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Aberrant DNA Hypermethylation Silenced LncRNA Expression in Gastric Cancer

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Abstract. *Background/Aim: Long noncoding RNAs (lncRNAs) are noncoding transcripts that are >200 nucleotides in length. However, the biological functions and regulation mechanisms of lncRNAs in gastric carcinogenesis remain unknown. Materials and Methods: The expression levels of Linc00472 were analyzed by real-time PCR. The DNA methylation status was assessed using Combined Bisulfite Restriction Analysis (COBRA). The biological role of Linc00472 was assessed in AGS cells with Linc00472 overexpression. Results: Using the next-generation sequencing approach, we identified DNA methylation-associated lncRNAs in gastric cancer cells. Among them, the expression level of Linc00472 significantly decreased in gastric cancer tissues compared to adjacent normal tissues. Furthermore, we observed a more frequent hypermethylation of CpG islands upstream of Linc00472 in gastric cancer tissues. Ectopic Linc00472 expression could significantly inhibit gastric cancer cell growth and migration. Conclusion: Epigenetically regulated Linc00472 expression plays a crucial role in modulating gastric cancer cell growth and motility.*

Gastric cancer (GC) is the most common cancer and the second leading cause of cancer-related death worldwide, especially in the Andean region of South America and in the Far East (1). Various factors contribute to the onset of GC, including *Helicobacter pylori*, smoking, and diet. Most patients are diagnosed with GC at an advanced stage; therefore, developing an early diagnosis marker and elucidating the detailed mechanisms of GC progression are beneficial.

In human tumors, global DNA hypomethylation is frequently observed, mostly in the DNA-repetitive region, and is accompanied by hypermethylation at particular promoter CpG islands of tumor suppressor genes (2). In GC, DNA methylation contributes to cancer progression and leads to aberrant silencing of tumor suppressor genes (or oncogene reactivation) (3). Park *et al.* (4) determined the global DNA methylation profile of GC by using a methylated DNA enrichment technique, and analyzed this profile by using a next-generation sequencing (NGS) approach. They reported that GC is associated with hypermethylation of 5' CpG islands and the 5' end of protein-coding genes as well as with hypomethylation of DNA-repetitive elements. Over the past few decades, the gain or loss of DNA methylation at the promoter of protein-coding genes in GC has been continually studied (5, 6).

An increasing amount of evidence has indicated that noncoding RNAs (ncRNAs) may play a critical role in regulating various cellular processes, including cell

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development and growth, the cell cycle, and cancer metastasis (7). Long noncoding RNAs (lncRNAs) are transcribed RNA molecules that are more than 200 nucleotides in length. In cellular processes, lncRNAs may function in regulating protein-coding gene expression, modifying epigenetic regulation, modulating alternative splicing processes, and titrating microRNAs (miRNAs) by serving as decoys (8). The human genome encodes approximately 15,000-17,000 potential lncRNAs; however, the biological functions of most lncRNAs remain unknown (9, 10).

Despite continued uncertainty regarding lncRNA function in normal tissues, increasing evidence has been obtained regarding the function of lncRNA in human GC. Previous studies have revealed that several dysfunctional lncRNAs are involved in the growth, migration, and invasion of GC cells. HOTAIR, an onco-lncRNA, exhibited increased expression in GC, leading to the promotion of cell growth and invasion through sponging miR-133a-3p (11-14). The overexpression of lncRNA H19 enhances carcinogenesis and metastasis by promoting GC cell proliferation and migration (15-18). The knockdown of ANRIL significantly repressed the proliferation of human GC cells by modulating the epigenetic modification of miR-99a/miR-499a (19). Hu *et al*. reported that GAPLINC regulates CD44 by serving as a molecular decoy for miR-211-3p and that it promotes cell migration and proliferation. The results of a tissue *in situ* hybridization assay suggested that GAPLINC overexpression defines a subgroup of GC patients with extremely poor survival (20).

Tumor-suppressive lncRNAs, namely BM742401, GAS5, FENDRR, and MEG3, were significantly down-regulated in GC tissues compared to adjacent normal tissues (21-25). DNA hypermethylation resulted in the low expression of MEG3 in GC patients with poor prognoses, including those with large tumors and a high depth of invasion. The ectopic expression of MEG3 could inhibit cell proliferation, promote cell apoptosis, and modulate p53 expression in GC cell lines (25). To date, few DNA methylation-regulated lncRNAs have been investigated in GC. Therefore, in this study, we identified DNA methylation-silenced lncRNA in GC by using high throughput approaches.

Materials and Methods

Cell lines. Six human cell lines, AGS, AZ-521, HR, TSGH, SNU-1, and NCI-N87, were obtained from the American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium supplemented with 10% inactivated FBS (Invitrogen, Carlsbad, CA, USA). Gastric cells were cultured in the presence or absence of 2.5 or 5 μM 5-Aza-dC for various periods (0, 1, 2, 3, and 4 days after treatment).

Analysis of strand-specific transcriptome sequence reads. Gastric cells were cultured in the presence or absence of 5 μM 5-Aza-dC for 4 days. The total RNA of GC cells was then prepared using TRIZOL (Invitrogen) according to the manufacturer's protocol. RNA samples were processed using the strand-specific preparation protocol for transcriptomes and were sequenced on the Illumina platform (Illumina, San Diego, CA, USA). Sequence reads were analyzed according to the method used in our previous study (26).

