LncRNA POT1-AS1 accelerates the progression of gastric cancer by serving as a competing endogenous RNA of microRNA-497-5p to increase PDK3 expression

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Background: Gastric cancer (GC) is the most common malignant tumor of the digestive system. Although progress has been reported in terms of treatment, it is still the second leading cause of cancer-related death. Long non-coding RNAs have been shown to play a key role in human cancers in recent investigations. However, the role of POT1-AS1 in GC is still unclear.

Methods: The relative POT1-AS1 level in GC tissues and paracancerous tissues was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The biological function of POT1-AS1 was studied by CCK8 and Transwell assays in vitro experiments. Moreover, the downstream target genes of POT1-AS1 were predicted by bioinformatics.

Results: In this study, high POT1-AS1 expression in GC cells was confirmed, and its elevated expression was linked to patients’ negative clinicopathological characteristics, as well as shorter disease-free survival (DFS) and overall survival (OS). POT1-AS1 was shown to serve as a competing endogenous RNA (ceRNA) by sponging miR-497-5p to increase PDK3 expression. The impact of POT1-AS1 silencing on GC malignant phenotypes could be reversed by suppressing miR-497-5p or restoring PDK3, according to rescue experiments.

Conclusions: In brief, POT1-AS1 acted as an oncogenic lncRNA in GC, facilitating GC development by affecting the miR-497-5p/PDK3 axis, implying that the POT1-AS1/miR-497-5p/PDK3 axis is a useful target in anticancer therapy.

Keywords: POT1-AS1; gastric cancer (GC); microRNA-497-5p; pyruvate dehydrogenase kinase 3 (PDK3)
Introduction

Gastric cancer (GC) is considered to be one of the common malignancy digestive carcinomas (1). This carcinoma is particularly widespread in China (2), where it is considered to be the second most prevalent and third deadliest carcinoma (3). While multiple detailed studies of GC have been undertaken to date, the processes driving the genesis and progression of the disease remain elusive, restricting patients' access to effective therapeutic options.

Long non-coding RNAs (lncRNAs) have been discovered to be a novel collection of sequences for researching the processes of different types of carcinomas (4-6). LncRNAs contain over 200 nucleotides and exist in the form of a linear transcript (7). As these linear transcripts cannot translate into proteins, they were once thought to be “junk” and “noise” in the genome (8). These non-coding RNAs are linked to various genetic phenomena, including gene expression at the transcriptional level, according to overwhelming evidence (9,10). Various human disorders, including neurodegenerative disorders, malignancies, heart-related complications, and endocrine diseases are linked to lncRNA dysfunction (11). According to the reported studies, in GC, abnormal lncRNA expression is common, and their dysfunction has been linked to GC progression (12-14). Multiple studies have suggested that abnormal lncRNA levels have tumor-promoting or suppressing activities. Furthermore, these RNAs play significant roles in the manipulation of a variety of carcinoma features (15-17). The functional association and expression pattern of POT1-AS1 in GC has remained elusive. In view of this fact, POT1-AS1 was considered as the subject of our investigation. This research may provide new insights into the pathophysiology of GC and may suggest novel therapeutic options. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/jgo-21-709).

Methods

Patients and tissue specimens

The current study included 132 patients suffering from GC who had never been treated with anticancer treatment. Following surgical removal, snap-freezing of GC tissues and nearby healthy tissues was carried out in liquid nitrogen, followed by storage at cryogenic temperature (−80 °C). Approval for this study was obtained from Changhai Hospital’s research ethics committee (No. 16ZR1400800). Each experiment was performed in accordance with the Declaration of Helsinki (as revised in 2013). Table 1 represents the patient demographics as well as clinical data. All patients provided informed consent.

