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LncRNA TRHDE-AS1 and MiR-1275 as Promising Prognostic Biomarkers and Effects on Tumor Cell Progression in Gastric Cancer

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Gastric cancer is a common malignant tumor with a relatively poor prognosis after surgery. Non-coding RNA may serve as biomarkers for the progression and prognosis of various cancers. The clinical significance and biological function of lncRNA TRHDE-AS1 and miR-1275 in gastric cancer were assessed in this study. 119 paired tissues were selected with adequate clinical information. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the expression level of lncRNA TRHDE-AS1 and miR-1275 in gastric cancer tissues and cells. The association between lncRNA TRHDE-AS1 or miR-1275 expression and the clinicopathological features of patients was analyzed by the Pearson Chi-square test. Kaplan-Meier analysis and Multi-variate Cox proportional regression analysis were utilized to evaluate the prognostic value of lncRNA TRHDE-AS1 and miR-1275. Finally, the effect of TRHDE-AS1 binding to miR-1275 on the gastric cancer cellular process was investigated by CCK-8 and Transwell assay. LncRNA TRHDE-AS1 was found to be downregulated in gastric cancer tissues and cells, but miR-1275 upregulated, which both showed significant associations with clinical pathology of gastric cancer patients (including TNM stage and lymph node metastasis) and a poor prognosis. LncRNA TRHDE-AS1 and miR-1275 can be considered two independent prognostic factors for gastric cancer. Furthermore, the upregulation of lncRNA TRHDE-AS1 inhibited cell proliferation, migration, and invasion of gastric cancer partly by miR-1275. LncRNA TRHDE-AS1/miR-1275 axis may be involved in the progression of gastric cancer and can be promising prognostic factors, which may provide new insights into the treatment of gastric cancer.

Keywords: gastric cancer; miR-1275; prognosis; progression; TRHDE-AS1
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Introduction

Gastric cancer is a high-recurrence-rate malignancy neoplasm that ranks third in cancer-related death cause worldwide (Smyth et al. 2020). The highest incidence and mortality rates of gastric cancer among both males and females are found in Eastern and Western Asia, including China (Torre et al. 2016; Machlowska et al. 2020). Gastric cancer incidence and mortality rates have been slightly declining attributable to the declining prevalence of *Helicobacter pylori* (HP) infection, the availability of fresh produce, and less salt (Ford et al. 2020; Smyth et al. 2020). However, it is noteworthy that rates of gastric cancer will be increasing though obesity has reached epidemic proportions worldwide (Garai et al. 2015). Due to the high incidence of recurrence, the survival and prognoses of gastric cancer patients are still unsatisfactory if diagnosed at an advanced stage, and efforts to prolong survival in metastatic gastric cancer have shown little improvement (Song et al. 2017; Ferlay et al. 2019). Thus, it is pivotal to discover promising prognostic factors and identify new reliable molecular prognostic factors of gastric cancer.

Benefited from modern molecular biological theory, the biogenesis and function of many non-coding RNAs

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(ncRNAs) are well described, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) (Guil and Esteller 2015; Hombach and Kretz 2016). MiRNAs are termed as a class of small cellular RNAs with 18 to 24 nucleotides, whose function is to bind the 3' untranslated region (3'UTR) of the target gene and regulate gene expression by impairing the translation (Catalanotto et al. 2016; Matsuoka and Yashiro 2018). MiRNAs are key players in regulating kinds of biological processes of the cell, such as proliferation, differentiation, migration, and invasion (Matsuoka and Yashiro 2018). To distinguish from small noncoding RNAs, lncRNAs are frequently defined as non-protein-coding transcripts larger than 200 nt. The molecular functions of lncRNAs are very diverse, and one of the best-studied aspects is their role in the transcriptional regulation of allelic expression at different levels (Boon et al. 2016). On account of the tissue-specific expression and genome-wide expression patterns in differential tissues, lncRNAs hold strong promise as novel biomarkers and therapeutic targets for cancers (Huarte 2015). LncRNA TRHDE-AS1 (lnc-TRHDE-AS1) has been selected as one of the factors in a risk score model for gastric cancer (Wu et al. 2021). However, the single prognosis value of lnc-TRHDE-AS1 and miR-1275 and the effects on gastric cancer have been elaborated.

In this article, we have investigated the expression of lnc-TRHDE-AS1 and its potential role in gastric cancer tissues and cells. The prognostic value of miR-1275 was estimated, too. With the investigation of the underlying mechanism of the cell function, their potential implications in cancer therapy were discussed.