Pathway enrichment analysis. We attempted to determine the functions of involved genes by investigating pathways. Differentially expressed genes were selected from NGS data; candidate genes were then mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways based on enzyme commission (EC) numbers by using the R package SubPathwayMiner v.3.1. Subsequently, the hypergeometric test was performed to identify significantly enriched pathways and calculate the false positive discovery rate by using the FDR-corrected q-value.

Clinical samples and DNA/RNA extraction. Human gastric carcinoma tissues were obtained from 42 patients who underwent gastric resection at the Department of Surgery, Veterans General Hospital, Kaohsiung, Taiwan. This study protocol was approved by the Institutional Review Board of Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan; IRB number: VGHKS13-CT4-09). All subjects or their guardians signed the informed consent. The total RNA and DNA of 42 paired tissues were extracted using the TRIzol reagent (Invitrogen). The detailed process is described in our previous study (27).

Real-time RT-PCR. Total RNA (2 μg; DNase I treatment) was reverse transcribed using oligo (dT)15 primers and SuperScript III Reverse Transcriptase according to manufacturer's instructions (Invitrogen). The reaction was performed at 42˚C for 1 h; the enzyme was subsequently inactivated by incubation at 85˚C for 5 min. cDNA was used for real-time PCR with gene-specific primers, and gene expression was detected using a SYBR Green I assay (Applied Biosystems, Foster City, CA, USA). The following primers were used: Linc00472-F: 5'-ACAACACAACACAGGAGGGG-3'; Linc 00472-R: 5'-CCATCCTTAAGCCCCTGCAA-3'; GAPDH-F: 5'-TGCACCACCAACTGCTTAGC-3'; GAPDH-R: 5'-GGCATGGA CTGTGGTCATGAG-3'; U6-F: 5'-CTCGCTTCGGCAGCACA-3'; and U6-R: 5'-AACGCTTC ACGAATTTGCGT-3.

DNA bisulfite conversion. Genomic DNA was extracted from cultured cells or gastric carcinoma tissues by using the TRIzol reagent (Invitrogen), and an aliquot (2 μg) was then subjected to bisulfite conversion by using the EZ DNA Methylation-Gold Kit (Zymo Research Corporation, Orange, CA, USA). The bisulfite conversion reaction was conducted in a PCR thermocycler at 98˚C for 10 min, then at 64˚C for 2.5 h, and finally at 4˚C for up to 20 h.

Combined bisulfite restriction analysis. Bisulfite-converted genomic DNA was used for the methylation analysis of promoters with specific methylation primers. PCR conditions were as follows: 94˚C for 10 min, followed by 35 cycles of 94˚C/1 min, 60˚C/1 min, and 72˚C/30 sec, and final extension at 72˚C for 10 min. PCR was conducted in the PCR thermocycler by using HotStart Taq DNA polymerase (Qiagen, Hilden, Germany). The methylation status of the genomic DNA of individual samples was also examined through BstuI digestion (New England Biolabs, MA, USA). Digested PCR fragments were then separated on 2% agarose gel. The following

Table I. *Summary of sequence read categories. The sequence reads were mapped to the sense and antisense strands of the known transcripts and genomic sequences. Control (without treatment) and 5-Aza-dC (with 5-Aza-dC treatment) libraries were derived from AGS cells with or without 5-Aza-dC treatment.*

	All Reads	Mapping to sense transcripts	Mapping to anti-sense transcripts	Mapping o genome	Un-mappable read
Control	85459111/100%	50351359/58.9%	7225240/8.5%	18788121/22.0%	9094391/10.6%
5-Aza-Dc	82523755/100%	48277134/58.5%	5813061/7.0%	18623195/22.6%	9810365/11.9%

primers were used: Linc00472-MF: 5'-TGAGGTTTTAGGAAA ATTTTAAAAA-3' and Linc00472-MR: 5'-CACTAACATACCAC TAACTTAAAAACC-3'.

Ectopic expression of Linc00472 genes. We constructed the full-length Linc00472 gene according to the NR_026807.1, which is 2945 bp in length. We used cDNA from GC cell lines as a template to construct the full-length expression vector. The sequence of Linc00472 was subcloned into the pcDNA4 expression vector. Stable GC cell lines expressing Linc00472 were generated by transfecting GC cells with pcDNA4-Linc00472 for 24 h, and zeocin (50 ng/ml) selection was conducted for 7-14 days. The pCDNA4 empty vector was used as the control. Expression of Linc00472 in GC cells was confirmed using real-time RT-PCR.

Cell proliferation and cell migration. For cell proliferation analysis, 5000 living cells were plated onto 96-well plates. Cell growth was determined at 0, 1, 2, 3, and 4 days by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The migration of cells was tested *in vitro* in transwell chambers (Costar, Lowell, MA, USA). Cells were added to the upper chamber of a transwell. After incubation for 24 h at 37˚C, cells on the lower side were subjected to Giemsa staining. The level of migration was determined using a microscope at 200× magnification. All experiments were repeated three times.