Cell culture

A healthy gastric epithelial cell line (RGM-1) and 4 types of GC cell lines (MKN-45, MGC-803, SGC-7901, and HGC-27) were provided by ATCC, Manassas, USA. Cells were then seeded in DMEM supplemented with 10% fetal bovine serum (FBS; heat-inactivated), 100 U/mL penicillin, and 100 mg/mL streptomycin. These antibiotics, DMEM, and FBS were obtained from Gibco. At 37 °C, cells were incubated in a humidified environment with a continuous supply of 5% CO₂.

Cell transfection

An siRNA targeting POT1-AS1 (si-POT1-AS1) and non-targeting control siRNA (si-NC) were procured from Guangzhou RiboBio Co., Ltd. for the loss-of-function study. The design and development of miR-497-5p agomir (agomir-497-5p) were carried out by GenePharma Co., Ltd. (Shanghai), and agomir (agomir-NC) was employed as a negative control. Endogenous miR-497-5p expression was silenced using miR-497-5p antagonir (antagomir-497-5p), and antagomir-NC was utilized as a control for antagonir-497-5p. GenePharma also produced the PDK3 overexpression vector pcDNA3.1-PDK3 (pc-PDK3) and the empty pcDNA3.1 vector. One day before transfection, cell seeding was carried out in 6-well plates, followed by transient transfection of the molecular products using Lipofectamine 2000TM reagent (Invitrogen). Si-NC: 5’-UUCUCCGAACGUGUCAGAGAC-3’; Si-POT1-AS1: 5’-UCCCGGGCTGCTGGUCAGAC-3’.

RT-qPCR

The content and quality of total RNA were determined via NanoDrop, Thermo Fisher Scientific, Inc. after isolation using TRIzol® reagent (Invitrogen). To assess the miR-497-5p expression, complementary DNA (cDNA) was obtained using a Mir-XTM miRNA First-Strand Synthesis Kit, followed by qPCR with a Mir-XTM miRNA qRT-PCR TB Green® Kit. For normalizing miR-497-5p expression, U6 snRNA was considered as an internal control. cDNA was obtained from total RNA using the PrimeScriptTM
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Pearson chi-square test was used for comparisons between subgroups. CA19-9, carbohydrate antigen 19-9.
RT Reagent Kit to measure POT1-AS1 and PDK3 mRNA expression, followed by amplification of the cDNA products via SYBR-Green PCR Master Mix. These kits and PCR Master Mix were procured from TaKaRa Bio Inc. Actin expression was used to normalize the levels of POT1-AS1 and PDK3 mRNA. The \(2^{-\Delta\Delta Cq}\) technique was used to examine the expression of each gene.

**POT1-AS1:** F: 5'AGATCTAGGCTTCGGAAGGAAAAGC-3', R: 5'CCCAAGATCTAGCGCACCGCCTA-3';

**PDK3:** F: 5'AGATCTAGATCTCGTCCACTGAAAGGCACAAAAGC-3', R: 5'CCCAAGCTTAACTTTCTCTCAAAAT-3';

**U1:** F: 5'GGCCACACTGACTTCTCAAAAT-3', R: 5'GGCTGTTGTCATACTTCTCATG-3';

**GAPDH:** F: 5'GGAGCGAGATCCCTCCAAAAT-3', R: 5'GGCTGTTGTCATACTTCTCATG-3';

**miR-497-3p:** F: 5'AACAGTGCAAACCACACTGTG-3', R: 5'GTCGTATCCAGTGCAGGGT-3';

**U6:** F: 5'GGCTTCAAGGAAACCTGGG-3', R: 5'GGCTTCAAGGAAACCTGGG-3'.

**CCK-8 assay**

To assess the proliferative ability of the cultured cell lines, a CCK-8 assay was employed. After overnight incubation, transfected cells were exposed to trypsin. Next, cells (2 × 10^3 cells/well) were grown in each well of a 96-well plate, followed by adding CCK-8 solution (10 µL), and then 2 h incubation was carried out at 37°C. A microplate reader (Bio-Rad) was employed for the measurement of optical density (OD) at 450 nm.