Materials and Methods

Patients and collection of tissues

Our study is comprised of 119 patients afflicted with gastric cancer, enrolled at our hospital. All patients underwent surgical resection by endoscope, laparotomy, or laparoscopy from 2012 to 2015. Prior to surgery, no one has received any treatment targeted at gastric cancer. Paired gastric samples, including cancerous tissues and normal tissues adjacent to cancer, were fresh-frozen and stored at −80°C until analysis. Patients' overall survival monitoring was performed via follow-up visits from the day of surgery. The staging of gastric cancer was classified according to the seventh edition of the American Joint Committee on Cancer (AJCC) Staging Manual.

The study protocol was approved by The Ethics Committee of Shandong Provincial Third Hospital, Shandong University. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written informed consent.

Cell lines and culture

For this study, a set of four gastric cancer cell lines namely AGS, NCI-N87, SNU-1, and KATO-III, and one immortalized normal gastric epithelial cell line namely GES-1 were utilized. AGS, NCI-N87, SNU-1, and KATO-III cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). GES-1 was purchased from Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All cell lines were tested and free of mycoplasma contamination using Mycoplasma Detection Kit (Roche, Mannheim, Germany). All cell lines were grown in RPMI 1640 medium (Gibco, San Francisco, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) in a humidified incubator (37°C, with 5% CO_2).

Cell transfection with pcDNA3.1- TRHDE-AS1 and/or miR-1275 mimic

The overexpression plasmid pcDNA3.1-TRHDE-AS1 and miR-1275 sequence (miR mimic) were designed and synthesized by RiboBio (Guangzhou, China), and the empty pcDNA3.1 vector or miR-1275 negative control (mimic NC) served as the control. Prior to transfection, cells were cultured in antibiotic-free medium to reach 80% cell confluence. Then cells were transfected with the addition of Lipofectamine 3000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) abiding by the application note. The transfection efficiency was confirmed by lnc-TRHDE-AS1 and miR-1275 expression using qRT-PCR.

Total RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Through TRIzol reagent (Invitrogen), total RNA isolation was conducted from gastric cancer tissues, adjacent normal tissues, and cultured cells. For analysis of miR-1275 expression, cDNA was synthesized using the MicroRNA Reverse Transcription Kit (Invitrogen, Foster City, CA, USA). For lnc-TRHDE-AS1 quantification, lnRcute lncRNA First-Strand cDNA Synthesis Kit (With gDNase) (Tiangen, China) was used for reverse transcription. The primer sequences were as follows: forward primer 5′-CGCTTGTGTACGGCGATGTG-3′ and reverse primer 5′-CTGCTGCGAGCACATTCCAC-3′ for lncRNA TRHDE-AS1; forward primer 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3' and reverse primer 5'-CATGTGGGCCATGAGGTCCACCAC-3' for Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*); stem-loop primer 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGACAGCCT-3', forward primer 5′-ACACTCCAGCTCAGGTGGGGGAGAGGCTGTC-3' and reverse primer 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAG-3' for miR-1275; forward primer 5′-CTCGCTTCGGCAGCACA-3' and reverse primer 5'-AACGCTTCACGAATTTGCGT-3' for *U6*. All qRT-PCR reactions were run on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) and Ct values were normalized firstly to those of their respective reference genes (*GAPDH* for lnc-TRHDE-AS1 and *U6* RNA for miR-1275 expression), secondly to the

mean of the control samples ($\Delta\Delta$Ct) and expressed using the result of $2^{-\Delta\Delta Ct}$.

CCK-8 assay

The proliferation of AGS and NCI-N87 cells was evaluated by the CCK-8 assay (Dojindo, Kumamoto, Japan), based on the manufacturer's instructions. After successful transfection, 1×10^4 transfected cells per well were seeded in 96-well plates, which were divided into an untreated group (only cells), pcDNA-TRHDE-AS1 group, pcDNA group, miR-1275 mimic group, and miR-NC group. The cells were cultured for 24, 48, and 72 h before the addition of CCK-8 to the culture medium in each well. After incubation for another 2 h at 37°C, absorbance at 450 nm was measured using a multifunctional microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Colony formation assay

AGS cells were resuspended sufficiently in the medium to get single-cell suspension. After counting the number of cells in the suspension, 800 cells per well were seeded into 6-well plates. The plates were then incubated for about 14 days. After washing, the cell colonies were fixed with ethanol, stained with crystalline violet, and taken photographs.

