell lines (A) qRT-PCR analysis of ABHD11-AS1 expression levels in siRNA transfected GC cells, compared with si-Scramble, *p<0.01. (B, C) CCK8 assays were conducted to determine the proliferation activity of in siRNA transfected GC cells compared with si-Scramble, *p<0.01 . (D, E) Colony-forming unit assays were performed to detect the proliferation of in siRNA transfected GC cells, compared with si-Scramble, *p<0.01. (F,G). qRT-PCR analysis of proliferation gene (cylin B1 and CDK2) expression levels in siRNA transfected GC cells, compared with si-Scramble, *p<0.01. (H□I) Annexin V-FITC staining assays were conducted to determine apoptosis percent of in siRNA transfected GC cells, compared with si-Scramble, *p<0.01. (J) ELISA assays were conducted to determine the apoptosis enzyme caspase-3 in siRNA transfected GC cells compared with si-Scramble, *p<0.01. (K) qRT-PCR analysis of apoptosis gene Bax expression in siRNA transfected GC cells compared with si-Scramble,#p <0.01. The statistical differences between samples were analyzed with paired samples t-test (n =63, *p<0.01).

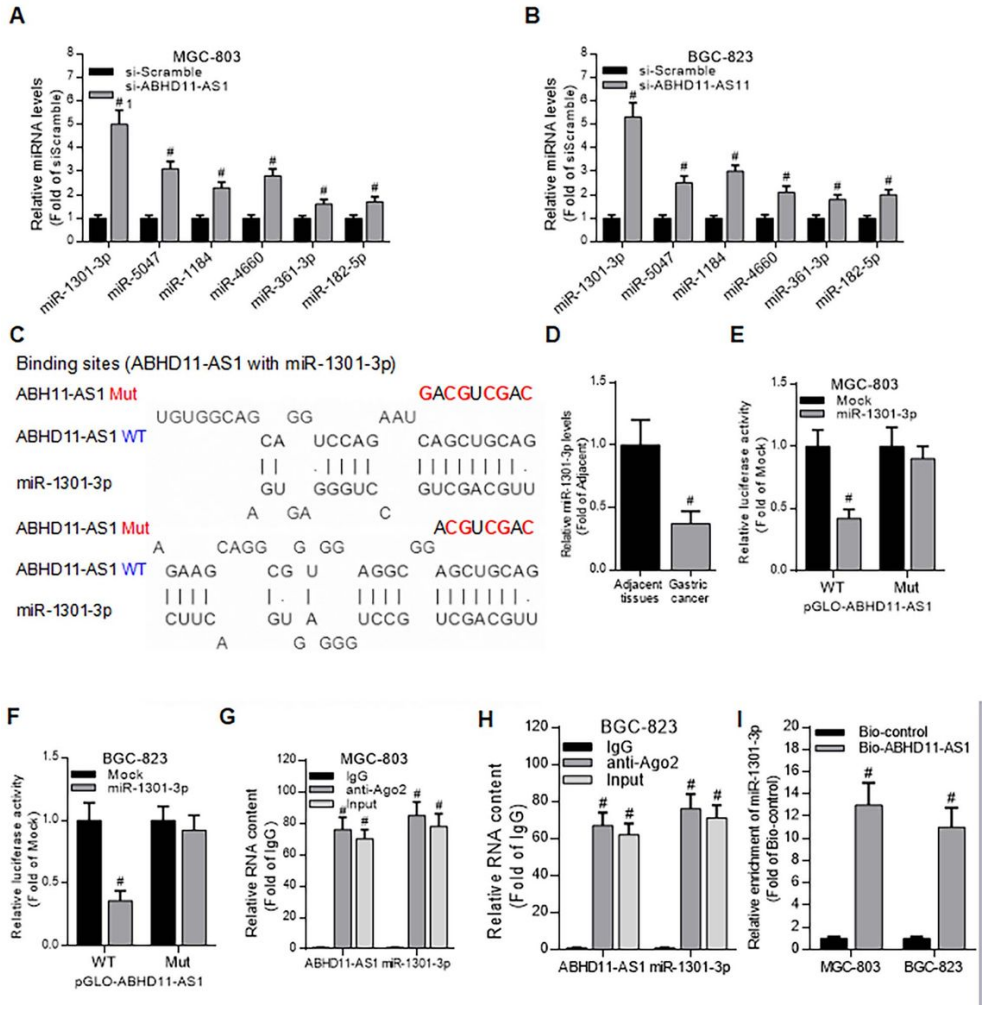

Figure 3

The effects of ABHD11-AS1 knocking-down is directly mediated miR-1301-3p (A,B) qRT-PCR analysis of some miRNA expression levels in the MGC-803 and BGC-823 cell lines pulled down by biotinylated ABHD11-AS1 probe compared with si-Scramble, *p<0.01. (C) Binding sites (ABHD11-AS1 with miR-1301-3p) and Mut ABHD11-AS1 site. (D) qRT-PCR analysis of miR-1301-3p expression levels in GC tissues, compared with adjacent tissues ,*p<0.01. (E、F) Luciferase assays were performed to measure the effect of miR-1301-3p on the activity of lncRNA-luciferase GC cell lines (MGC-803 and BGC-823) transfected