**Transwell assays**

Cell digestion was carried out using trypsin 48 h post transfection, followed by washing the cells (twice) with PBS. For the evaluation of the invasive properties of cells, transfected cells (5 × 10^5) were re-suspended in DMEM (200 µL) and then added into each well of the upper portion of a Transwell insert (8-µm pore; Corning) with Matrigel® precoated membranes (BD Biosciences). Next, 600 µL of 10% FBS cultured media was added to the lower part. For 24 h, the Transwell inserts were incubated in a humidified environment with a continuous supply of 5% CO₂ (at 37°C). The noninvasive cells (on the upper insert portion) were then cleaned (using a cotton swab), while fixation of the invasive cells was carried out in pure methanol, followed by staining with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature. The method for assessing cell migration was the same as above, however, Matrigel® was not used for membrane precoating. An Olympus microscope (Olympus Corporation) was used to image the migratory as well as invasive cells, followed by evaluating their migratory and invasive potential.

**Bioinformatics evaluations**

StarBase-3.0 (http://starbase.sysu.edu.cn/) was employed for predicting the possible POT1-AS1 targets.

**RNA Immunoprecipitation (RIP) assay**

RIP assay was utilized to examine the binding between POT1-AS1 and miR-497-5p in GC cells via the RIP Kit (Millipore). Next, the cells were exposed to lysis buffer, and then the cell lysates were treated with magnetic beads coated with human antibodies AGO2 or IgG (as a negative control) for 24 h (at 4°C). Subsequently, the collection of magnetic beads was carried out and then immunoprecipitated RNA was isolated. Finally, RT-qPCR was performed to evaluate the enrichment of POT1-AS1 and miR-497-5p.

**lncRNA localization**

The isolation and extraction of nuclear and cytoplasmic RNA of the indicated GC cell lines were carried out using the Cytoplasmic and Nuclear RNA Purification Kit (Amyjet) to localize lncRNA. Next, qRT-PCR was conducted to identify the expression of POT1-AS1. The cytoplasmic and nuclear controls were GAPDH and U1, as respectively performed (18).

**Luciferase reporter assay (LRA)**

Shanghai GenePharma developed and produced POT1-AS1 fragments with wild-type (WT) and mutant (MUT) miR-497-5p interacting sites. The WT-POT1-AS1 and MUT-POT1-AS1 reporter plasmids were generated and then the reporter plasmids for WT-PDK3 and MUT-PDK3 were also made in the same way, followed by growing the cells in 24-well plates to 60–70% confluency. In the presence of agomir-NC or agomir-497-5p, cultured cells were treated with WT or MUT reporter plasmids, followed by collecting the plasmid-treated cells 48 h post transfection, and their activity was then assessed with a DLR Assay System (Promega Corporation). The normalization of data
was carried out with Renilla luciferase activity.

**Western blotting (WB)**

Using RIPA buffer, proteins were extracted from cultured cells, followed by measuring the protein concentration via a bicinchoninic acid assay kit. The above buffer and kit were procured from the Beyotime Institute of Biotechnology. For the separation of an equal amount of protein, the obtained proteins were processed on SDS-PAGE, followed by transferring these proteins to PVDF membranes (Millipore). Next, skim milk powder (5% solution) was used to block the membrane (for 2 h) at ~25°C. Overnight incubation of the membrane was carried out with mouse anti-human PDK4 (1:1,000; ab182408, Abcam) and Actin (1:1,000; ab128915, Abcam) primary antibodies at 4°C, followed by incubating the membrane with secondary antibodies conjugated to HRP (1:5,000; cat. no. ab205718, Abcam) for 2 h. Protein signals were identified using an HRP Substrate kit (EMD Millipore).