Transwell assay

Migration and invasion of AGS and NCI-N87 cells were quantified utilizing 24-well-8-μm transwell chamber (Thermo Fisher Scientific). Transfected cells were seeded in 24-well plates at a density of 5×10^4 cells per well. In the migration assay, the upper chamber was filled with medium without serum, while the lower chamber was filled with 10% FBS medium. After 48 h, cells were fixed with 4% formaldehyde and methanol. After scraping off non-migrated cells with cotton swabs, the number of migrated cells was counted under an inverted microscope. In the invasion assay, the chambers were coated with Matrigel (BD Biosciences, San Jose, CA, USA), the other steps were the same as the migration assay.

Dual-luciferase reporter assay

The luciferase reporter assay was performed to clarify the binding between lnc-TRHDE-AS1 and miR-1275. WT-TRHDE-AS1 (The fragment from wild-type lncRNA TRHDE-AS1 containing the predicted miR-1275 binding site, 5'-UAGCAAGCCUCAUCAUCAUAAGCCCCCAA-3') and MUT-TRHDE-AS1 (the fragment containing the potential miR-1275 binding site mutations, 5'-UAGCAUCGGAAUCAUCAUAAGGGGGGUA-3') were synthesized by RiboBio and subcloned into the pmirGLO luciferase vector (Promega, Madison, WI, USA). AGS cells were co-transfected with the above luciferase reporter vectors containing WT-TRHDE-AS1 (or MUT-TRHDE-AS1) together with the miR-1275 mimic, or their negative controls (miR-NC) using Lipofectamine 3000 (Invitrogen). After transfection for 48 h, the relative luciferase activity was analyzed by the Dual-Luciferase Reporter Assay System (Promega).

Statistical analysis

The data are represented as mean ± standard deviation (SD) from triple experiments, and the statistical analysis was performed with IBM SPSS Statistics 23 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software Inc., Boston, MA, USA). Assessment of the difference between the two groups was carried out with a student's t-test. Pearson Chi-square test was used to analyze the relationship between lnc-TRHDE-AS1 expression and clinicopathological features of patients, as well as to associate miR-1275 expression with patients' characteristics. Survival analysis was depicted by Kaplan-Meier curves using log-rank test. Multi-variate Cox proportional regression analysis was applied to analyze the prognostic value of parameters in gastric cancer. Pearson Correlation Coefficient was adopted for the correlation between lnc-TRHDE-AS1 expression and miR-1275 expression. $P < 0.05$ was statistically significant.

Results

Aberrant levels of lnc-TRHDE-AS1 and miR-1275 expression are detected in gastric cancer tissues and cells

Through the retrieval of relevant databases, we found that lnc-TRHDE-AS1 was downregulated while miR-1275 was upregulated in stomach adenocarcinoma tissues compared with that in normal tissues, respectively (Supplementary Fig. S1). To verify the expression of lnc-TRHDE-AS1 and miR-1275 expression in human gastric cancer tissue and cells, qRT-PCR was performed. The expression of lnc-TRHDE-AS1 is significantly reduced in gastric cancer tissues compared to the matched adjacent normal tissues, as well as in cells ($P < 0.01$, Fig. 1A, B). While the miR-1275 level in gastric cancer tissues and cells was significantly higher than that in gastric para-cancer normal tissues and cells ($P < 0.001$, Fig. 1C, D).

lnc-TRHDE-AS1 could bind to miR-1275

LncBase Predicted v.2 database (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2%2Findex-predicted) showed that lnc-TRHDE-AS1 carries putative miR-1275 targeting sites (Fig. 2A). The expression level of miR-1275 was significantly negatively associated with lnc-TRHDE-AS1 expression level in paired tumor tissues and normal tissues (Pearson $r = -0.8233$, $P < 0.0001$, Fig. 2B). Transfection of pcDNA-TRHDE-AS1 can upregulate miR-1275 level in AGS and NCI-N87 cells ($P < 0.001$, Fig. 2C, D). Besides, we constructed luciferase reporters containing WT-TRHDE-AS1 or MUT-TRHDE-AS1. We found that transfection of miR-1275 mimics reduced the luciferase activities of the wild-type lnc-TRHDE-AS1 reporter vector, but not empty vector or mutant reporter vector in AGS and NCI-N87 cells ($P <$