with the reporter vector WT-ABHD11-AS1 and Mut-ABHD11-AS1 reporter gene compared with mock, *p<0.01. (G,H) qRT-PCR analysis of miR-1301-5p and ABHD11-AS1 bound to ago2, (RNA immunoprecipitation assay assays), compared with IgG group, *p<0.01. (I) qRT-PCR analysis expression of miR-1301-3p transfected with si-ABHD11-AS1 compared with Bio-control, #p <0.01.

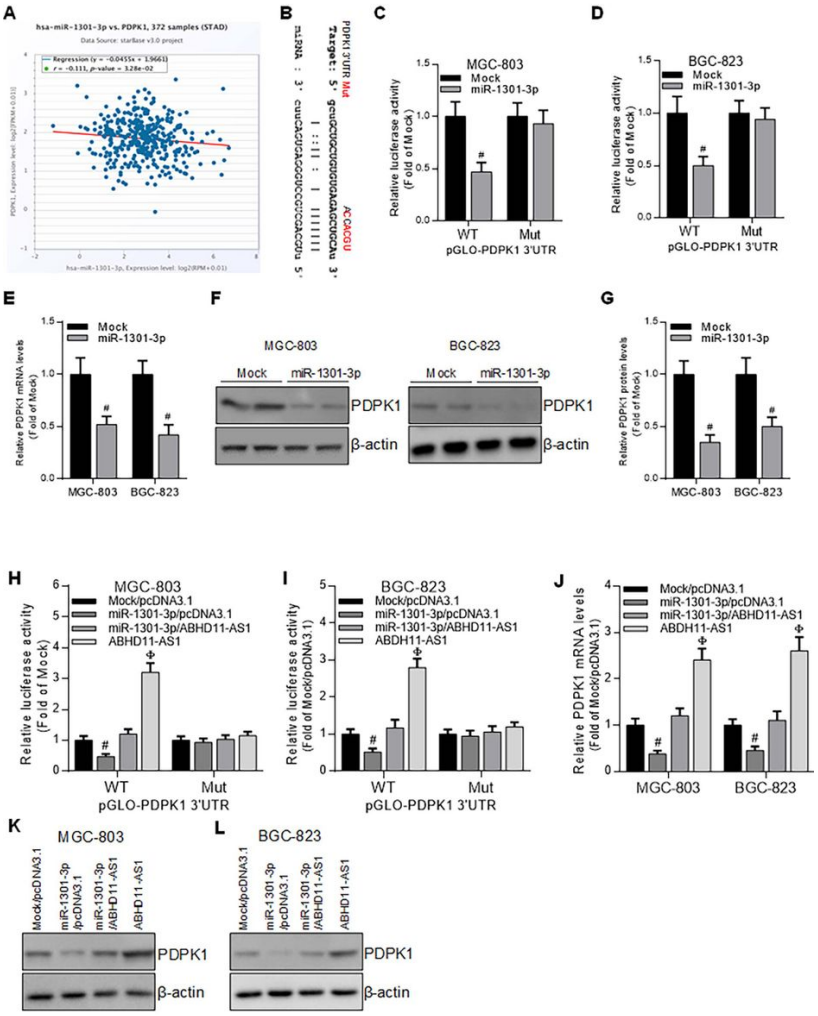

Figure 4

The expression of PDPK1in GC cell lines is regulated by the antagonism effect between miR-1301-3p and ABHD11-AS1 The correlation between PDPK1 and Mir-1301-4p by Pearson correlation analysis, data from Starbase. Binding sites (PDPK1 with Mir-1301-4p), predicted by targets can. (C,D) Luciferase assays were performed to evaluate the effect of miR-1301-3p on the activity of PDPK1 3'UTR in GC cell lines (BGC-823 and MGC-803) transfected with the reporter vector WT-PDPK1 and Mut-PDPK1 reporter gene compared with mock, *p<0.01. (E) qRT-PCR analysis of the expression of PDPK1 mRNA mediated by miR-1301-3p in gastric cancer cell lines compared with mock, *p<0.01. (F,G) Western blotting analysis to evaluate the regulation of PDPK1 protein level mediated by miR-1301-3p in gastric cancer cell lines compared with mock , *p<0.01. (H, I) the combined effect of miR-1301-3p and ABHD11-AS1 on the activity of PDPK1 3'UTR reporter gene was evaluated by Luciferase assays compared with mock, *p<0.01. (J) qRT-PCR analysis of the expression of PDPK1 mRNA mediated by ABHD11-AS1 and miR-1301-3p simultaneously, compared with mock/pcDNA3.1, *p<0.01. (K, L) the expression of PDPK1 protein co-mediated by miR-1301-3p and ABHD11-AS1 in GC cell lines was measured by western blot analysis compared with mock/pcDNA3.1, *p<0.01 .