**Statistical analysis**

The obtained data were represented as mean ± SD. Each experimental procedure was conducted 3 times in an independent manner. The relationships between POT1-AS1 expression and clinical parameters in GC patients were assessed with the χ² test, followed by comparing groups via Student’s t-test or one-way ANOVA. Next, Tukey’s post hoc test was performed for the comparison of variations among groups. The Kaplan–Meier (K-M) method was used to calculate the overall survival (OS) rate of GC patients, and the log-rank (L-R) test was used to compare the results. Spearman’s correlation analysis was performed to examine the link between the expressions of 2 genes in GC tissues. P value <0.05 was considered to be statistically significant.

**Results**

**POT1-AS1 is increased in GC**

We used RT-qPCR to examine the expression profile of POT1-AS1 in 132 pairs of GC tissues as well as neighboring normal tissues to identify the role of POT1-AS1 in GC. The obtained data indicated high expression of POT1-AS1 in GC tissues compared with nearby healthy tissues, as depicted in Figure 1A (P<0.05). RT-qPCR was conducted to evaluate the expression of POT1-AS1 in 4 GC cell lines (MGC-803, SGC-7901, MKN-45, and HGC-27) and a healthy gastric epithelial cell line (RGM-1) to further support the aforementioned data. In GC cells, POT1-AS1 expression was elevated compared with RGM-1 cells, as depicted in Figure 1B (P<0.05 vs. RGM-1). We evaluated the clinical implications of POT1-AS1 in GC after confirming the abnormal upregulation of POT1-AS1. The median value of POT1-AS1 expression in GC tissues was used as the cut-off value, and each GC patient in this investigation was separated into 2 groups: low POT1-AS1 expression and high POT1-AS1 expression. Elevated expression of POT1-AS1 was significantly linked with larger tumor size (P=0.001), lymph node metastasis (P=0.001), and better TNM stage (P=0.008) in GC patients, as represented in Table 1. The GEPIA software program was employed to validate the link between the expression of POT1-AS1 and the outcomes of 358 patients with GC from TCGA, and the obtained data revealed that elevated expression of POT1-AS1 was significantly linked with poor OS [n=373, HR =1.6, P(HR) =0.0057, L-R P=0.0054] and disease-free survival (DFS) [n=373, HR =2.1, P(HR) =0.00021, L-R P=0.00015] in CRC, as depicted in Figure 1C,1D. These findings reveal that POT1-AS1 may have a role in GC progression.

**Silencing of POT1-AS1 attenuates the progression of GC cells in vitro**

POT1-AS1 expression was elevated in MKN-45 and HGC-27 cells relative to MGC-803 and SGC-7901 cells. To identify the function of POT1-AS1 in GC development, POT1-AS1 was depleted in cells (MKN-45 and HGC-27) via transfection with si-POT1-AS1, as represented in Figure 2A. A control group of cells was transfected with si-NC. The obtained data of the CCK-8 assay revealed that MKN-45 and HGC-27 cells transfected with si-POT1-AS1 had considerably decreased proliferative potential than cells transfected with si-NC, as depicted in Figure 2B. Furthermore, a considerable decrease was found in the migratory and invasive potential of POT1-AS1-silenced MKN-45 and HGC-27 cells relative to si-NC-transfected cells, as represented in Figure 2C,2D. These data suggest that POT1-AS1 is a cancer-enhancing IncRNA in GC.