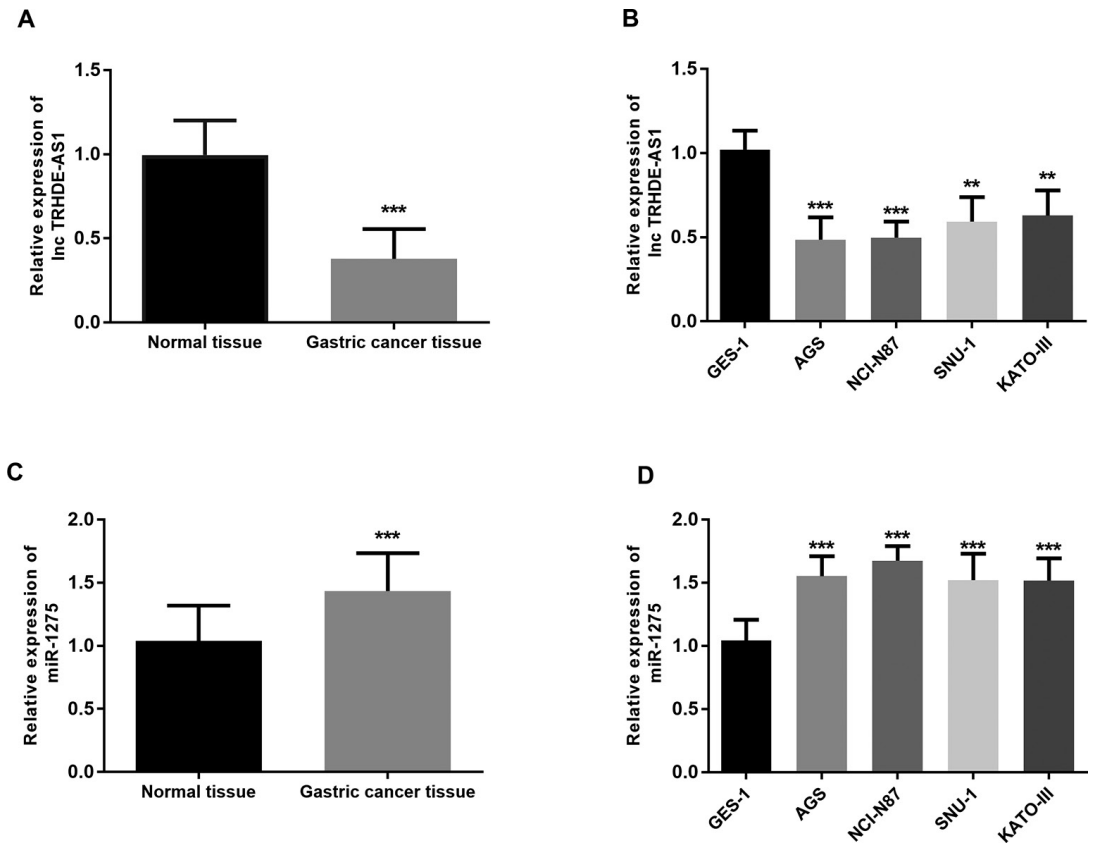

Fig. 1. Lnc-TRHDE-AS1 with a decreased expression and miR-1275 increased in gastric cancer tissues and cell lines. A. The expression of lnc-TRHDE-AS1 in gastric cancer tissues and adjacent normal tissues (***$P < 0.001$). B. The expression of lnc-TRHDE-AS1 in gastric cancer cell lines (AGS, NCI-N87, SNU-1 and KATO-III) and normal gastric cell (GES-1) (**$P < 0.001$, ***$P < 0.001$). C. The expression of miR-1275 in gastric cancer tissues and adjacent normal tissues (***$P < 0.001$). D. The expression of miR-1275 in gastric cancer cell lines (AGS, NCI-N87, SNU-1 and KATO-III) and normal gastric cell (GES-1) (***$P < 0.001$). Data were shown as the mean of triple samples with the error bar (SD).

0.001, Fig. 2E, F). So, lnc-TRHDE-AS1 could bind to miR-1275.