Results

lncRNA expression profiles in epigenetic modulator-treated GC cells. In cancer biology, some specific dysfunctional lncRNAs have been found to be effective markers of several cancers. DNA methylation plays a vital role in regulating gene expression by establishing and maintaining the DNA methylation status of gene promoters. Therefore, we assumed that abnormal methylation patterns lead to gastric carcinogenesis through the hyper- or hypomethylation of promoters of lncRNAs. We used 5-Aza-dC treatment to achieve overall genomic DNA hypomethylation in AGS cells. Using the Illumina NGS platform, we applied a strand-specific protocol to generate strand-specific transcriptome data from AGS cells following treatment without and with 5-Aza-dC. In contrast to conventional transcriptome sequencing, the strand-specific protocol enabled distinguishing between sense and antisense transcripts. Hence, we determined the number of reads that were located individually in the sense and antisense strands of known transcripts. After analysis of strand-specific transcriptome sequence reads, more than 80 million clean sequence reads were generated, and nearly 88% of all the generated sequence reads could be mapped back to either transcripts or the genome, demonstrating the high quality of sequencing (Table I). The reads matching the sense or antisense strands of the known transcripts were used to evaluate the expression levels of known genes (70% of the total reads). Based on these transcriptome profiles, we identified 2403 protein-coding genes, 213 long non-coding genes (lncRNA), and 37 antisense long-coding genes (AsRNA). 5-Aza-dC treatment in AGS cells restored (>2-fold change) the expression of the aforementioned genes (Figure 1A). As shown in Figure 1B, the expression of only a small fraction of lncRNAs changed by two-fold or higher (fold change ≥2 or ≤2) between AGS cells treated with and without 5-Aza-dC.

Differentially expressed genes and pathway enrichment analysis. Our results showed that 2403 known protein-coding genes showed increased expression levels after 5-Aza-dC treatment (Figure 1A). Through pathway enrichment analysis, these DNA-associated protein-coding genes were shown to be significantly involved in cancer-related pathways, including the p53 pathway, focal adhesion, transcriptional misregulation in cancer, and TGF-beta, PI3K, and Wnt signaling (Figure 1C). These results revealed the function of DNA methylation-silencing of protein-coding genes in cancer-related signaling pathways and showed the high quality of NGS data from AGS control cells and AGS cells treated with 5-Aza-dC. Therefore, we further identified additional DNA-associated lncRNAs or antisense RNA from our NGS libraries and elucidated their biological function.

Identification of DNA hypermethylation-silencing lncRNA candidates in GC. The transcription mechanism of lncRNA is fundamentally similar to that of classic protein-coding genes; hence, the hypermethylation of the promoter region of tumor-suppressive lncRNAs may result in silencing of their expression during GC progression. Subsequently, we selected only lncRNA candidates that