**POT1-AS1 directly binds with miR-497-5p in GC cells as a molecular miRNA sponge**

Several biological activities have been linked with IncRNAs,
including acting as competing endogenous RNAs (ceRNAs) for miRNAs, according to new findings. The subcellular location of POT1-AS1 was initially determined to evaluate whether it could be a ceRNA. The results of the nuclear/cytoplasmic fractionation experiment revealed that POT1-AS1 is preferentially localized in the cytoplasm (Figure 3A). Hence, it was speculated that POT1-AS1 might be a ceRNA. The directly interacting miRNAs of POT1-AS1 were then predicted using the publicly accessible database starBase 3.0, followed by selecting miR-497-5p for further evaluations, as it shares complementary binding sites with POT1-AS1 and suppresses tumors in GC, as depicted in Figure 3B (19). The binding between miR-497-5p and POT1-AS1 in GC cells was evaluated by LRA. In the presence of agomir-497-5p or agomir-NC, the transfection of WT-POT1-AS1 or MUT-POT1-AS1 was carried out into cells (HGC-27 and MKN-45). The miR-497-5p expression was considerably elevated in these cells post transfection with agomir-497-5p, as represented in Figure 3C. The obtained data indicated that the luciferase activity of WT-POT1-AS1 cells was considerably lowered (P<0.05), however, variations were not observed in the activity of MUT-POT1-AS1 with high expression of miR-497-5p (Figure 3D). The RIP assay was performed to investigate the link between miR-497-5p and POT1-AS1, and the data indicated that POT1-AS1 and miR-497-5p were enriched in immunoprecipitates (Ago2-containing) relative to the IgG control, as depicted in Figure 3E. We used RT-qPCR to evaluate the expression of miR-497-5p in 132 pairs of GC tissues and their surrounding

**Figure 1** Elevated expression of POT1-AS1 in gastric cancer and its relationship with poor prognosis. (A) The expression of POT1-AS1 in 132 pairs of GC tissues and nearby healthy tissues was assessed by RT-qPCR. *P<0.05 compared with nearby healthy tissues. (B) To evaluate POT1-AS1 expression by RT-qPCR, RNA was isolated from 4 GC cell lines (SGC-7901, MGC-803, HGC-27, and MKN-45) and a healthy gastric epithelial cell line (RGM-1). *P<0.05 relative to RGM-1. (C,D) The K–M method and the L-R test were used to examine the link between POT1-AS1 and OS and DFS in GC patients using GEPIA data. Data are shown as mean ± SD and were derived from n=3 independent experiments. ***P<0.001.
Figure 2 Decreased expression of POT1-AS1 attenuates the proliferative, migratory, and invasive abilities of MKN-45 and HGC-27 cells and activates the apoptotic process of the cells in vitro. (A) RT-qPCR was conducted to evaluate the expression of POT1-AS1 in MKN-45 and HGC-27 cells post transfection of si-POT1-AS1 or si-NC; (B) the CCK-8 assay was used to identify the proliferation of POT1-AS1 deficient cells (HGC-27 and MKN-45); (C, D) HGC-27 and MKN-45 cells were transfected with si-POT1-AS1 or si-NC, and Transwell assays were used to determine their migratory and invasive capacities. Magnification, ×200. 0.1% crystal violet was used to stain the cells. Data are shown as mean ± SD and were derived from n=3 independent experiments. *P<0.05, **P<0.001.
Figure 3 Direct binding of POT1-AS1 with miR-497-5p for sponging its expression in GC cells. (A) POT1-AS1 was mostly detected in the cytoplasm of HGC-27 and MKN-45 cells, according to nuclear RNA fractionation and cytoplasmic studies. (B) POT1-AS1 binding sites for WT and MUT miR-497-5p were discovered using bioinformatics analysis. (C) Expression of miR-497-5p in HGC-27 and MKN-45 cells post transfection with agomir-497-5p or agomir-NC. (D) Cells including MKN-45 or HGC-27 and WT-POT1-AS1 or MUT-POT1-AS1 were co-transfected with agomir-497-5p or agomir-NC. LR activity was evaluated 48 h post co-transfection. (E) Compared to the IgG control, POT1-AS1 and miR-497-5p were enriched in immunoprecipitates (Ago2-containing). (F) RT-qPCR was used to evaluate the miR-497-5p expression in 132 pairs of GC tissues as well as neighboring healthy tissues. (G) Spearman’s correlation analysis was conducted to confirm the link between POT1-AS1 and miR-497-5p expression in the same GC tissues. (H) RT-qPCR was employed to evaluate the miR-497-5p expression in POT1-AS1-silenced cells. Data are shown as mean ± SD and were derived from n=3 independent experiments. ***P<0.001, #P>0.05.