Expression levels of lnc-TRHDE-AS1 and miR-1275 are correlated with clinicopathological factors of gastric cancer

A total of 119 gastric cancer patients were entered into this study, and complete clinicopathological characteristics of all these patients can be obtained. Patients were divided into two groups based on the median value of the lnc-TRHDE-AS1 relative expression (0.340) or miR-1275 relative expression (1.457) in gastric cancer tissues. Chi-square analyses in Table 1 showed a significant association between lnc-TRHDE-AS1 expression levels and clinical pathology of gastric cancer patients, including TNM stage ($P = 0.028$) and lymph node metastasis ($P = 0.012$). Moreover, miR-1275 levels in cancer tissues were also significantly associated with TNM stage ($P = 0.031$) and lymph node metastasis ($P = 0.034$).

lnc-TRHDE-AS1 and miR-1275 predict poor prognosis of gastric cancer

To determine the clinical significance of lnc-TRHDE-AS1 in gastric cancer, Kaplan-Meier curves using the log-rank test and multi-variate Cox proportional regression analysis were employed. Notably, the low expression of lnc-TRHDE-AS1 in gastric cancer tissues significantly lowered the five-year overall survival rate of gastric cancer patients ($P = 0.006$, Fig. 3A). The group with low expression of lnc-TRHDE-AS1 had a nearly 35% disadvantage in five-year overall survival. Furthermore, multivariate analyses of the related parameters also revealed that lnc-TRHDE-AS1 levels in cancer tissues were with unfavorable significance for the gastric cancer patient's survival (95% CI = 1.301-5.924, $P = 0.008$, Table 2). Therefore, a low level of lnc-TRHDE-AS1 may predict a poor prognosis of gastric cancer and has the potential to be an independent prognostic predicted factor.

The prognostic significance of miR-1275 level for gastric cancer was also studied. From the obtained survival information of the patients, the Kaplan-Meier curve was conducted and shown in Fig. 3B ($P = 0.021$). Patients with higher miR-1275 levels suffered from significantly shorter overall survival. The correlation of high expression of miR-1275 with the poor overall survival of gastric patients was also confirmed by Cox proportional regression analysis in Table 3 (95% CI = 1.325-6.416, $P = 0.008$). So, miR-