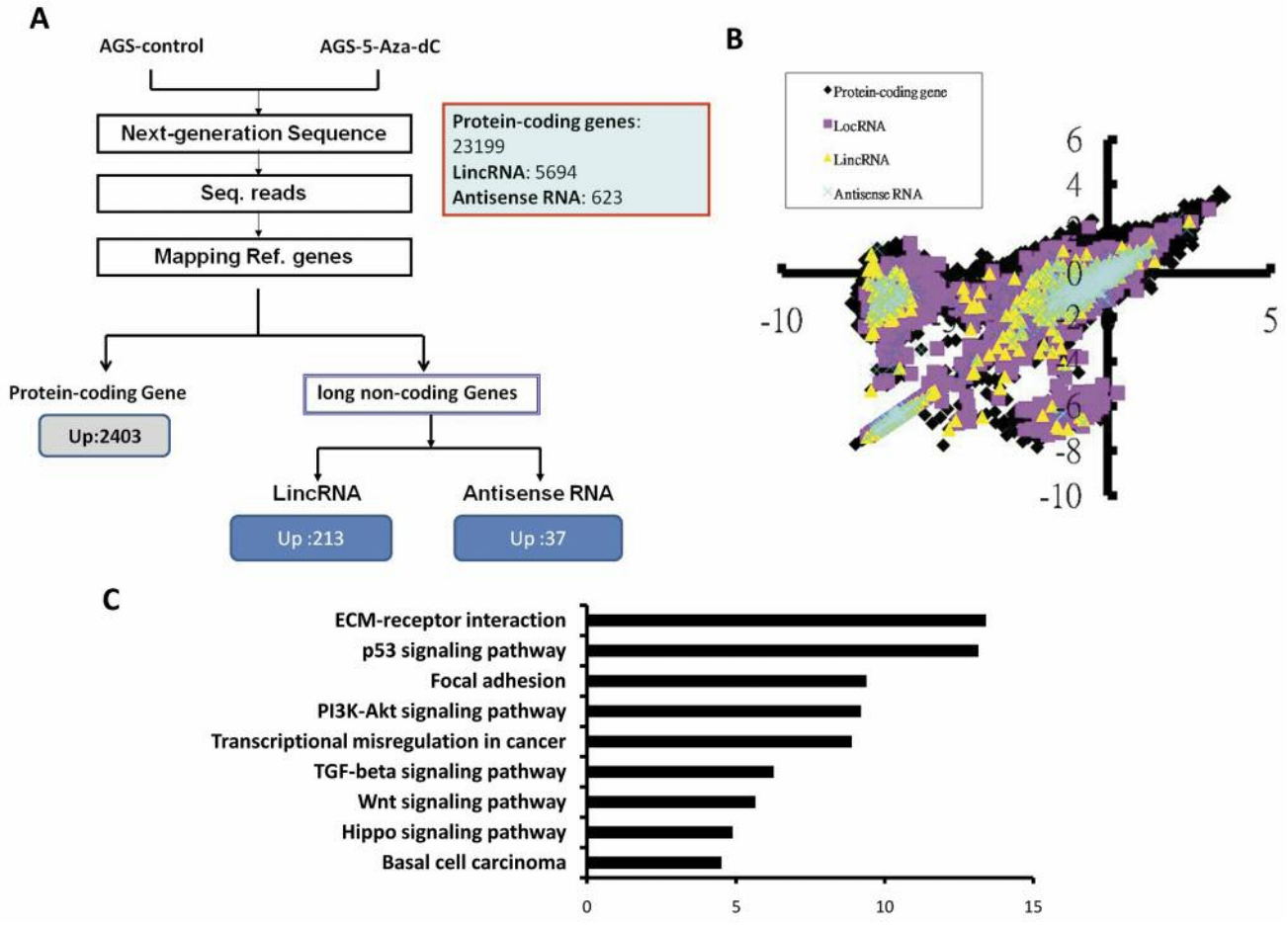

Figure 1. *Overall workflow analysis of DNA-associated long noncoding RNAs (lncRNAs) through next-generation sequencing (NGS) in AGS treated with 5-Aza-dC. (A) Flowchart to identify DNA methylation-associated lncRNA candidates through NGS. (B) Scatter plot of lncRNA distribution in AGS cells with 5-Aza-dC treatment versus control treatment. (C) Differentially expressed genes were identified and subjected to pathway enrichment analysis.*

exhibited ≥1 reads per kilobase per million reads (RPKM) in one of the libraries and the candidates that were up-regulated >2-fold in AGS cells treated with 5-Aza-dC in comparison with control cells. Furthermore, these lncRNAs must have had a CpG island in their upstream region. Based on these criteria, we identified 78 DNA methylation-silencing lncRNA candidates in GC, including 53 lncRNAs and 25 antisense RNAs (Table II). A previous study reported that Linc00472 plays a tumor suppressive role in several cancers, including colon cancer, breast cancer, and lung cancer (28-30). However, the detailed mechanism and biological function of Linc00472 in GC remain unknown. Therefore, we selected Linc00472 for further examination.

Experimental validation of Linc00472 expression silencing through DNA hypermethylation in GC cell lines. By searching the University of California Santa Cruz (UCSC) database, we identified CpG islands located in the putative transcription start sites of the linc00472 loci, suggesting that DNA methylation might control their transcriptional activities (Figure 2A). We also confirmed that Linc00472 was re-expressed in AGS cells after DNA demethylation treatment with 5-Aza-dC at various time points (0, 1, 2, 3, and 4 days after treatment). Our results showed that the expression of Linc00472 could be restored by treating AGS cells with 5-Aza-dC (Figure 2B). The methylation status of this CpG-rich region in six human GC cell lines was analyzed using the Combined Bisulfite Restriction Analysis (COBRA) approach. As shown in Figure 2C, CpG islands upstream of Linc00472 were hypermethylated in AGS, TSGH, and SUN-1 cells. After 4 days of 5-Aza-dC treatment, the transcriptional activity of Linc00472 was reactivated in AGS, TSGH, NCI-N87, and SUN-1 cells