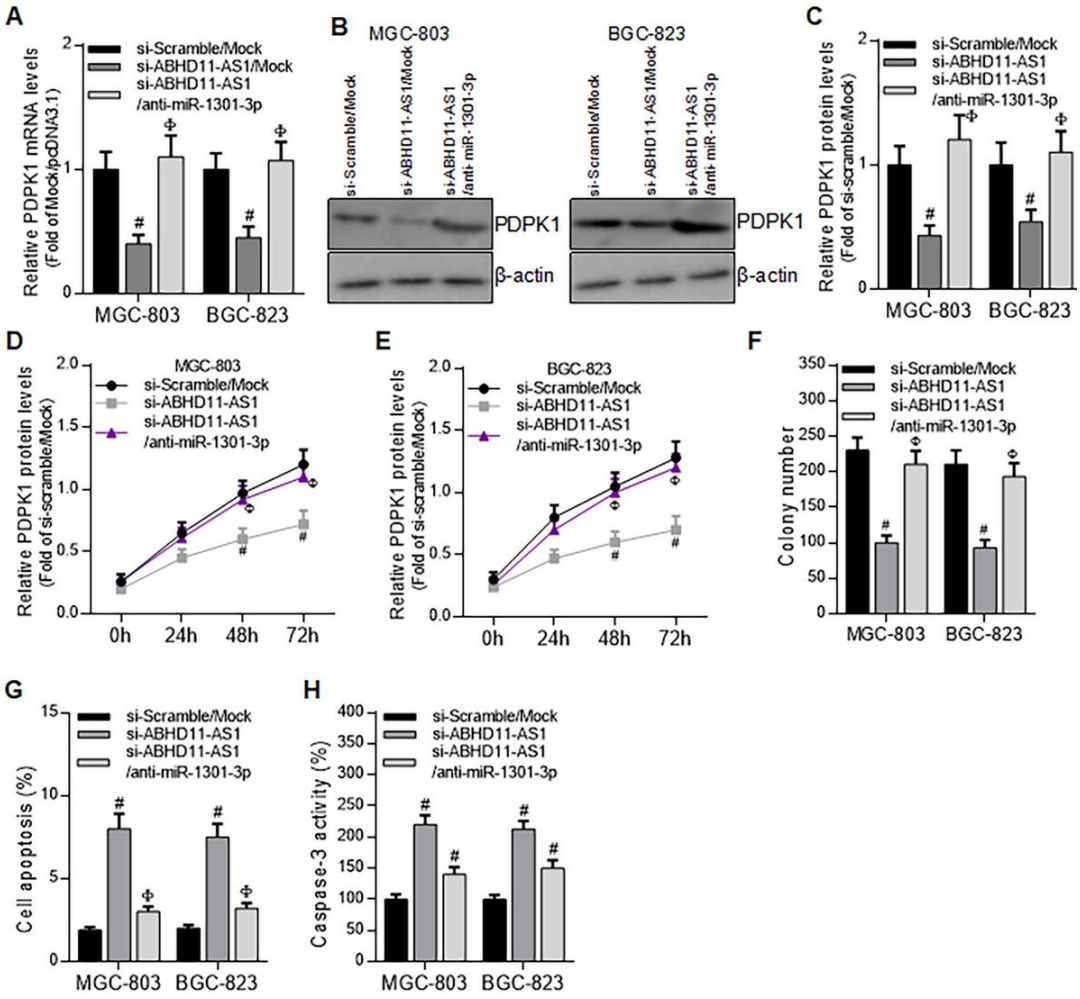

Figure 5

The activity of gastric cancer cells was co-mediated by miR-1301-3p and ABHD11-AS1. (A,B) The expression of PDPK1 in GC cell lines was measured by qRT-PCR analysis after both ABHD11-AS1 and miR-1301-3p were suppressed, compared with si-Scramble/Mock and si-ABHD11-AS1/Mock, *p<0.01. (B, C) The expression of PDPK1 protein was determined by western blot analysis in GC cell lines after both ABHD11-AS1 and miR-1301-3p were suppressed, compared with si-Scramble/Mock, #p <0.01. (D, E) Proliferation activity were evaluated by CCK8 assays in GC cell lines after both ABHD11-AS1 and miR-1301-3p were suppressed, compared with si-ABHD11-AS1/Mock, #p <0.01. (F) Proliferation activity was evaluated by clone formation assay in GC cell lines after both ABHD11-AS1 and miR-1301-3p were suppressed, compared with si-ABHD11-AS1/Mock, #p <0.01. (G, H) Annexin V-FITC staining assays were conducted to determine apoptosis percent of GC cell lines after both ABHD11-AS1 and miR-1301-3p were suppressed , compared with si-ABHD11-AS1/Mock and si-ABHD11-AS1/Mock, #p <0.01.