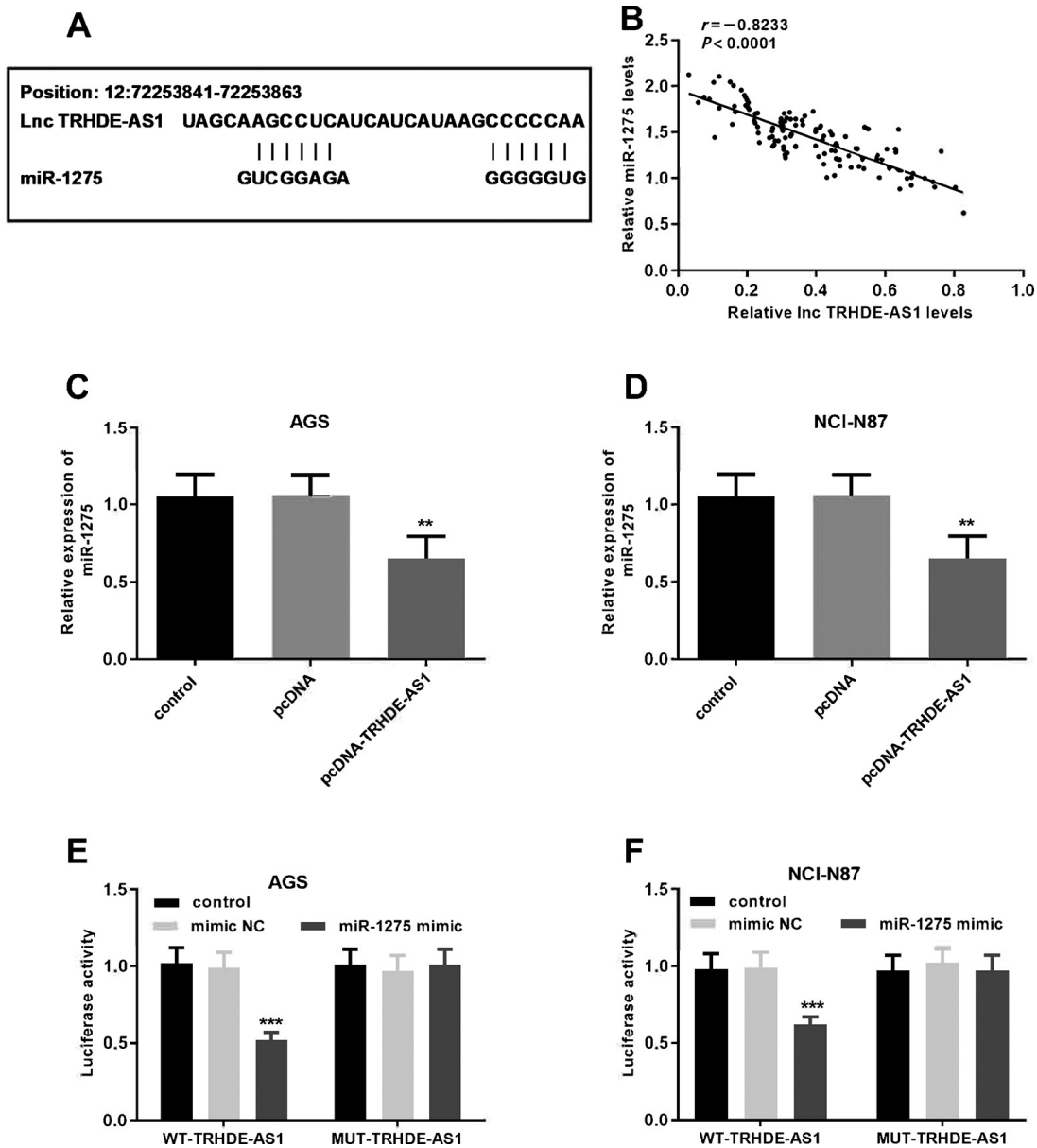

Fig. 2. Lnc-TRHDE-AS1 directly targets miR-1275 in gastric cancer.
A. Binding sites between the lnc-TRHDE-AS1 and miR-1275. B. The reverse correlation between lnc-TRHDE-AS1 expression and miR-1275 expression (r = −0.8233, $P < 0.0001$). C and D. Overexpression of lnc-TRHDE-AS1 inhibited the expression of miR-1275 (**$P < 0.01$). E and F. The luciferase activity of lnc-TRHDE-AS1 was significantly suppressed by the overexpression of miR-1275 (***$P < 0.001$). Data were shown as the mean of triple samples with the error bar (SD).

1275 can be a powerful and independent prognostic marker for gastric cancer.

Overexpression of lnc-TRHDE-AS1 suppressed cell proliferation of gastric cancer cells, but miR-1275 can revoke it

Furthermore, we tried to explore the regulatory role of lnc-TRHDE-AS1 in the proliferation of gastric cancer cells. The pcDNA-TRHDE-AS1 can successfully upregulate the expression of lnc-TRHDE-AS1 and downregulated the expression level of miR-1275, while miR-1275 mimic can successfully recover the suppressed expression caused by pcDNA-TRHDE-AS1 ($P < 0.001$, Fig. 4A, B). CCK8 assays showed that the overexpression of lnc-TRHDE-AS1 by pcDNA-TRHDE-AS1 significantly hindered the proliferation rate of gastric cancer cells in 72 h, however, upregulated expression of miR-1275 can offset this inhibition ($P < 0.001$, Fig. 4C, D). In addition, colony formation assay was performed in pc-DNA, pcDNA-TRHDE-AS1, and pcDNA-TRHDE-AS1 + miR-1275 groups. It is found that lnc-TRHDE-AS1 overexpression suppressed the colony formation of AGS cells, but miR-1275 mimic can offset that (Fig. 4E). These results implied lnc-TRHDE-AS1 can inhibit the proliferation of gastric cancer cells but miR-1275 promotes it.

Table 1. The association between lnc-TRHDE-AS1 expression and clinicopathological characteristic of patients.