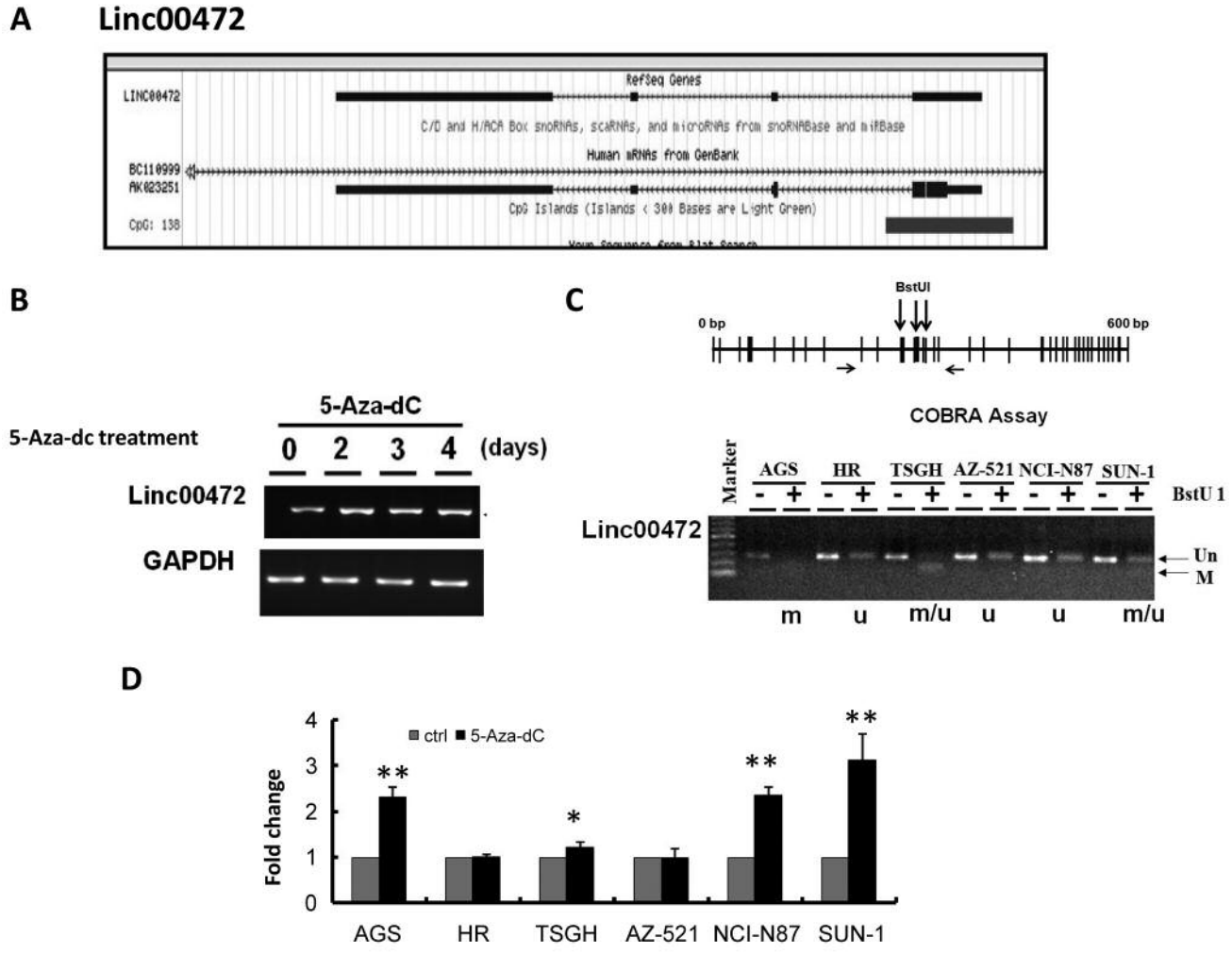

Figure 2. *Linc00472 expression was regulated epigenetically in gastric cancer (GC) cells. (A) Schematic representation of the location of linc00472; transcripts of its expressed sequence tags were identified from the UCSC website (http://genome.ucsc.edu/). The asterisk indicates the positions of the individual, neighboring CpG-rich regions of Linc00472. (B) The expression level of Linc00472 was examined in AGS cells with or without 5-Aza-dC treatment (5.0 μM) for 1, 2, 3, and 4 days. (C) The methylation status of CpG islands of Linc00472 was examined in six human GC cell lines by using the COBRA approach. The arrows indicate the unmethylated (u) and methylated (m) alleles. (D) The expression of Linc00472 was reactivated after 5-Aza-dC treatment in the six human GC cell lines.*

Table II. *DNA methylation-associated long noncoding RNAs were identified using the next-generation sequencing approach.*

LncRNA	Antisense RNA
LINC00925, LINC00200, LOC101927167, LOC100996662, LOC101927305, LINC00472, LOC100129520, LOC644554, LINC00221, LOC101930288, LINC00668, LOC101929151, LINC00452, LINC00960, LOC729083, LOC100507377, LOC730102, LOC284023, LOC101928595, LOC254896, LOC101929232, LOC101927476, LOC100132111, LOC728752, LINC00674, LOC101927929, LOC100505715, LOC100506634, LOC101928371, LOC648987, LOC101928844, LOC101928803, LOC642236, LOC100506282, LINC00884, LOC284561, LOC100289097, LOC100268168, LINC00337, LINC00665, LINC00842, LOC100379224, LOC100130093, LOC101928076, LINC00984, LOC101928605, LOC101927841, LOC101927974 LOC642533, LOC100506639, LOC100996246, LOC401010, LOC101927703	CELF2-AS1, PSMD5-AS1, HOXD-AS1, MYLK-AS1, ZNF571-AS1, HOXC-AS5, HEXA-AS1, GAS6-AS1, ZNF582-AS1, ZNF790-AS1, ZNF667-AS1, NDUFB2-AS1, NR2F1-AS1, GATA6-AS1, TP73-AS1, BOLA3-AS1, NDUFA6-AS1, GPR75-ASB3, GAS6-AS2, UBL7-AS1, PROSER2-AS1, FBXL19-AS1, SOCS2-AS1, ARHGAP5-AS1, NOP14-AS1