healthy tissues to back up our findings. In comparison to surrounding healthy tissues, miR-497-5p was only modestly expressed in GC tissues, as depicted in Figure 3F. Furthermore, Spearman’s correlation analysis indicated an inverse expression association between POT1-AS1 and miR-497-5p in the 132 GC tissues (Figure 3G; Spearman r=0.443, P<0.001). The impact of POT1-AS1 on miR-497-5p expression was further evaluated in GC cells and the data revealed that miR-497-5p expression was considerably increased by POT1-AS1 silencing in HGC-27 and MKN-45 cells, as indicated in Figure 3H. According to these findings, POT1-AS1 may function as a molecular sponge...
The impact of POT1-AS1 on PDK3 expression in GC cells by miR-497-5p sponging

In GC cells, PDK3 appears to be a direct target gene of miR-497-5p, according to a prior study (20). We investigated whether POT1-AS1 plays a role in the regulation of PDK3 in GC cells after discovering that it sponges miR-497-5p. To accomplish this, HGC-27 and MKN-45 cells were transfected with si-POT1-AS1 or si-NC, and PDK3 expression was examined. POT1-AS1 silencing significantly reduced PDK3 expression in HGC-27 and MKN-45 cells at both the transcriptional (Figure 4A) and translational (Figure 4B) levels. Using RT-qPCR, PDK3 expression was evaluated in 132 pairs of GC tissues as well as neighboring healthy tissues. In comparison to the nearby normal tissues, PDK3 expression was considerably elevated in GC tissues, as shown in Figure 4C. Furthermore, Spearman’s correlation analysis revealed a positive link between PDK3 mRNA expression and POT1-AS1 expression in the 132 GC tissues (Figure 4D; Spearman ρ=0.329, P<0.001). Si-POT1-AS1 plus antagonir-497-5p or antagonir-NC was transfected into MKN-45 and HGC-27 cells to observe whether POT1-AS1 influences PDK3 expression by sponging miR-497-5p. RT-qPCR was conducted to confirm the effectiveness of the antagonir-497-5p transfection. Antagonir-497-5p transfection led to a substantial reduction in miR-497-5p expression in MKN-45 and HGC-27 cells (Figure 4E). In MKN-45 and HGC-27 cells, antagonir-497-5p re-introduction reversed the downregulation of PDK3 at the transcriptional (Figure 4F) and translational level (Figure 4G, P<0.05) caused by POT1-AS1. These findings revealed that POT1-AS1 sponging miR-497-5p is used to favorably control PDK3 expression in GC cells.

The effects of POT1-AS1 silencing in GC are partially rescued by attenuating miR-497-5p

Rescue experiments were conducted to identify the effect of the miR-497-5p/PDK3 axis on POT1-AS1 in GC cells. The proliferative, apoptotic, migratory, and invasive potential of MKN-45 and HGC-27 cells was evaluated post si-POT1-AS1 co-transfection with antagonir-497-5p or antagonir-NC. The proliferative, migratory, and invasive potential of MKN-45 and HGC-27 cells was decreased by POT1-AS1 silencing, as indicated in Figure 5A–5C, respectively. In these cells, however, suppression of miR-497-5p partially neutralized the impact of POT1-AS1 silencing.

By restoring PDK3 expression, the impact of POT1-AS1 silencing on the progression of GC cells was reversed

Si-POT1-AS1 and pc-PDK3 or the empty pcDNA3.1 vectors were co-transfected into MKN-45 and HGC-27 cells. The transfection capacity of pc-PDK3 was determined using WB. Following pc-PDK3 transfection, PDK3 protein expression was considerably enhanced in MKN-45 and HGC-27 cells, as indicated in Figure 6A. Co-transfection of pc-PDK3 in MKN-45 and HGC-27 cells eliminated the decrease in the proliferative rate (Figure 6B) caused by POT1-AS1 silencing, as confirmed by the CCK-8 assay. Furthermore, suppressing POT1-AS1 elevated the rate of apoptosis in MKN-45 and HGC-27 cells, whereas elevated PDK3 expression reversed this impact (Figure 6B). The migration and invasion of MKN-45 and HGC-27 cells (Figure 6C, 6D) were similar to the aforementioned results. Overall, these findings suggest that POT1-AS1 has a pro-oncogenic impact on GC development by acting as a ceRNA for miR-497-5p, thereby enhancing PDK3 expression.