	Patients (n = 119)	Low lnc-RHDE-AS1 expression (n = 63)	High lnc-TRHDE-AS1 expression (n = 56)	*P* value	Low miR-1275 expression (n = 58)	High miR-1275 expression (n = 61)	*P* value
Age							
≤ 65	60 (50.4%)	30 (47.6%)	30 (53.6%)	0.517	26 (44.8%)	34 (55.7%)	0.234
> 65	59 (49.6%)	33 (52.4%)	26 (46.4%)		32 (55.2%)	27 (44.3%)	
Sex							
Male	66 (55.5%)	33 (52.4%)	33 (58.9%)	0.473	31 (53.4%)	35 (57.4%)	0.666
Female	53 (44.5%)	30 (47.6%)	23 (41.1%)		27 (46.6%)	26 (42.6%)	
Tumor size							
< 3cm	65 (54.6%)	30 (47.6%)	35 (62.5%)	0.104	34 (58.6%)	31 (50.8%)	0.393
⩾ 3cm	54 (45.4%)	33 (52.4%)	21 (37.5%)		24 (41.4%)	30 (49.2%)	
Invasion depth							
T1/T2	57 (47.9%)	25 (39.7%)	32 (57.1%)	0.057	33 (56.9%)	24 (39.3%)	0.055
T3/T4	62 (52.1%)	38 (60.3%)	24 (42.9%)		25 (43.1%)	37 (60.7%)	
Histological type							
Differentiated	59 (49.6%)	27 (42.9%)	32 (57.1%)	0.120	34 (58.6%)	25 (41.0%)	0.054
Undifferentiated	60 (50.4%)	36 (57.1%)	24 (42.9%)		24 (41.4%)	36 (59.0%)	
TNM Stage							
I-II	66 (55.5)	29 (46.0%)	37 (66.1%)	0.028	38 (65.5%)	28 (45.9%)	0.031
III-IV	53 (44.5%)	34 (54.0%)	19 (33.9%)		20 (34.5%)	33 (54.1%)	
Lymph node metastasis							
Negative	62 (52.1%)	26 (41.3%)	36 (64.3%)	0.012	36 (62.1%)	26 (42.6%)	0.034
Positive	57 (47.9%)	37 (58.7%)	20 (35.7%)		22 (37.9%)	35 (57.4%)	
HP infection							
Negative	50 (42.0%)	25 (39.7%)	25 (44.6%)	0.584	25 (43.1%)	25 (41.0%)	0.815
Positive	69 (58.0%)	38 (60.3%)	31 (55.4%)		33 (56.9%)	36 (59.05)	

Data were shown as n (%).
HP, *Helicobacter pylori*.

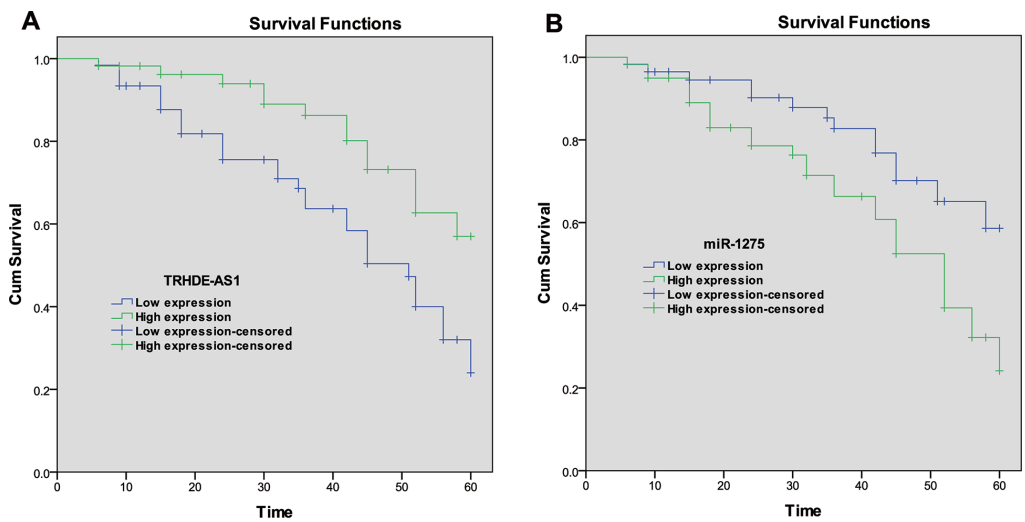

Fig. 3. Kaplan-Meier curves.
A. Kaplan-Meier curve of patients based on lnc-TRHDE-AS1 expression (Log rank $P = 0.006$). B. Kaplan-Meier curve of patients based on miR-1275 expression (Log rank $P = 0.021$).

Upregulated lnc-TRHDE-AS1 inhibits cell migration and invasion of gastric cancer cells, but miR-1275 can resume it

From transwell assay, the migratory abilities were attenuated by pcDNA-TRHDE-AS1 transfection in AGS and NCI-N87 cells but resumed by co-transfection with pcDNA-TRHDE-AS1 and miR-1275 mimic ($P < 0.01$, Fig. 5A, B). Furthermore, the invasion exhibited a depression when lnc-TRHDE-AS1 was upregulated by transfecting

Table 2. Cox regression analysis of lnc-TRHDE-AS1 expression and the clinicopathological features of patients.