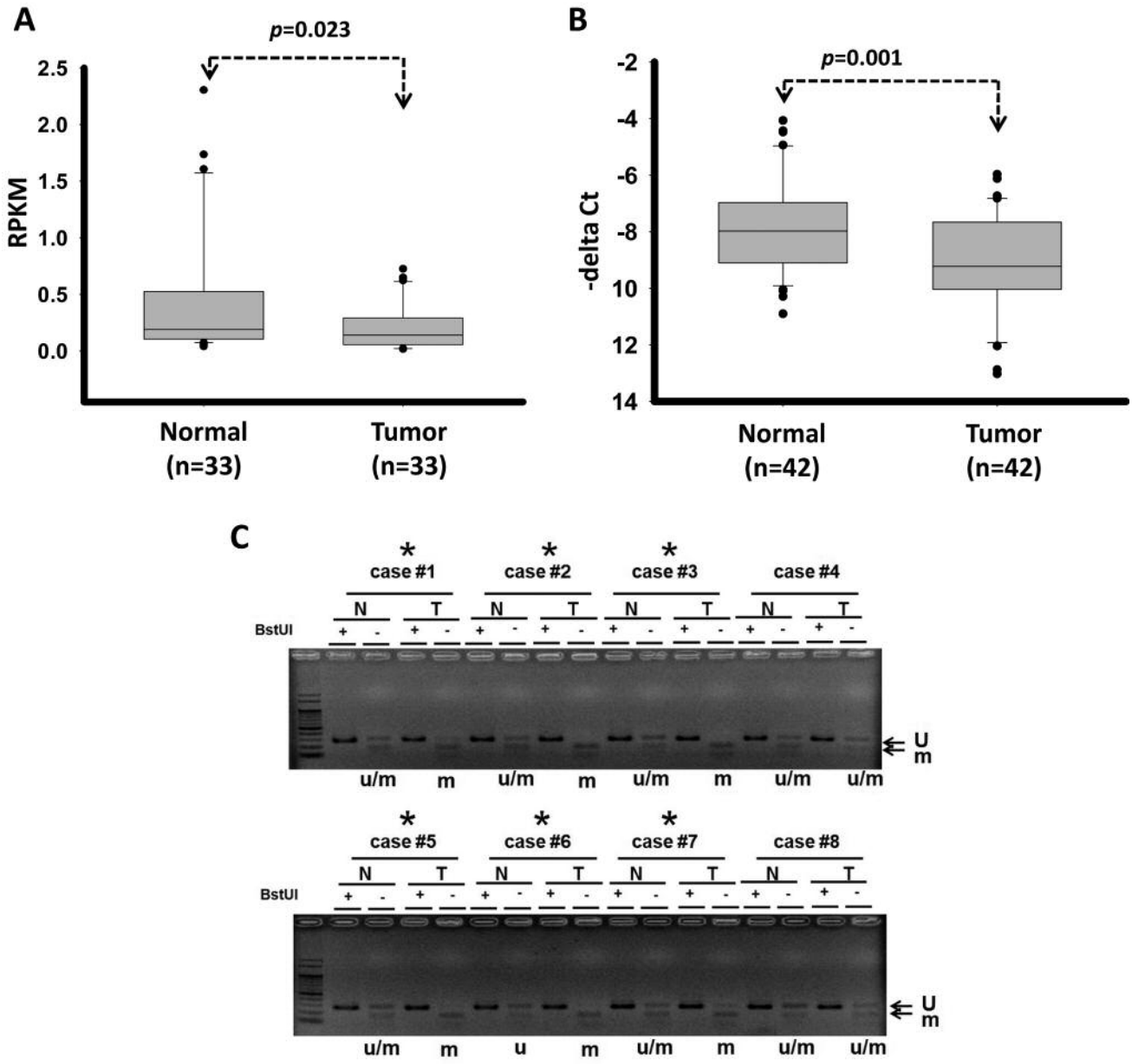

Figure 3. *Linc00472 expression level was analyzed in gastric cancer. (A) The expression level of Linc00472 was analyzed through RNA sequencing analysis using TCGA database. (B) Using the real-time PCR approach, the expression level of Linc00472 was examined in gastric cancer tissues and adjacent normal tissues from 42 gastric cancer patients. (C) The methylation status of CpG-rich regions upstream of Linc00472 was analyzed in gastric cancer tissues using COBRA. The arrows indicate the unmethylated (u) and methylated (m) alleles. Asterisks indicate tumor-specific methylation in gastric cancer.*

(Figure 2D). These results indicate that the Lnc00472 gene can be regulated epigenetically in GC cells through DNA methylation.

Linc00472 expression silencing through DNA hyper-methylation in GC. Next, we examined the expression level of Linc00472 in GC by analyzing The Cancer Genome Atlas (TCGA) database, Gene Expression across Normal and Tumor tissue database (GENT), and our clinical samples. According to the TCGA database, the expression level of Linc00472 was significantly decreased in GC (Figure 3A). Using real-time PCR, we examined the expression level of Linc00472 in clinical tissues from 42 GC patients. Our data revealed that Linc00472 expression was significantly