Discussion

Because of their critical roles in the progression of cancer and their significant clinical uses, IncRNAs have now generated great interest (20,21). Abnormal expression of IncRNAs in GC has been reported in several studies and plays a role in GC metastasis and progression (22). As a result, investigating the functions of IncRNAs in the carcinogenesis of GC may lead to the discovery of novel treatment targets for patients with GC. The goal of the present study was to reveal the expression of POT1-AS1 in GC and to determine its therapeutic relevance in patients suffering from GC. We extensively studied the involvement of POT1-AS1 in GC cell progression. We also identified the exact processes behind POT1-AS1’s pro-oncogenic involvement in GC development. POT1-AS1 was substantially expressed in GC cells according to our findings. In patients suffering from GC, elevated POT1-AS1 expression was associated with increased tumor volume, the presence of metastases in lymph nodes, and a better TNM stage, with P values of 0.001, 0.001, and 0.008, respectively. Furthermore, GC patients with elevated expression of POT1-AS1 exhibited lower OS and
Figure 4 POT1-AS1 regulates PDK3 expression by acting as a ceRNA for miR-497-5p. (A,B) The transcriptional and translational level of PDK3 was evaluated using RT-qPCR and WB, respectively, post transfection of si-POT1-AS1 or si-NC into MKN-45 and HGC-27 cells. (C) RT-qPCR was employed to examine the expression of PDK3 mRNA in 132 pairs of GC tissues as well as nearby normal tissues. (D) Spearman’s correlation analysis was conducted to find a link between POT1-AS1 and PDK3 mRNA expression in 132 GC tissues. The miR-497-5p expression in MKN-45 and HGC-27 cells (transfected with antagomir-497-5p or antagomir-NC) were evaluated via RT-qPCR. (F,G) The transfection of MKN-45 and HGC-27 cells was carried out with antagomir-497-5p or antagomir-NC in the presence of si-POT1-AS1. To determine the transcriptional as well as the translational level of PDK3, RT-qPCR and WB were conducted to identify PDK3 mRNA and protein expression, respectively. Data are shown as mean ± SD and were derived from n=3 independent experiments. ***P<0.001.
Figure 5 The attenuation of miR-497-5p partially reverses the impact of POT1-AS1 silencing in MKN-45 and HGC-27 cells. (A) HGC-27 and MKN-45 cells were used in experiments after being co-transfected with antagomir-497-5p and si-POT1-AS1 or antagomir-NC. The CCK-8 assay was conducted to investigate cell proliferation. (B,C) The Transwell assay was carried out to evaluate the migratory and invasive potential of MKN-45 and HGC-27 cells. Magnification, ×200. 0.1% crystal violet was used to stain the cells. Data are shown as mean ± SD and were derived from n=3 independent experiments. *P<0.05. **P<0.001. *P>0.05.
Figure 6 The restoration of PDK3 expression reversed the influence of POT1-AS1 silencing on the malignant phenotypes of MKN-45 and HGC-27 cells. (A) WB was employed to evaluate PDK3 protein expression in HGC-27 and MKN-45 cells transfected with pc-PDK3 or empty pcDNA3.1 plasmid. (B-D) The co-transfection of cells (MKN-45 and HGC-27) was carried out with si-POT1-AS1 and pc-PDK3 or pcDNA3.1. The CCK-8 and Transwell assays were used to evaluate the proliferative, migratory, and invasive potential of the cells post transfection. Magnification, ×200. 0.1% crystal violet was used to stain the cells. Data are shown as mean ± SD and were derived from n=3 independent experiments, *P<0.05; **P>0.05.
DFS compared with patients with decreased expression of POT1-AS1. The functional assays conducted in this work demonstrated that silencing POT1-AS1 inhibits cellular proliferation, colony formation, migration, and invasion.