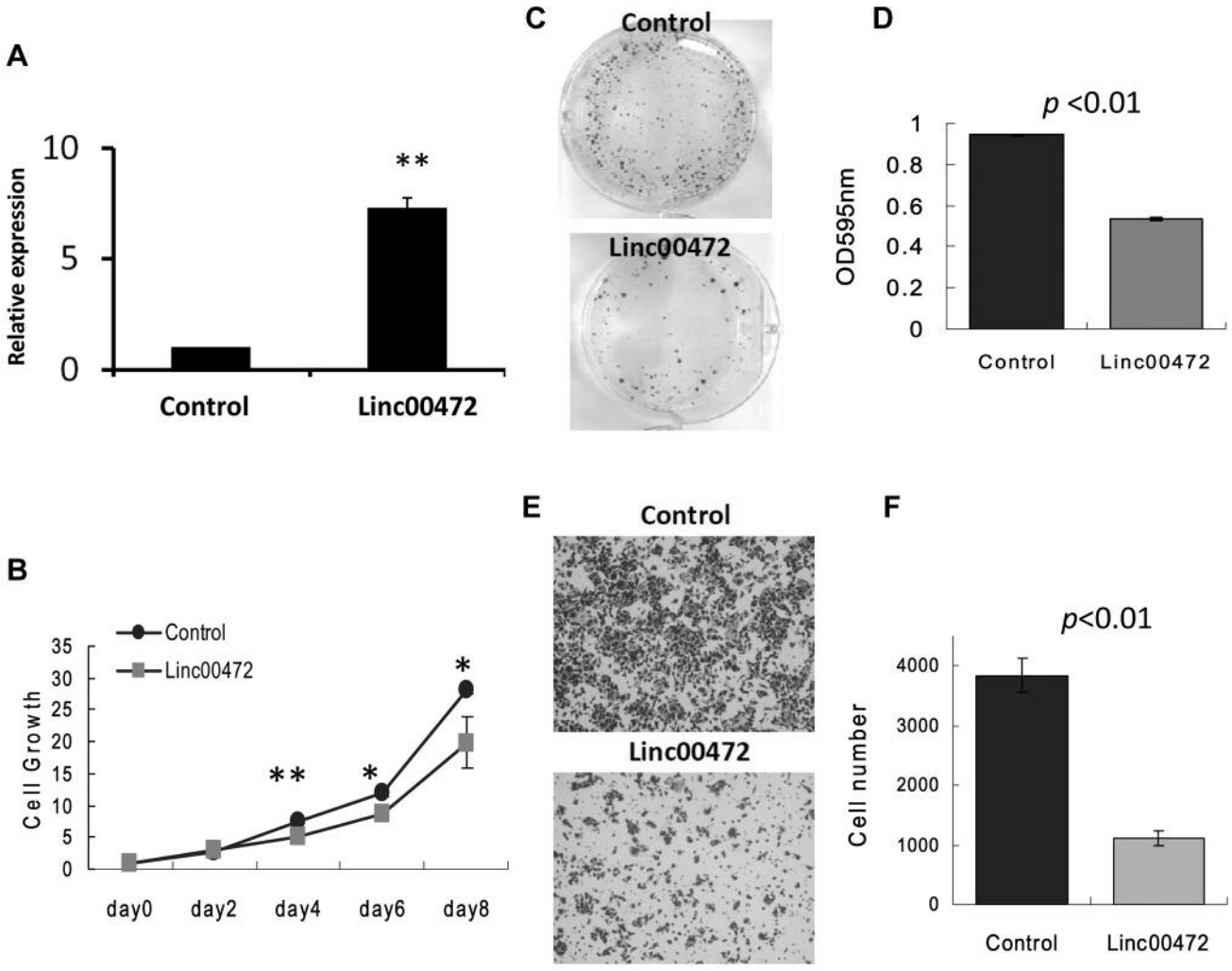

Figure 4. *Linc00472 played a tumor suppressive role through suppressing gastric cancer cell growth and motility. (A) The expression level of Linc00472 was examined in AGS cells with or without Linc00472 stable expression by using real-time PCR. (B) Cell proliferation was measured at various points (0, 1, 2, 3, and 4 days) in AGS cells with Linc00472 expression and was compared with the scramble control. (C) The colony formation assay was performed in AGS cells with or without Linc00472 stable expression for 2 weeks. These cells were fixed and stained with crystal violet solution, and relative colony formation was quantified. (D) The migration abilities of cells were examined using the transwell assay. Migrating cells were stained with crystal violet solution and were enumerated by counting three fields under a phase-contrast microscope. (E) Relative migration abilities were quantified in AGS cells with and without Linc00472 stable expression.*

reduced in GC tissues compared with adjacent normal tissues (linc00472: 37 out of 42, p=0.001) (Figure 3B). We further examined the methylation status of the CpG islands of Linc00472 in GC tissues and adjacent normal tissues from eight patients. The results revealed that DNA methylation was frequently observed in GC tissues (Linc00472: 8 out of 8). The tumor-specific hypermethylation of the CpG region upstream of Linc00472 was frequently observed in GC tissues (Linc00472: 6 out of 8), which may result in its decreased expression in GC tissues compared with adjacent normal tissues (Figure 3C).

Linc00472 expression suppresses GC cell growth. To examine the biological function of Linc00472 in GC cells, we established a stable Linc00472-expressing AGS cell line through transfection with the pLinc00472 expression vector. As shown in Figure 4A, the expression level of Linc00472 significantly increased in AGS cells with Linc00472 stable expression compared with control cells. The ectopic expression of Linc00472 significantly suppressed AGS cell proliferation, colony formation, and cell migration ability (Figure 4B-F). These results revealed that Linc00472 plays a tumor suppressive role in GC.

Discussion

Previous studies have reported that several protein-coding genes in GC cells are modulated through DNA methylation. Using a microarray, Mikata *et al*. identified elevated expression levels of DKK1, ZNF33A, NMU, EMP3, DHRSX, LEPR, SLAMF7, TKTL1, VCX, GAGE7, SSX3, PAGE5, BCL2L10, and XCR1 in GC cells with DNA demethylation treatment (31). Similarly, using the microarray approach, Yamashita *et al*. revealed that 42 genes (*ABHD9, ADFP, ALDH1A3, ANXA5, AREG, BDNF, BMP7, CAV1, CDH2, CLDN3, CTSL, EEF1A2, F2R, FADS1, FSD1, FST, FYN, GPR54, GREM1, IGFBP3, IGFBP7, IRS2, KISS1, MARK1, MLF1, MSX1, MTSS1, NT5E, PAX6, PLAGL1, PLAU, PPIC, RBP4, RORA, SCRN1, TBX3, TFAP2C, TNFSF9, ULBP2, WIF1, ZNF177*, and *ZNF559*) were methylated in AGS cells (32). In our study, the restored expression of lncRNA candidates was consistently detected in AGS cells following 5-Aza-dC treatment (>2-fold change; data not shown). These results support the protein-coding gene data of previous studies and showed the high quality of NGS data from AGS control cells and AGS cells treated with 5-Aza-dC in our study. Except for protein-coding genes, we identified several lncRNAs that were modulated in AGS cells through DNA hypermethylation. In our previous study, SOX21 and its antisense RNA SOX21-AS1 were co-silenced in oral cancer cells through DNA hypermethylation (27). In the present study, we identified several antisense RNA transcripts that might share bidirectional promoters with their sense RNA transcripts. Previous studies have reported that the sense transcripts of some of the lncRNA candidates identified in the present study, including GAS6, GATA6 MYLK, ZNF582, GPR75, GAS6, and SOCS2, could be regulated in human cancer through DNA methylation (33-38). In addition, HOTAIR is a commonly applied lncRNA transcribed from the HOX locus; it affects the chromatin methylation state of the HOXD locus through recruitment of the polycomb repressive complex (PRC2). Other HOX gene regions also display myogenic DNA hypermethylation despite their moderate expression in human cancer cells (39). In the present study, 5-Aza-dC treatment restored antisense RNA expression within HOX locus regions such as HOXC-AS5 and HOXD-AS1.

Based on our profiles, we identified two antisense RNA candidates, namely ANF582-AS1 and ZNF-667-AS1, which had been reported to be silenced by DNA hypermethylation in human cancers (40, 41). ZNF582-AS1 plays a tumor suppressive role by suppressing the colony formation of colon cancer cells, and its expression is frequently silenced through the DNA hypermethylation of promoters in colon cancers. The methylation status of ZNF582-AS1 and its ectopic expression are associated with poor survival of CRC patients (40). Meng *et al*. observed the aberrant hypermethylation of CpG sites of ZNF667-AS1 in laryngeal squamous cell carcinoma, resulting in its gene expression silencing, and its expression level was well associated with moderate/poor pathological differentiation (41). However, the detailed function and mechanism of long noncoding antisense RNAs in GC remains unclear. Except for antisense RNAs, we identified several intergenic long noncoding RNA candidates that might be regulated in GC through DNA methylation. Among them, Linc00472 expression is silenced in breast cancer and colon cancer through DNA methylation (28, 29). This is the first study to report that Linc00472 expression significantly decreased after promoter hypermethylation in GC tissues compared with adjacent normal tissues. Similarly, previous studies have revealed that Linc00472 was significantly down-regulated in breast cancer, ovarian cancer, colon cancer, and hepatocellular carcinoma (29, 30, 42-44). Furthermore, the low expression of Linc00472 was significantly associated with poor prognosis of patients with breast cancer, lung cancer, colon cancer, or hepatocellular carcinoma ovarian cancer (28, 29, 42, 43). In the present study, our data revealed that Linc00472 could be silenced in GC through DNA hypermethylation. However, according to TCGA database analysis, the expression level of linc00472 was not correlated with GC prognosis (data not shown). Ye *et al*. reported that Linc00472 could suppress colon cancer cell proliferation and induce cell apoptosis by elevating PDCD4 expression through sponging miR-196a (30). In the present study, we observed similar effects: Linc00472 plays a tumor suppressive role in GC cell growth and motility. In addition, our previous study indicated that miR-196a was significantly overexpressed and played an oncogenic role in regulating GC cell proliferation and motility (45). Su *et al*. also indicated that Linc00472 inhibits lung cancer cell proliferation and promotes lung cancer cell apoptosis by regulating the miR-24-3p–DEDD axis (43). Chen *et al*. also reported that Linc00472 is an important tumor suppressor that modulates the growth and motility of human HCC through the miR-93-5p–PDCD4 axis (42). Furthermore, miR-93 expression may promote GC cell growth and motility by modulating the cancer-related pathway (46-48). Taken together, these results indicate that Linc00472 suppresses GC cell growth and motility potentially through sponging oncogenic miRNA expression, such as miR-196a, miR-93, and miR-24-3p. In addition, our present results revealed that Linc00472 was mainly localized to the nucleus of GC cells. In general, the lncRNA–miRNA interaction frequently occurred in the cytoplasm of cells. Therefore, Linc00472 exerts its tumor suppressive function through the protein–Linc00472 or DNA–Linc00472 interaction. More experiments should be conducted in the future to elucidate the detailed mechanism.

Conclusion

Epigenetically regulated Linc00472 expression plays a crucial role in modulating gastric cancer cell growth and motility. Our finding provided the new insight that Linc00472 is a potential therapeutic target for GC.

Availability of data and materials

All NGS data were submitted to NCBI GEO.

Conflicts of Interest

The Authors declare that they have no competing interests regarding this study.

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Authors' Contributions

KWT executed this study and wrote the draft of this manuscript. SCL performed analysis of NGS data. CYT, NHC, KCW and CHK performed clinical samples collection and analysis. YHL performed the statistical analysis. CYT, NHC, KCW and CHK helped prepare manuscript. KWT and HTC supervised the study and edited the manuscript.

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