Through multiple mechanisms, lncRNAs can contribute significantly to tumorigenesis and the progression of cancer. However, the most studied and dominant mechanism is the ceRNA regulatory mechanism, in which lncRNAs compete with miRNAs and consequently elevate the expression of specific miRNA target genes (23). We aimed to explore the pathways underlying POT1-AS1 enhanced GC cells migration and invasion. Firstly, miR-497-5p has been defined to contain a complementary binding site (CBS) for POT1-AS1. RIP and the LRA were carried out for the validation of the interaction between miR-497-5p and POT1-AS1. Secondly, the expression of miR-497-5p was found to be comparatively lower in GC tissues and was inversely linked with the expression of LINC00577. Thirdly, decreased expression of POT1-AS1 enhanced the miR-497-5p level, which led to reduce a PDK3 expression level. Finally, restoring PDK3 or suppressing miR-497-5p could reverse the effects of POT1-AS1 suppression on GC oncogenic phenotypes. Our obtained results indicated a ceRNA model in GC cells that included POT1-AS1, miR-497-5p, and PDK3. In terms of the mechanism, the PDK family is comprised of 4 members, namely PDK1, PDK2, PDK3, and PDK4. The fact that these kinases are expressed differentially in various tissues suggests that their physiological and pathological functions are tissue specific (23,24). For example, PDK4 is implicated in metabolic alterations due to fat-enriched diet and diabetes (24,25), but most studies have revealed that PDK1 and PDK3 play roles in cell survival and cellular metabolic switching when cells are exposed to hypoxia (26-28). In contrast, PDK3 is involved in carcinoma development. Elevated PDK3 expression is linked to poor prognosis in acute myelogenous leukemia (29). The apoptotic process of glioblastoma multiforme (30) was facilitated when PDK3 expression was downregulated. Furthermore, dichloroacetate inhibits PDK3 activity, which increases the anticancer effects of elesclomol (31) in melanoma. Taken together, these findings reveal that PDK3 can serve as a candidate target against the progression of carcinomas. Our obtained results revealed a unique upstream mechanism that controls the miR-497-5p/PDK3 interaction in GC cells through in vitro experiments. POT1-AS1 has an miR-497-5p interacting site which acts as a molecular ceRNA, elevating PDK3 expression via the increased level of miR-497-5p expression in GC cells. The discovery of the POT1-AS1/miR-497-5p/PDK3 regulation cascade may aid in furthering the understanding of GC pathophysiology and may provide potential treatment options for patients suffering from GC.

The following are some of the limitations of this study: (I) we did not evaluate onco-POT1-AS1 targets in GC cells, including miR-122-5p, miR-140-3p, or miR-552-3p. To fully understand the role of POT1-AS1 in GC, more research is needed in the future; (II) in this work, we used an arbitrary cut-off value for the POT1-AS1 expression level, and future research should focus on determining the best clinically relevant cut-off value; (III) furthermore, because our study sample size was small, more research is needed to demonstrate that POT1-AS1 is a useful biomarker for GC patients.

The present study discovered a unique method through which POT1-AS1 contributes to GC carcinogenesis. Silencing of POT1-AS1 inhibited the malignancy of GC cells. POT1-AS1 strongly regulated PDK3 expression in GC cells by sponging miR-497-5p, and the cancer-promoting effects of POT1-AS1 during GC progression were attributed to the validated ceRNA model. The POT1-AS1/miR-497-5p/PDK3 cascade could be a candidate target for GC treatment.

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Footnote

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