	HR	95% CI	*P* value
Age (> 65 vs. ≤ 65)	1.041	0.525-2.065	0.908
Sex (Male vs. female)	1.228	0.626-2.410	0.549
Tumor size (≥ 3cm vs. < 3cm)	1.426	0.699-2.908	0.329
Invasion depth (T3/T4 vs. T1/T2)	1.982	1.018-3.859	0.044
Histological type (Undifferentiated vs. Differentiated)	1.219	0.635-2.340	0.552
TNM Stage (III-IV vs. I-II)	2.174	1.069-1.421	0.032
Lymph node metastasis (Positive vs. Negative)	2.184	1.075-4.436	0.031
HP infection (Positive vs. Negative)	1.659	0.818-3.366	0.160
Lnc-TRHDE-AS1 (Low vs. High)	2.777	1.301-5.924	0.008

HR, hazard ratio; CI, confidence interval; HP, *Helicobacter pylori*.

Table 3. Cox regression analysis of miR-1275 expression and the clinicopathological features of patients.

	HR	95% CI	*P* value
Age (> 65 vs. ≤ 65)	1.304	0.648-2.622	0.457
Sex (Male vs. female)	1.004	0.528-1.911	0.990
Tumor size (≥ 3cm vs. < 3cm)	1.077	0.554-2.095	0.827
Invasion depth (T3/T4 vs. T1/T2)	1.222	0.620-2.409	0.563
Histological type (Undifferentiated vs. Differentiated)	1.388	0.704-2.737	0.344
TNM Stage (III-IV vs. I-II)	2.075	1.088-3.958	0.027
Lymph node metastasis (Positive vs. Negative)	2.178	0.990-4.790	0.053
HP infection (Positive vs. Negative)	1.365	0.708-2.631	0.353
miR-1275 (High vs. Low)	2.915	1.325-6.416	0.008

HR, hazard ratio; CI, confidence interval; HP, *Helicobacter pylori*.

with pcDNA-TRHDE-AS1, but restored by miR-1275 ($P < 0.01$, Fig. 5C, D). These results implied lnc-TRHDE-AS1 can weaken the migration and invasion of gastric cancer cells but miR-1275 boosts them.

Discussion

Being the fourth most common malignancy, the morbidity and mortality rates of gastric cancer in China increased from the age of 40 years and peaked in the age group of 80 years (Wang et al. 2019b). Gastric cancer has been a great healthy burden worldwide, especially in China as the population gets aged. Adequate surgical resection is the only curative therapeutic option for gastric cancer (Wang et al. 2019a). However, the overall survival outcome after surgery remains undesirable. The current AJCC TNM stage system or Union International Committee on Cancer (UICC) has shown valuable but sometimes inadequate prediction for the prognosis of gastric cancer patients (Son et al. 2014; Marano et al. 2015). The increasing amount of evidence implied that the discovery of molecular biomarkers will be beneficial to the prognostic evaluation and even identification of potential high-risk gastric cancer patients (Min et al. 2017). Benefiting from an increasing number of studies pointing to the essential regulatory role of ncRNAs over the last few years, ncRNAs are now known to be implicated in the biological complexity of many diseases including cancers (Lekka and Hall 2018).

Growing publications have demonstrated that the aberrant expressions of specific lncRNAs in cancer samples can indicate the spectrum of tumor progression and may serve as independent biomarkers for diagnosis and prognosis (Lorenzi et al. 2019). Recently, lncRNAs have been associated with the biology of gastric cancer (Yang et al. 2016; Zhu et al. 2016). However, the prognostic values of lnc-TRHDE-AS1 in gastric cancer have not been clarified clearly. In this study, the expression level of lnc-TRHDE-AS1 in gastric cancer tissues and cells was determined and compared with normal ones. A decreased expression level of lnc-TRHDE-AS1 was found in tumor tissues and cells. Furthermore, lnc-TRHDE-AS1 expression level was related to unfavorable prognostic parameters including TNM stage and lymph node metastasis. Moreover, the low expression of lnc-TRHDE-AS1 in gastric cancer tissues implied a shorter five-year overall survival and was an unfavorable prognostic factor for the gastric cancer based on the Multivariate Cox proportional regression analysis result. Our findings are in line with the well-documented prognostic predictor role of lnc-TRHDE-AS1, which constructed a risk

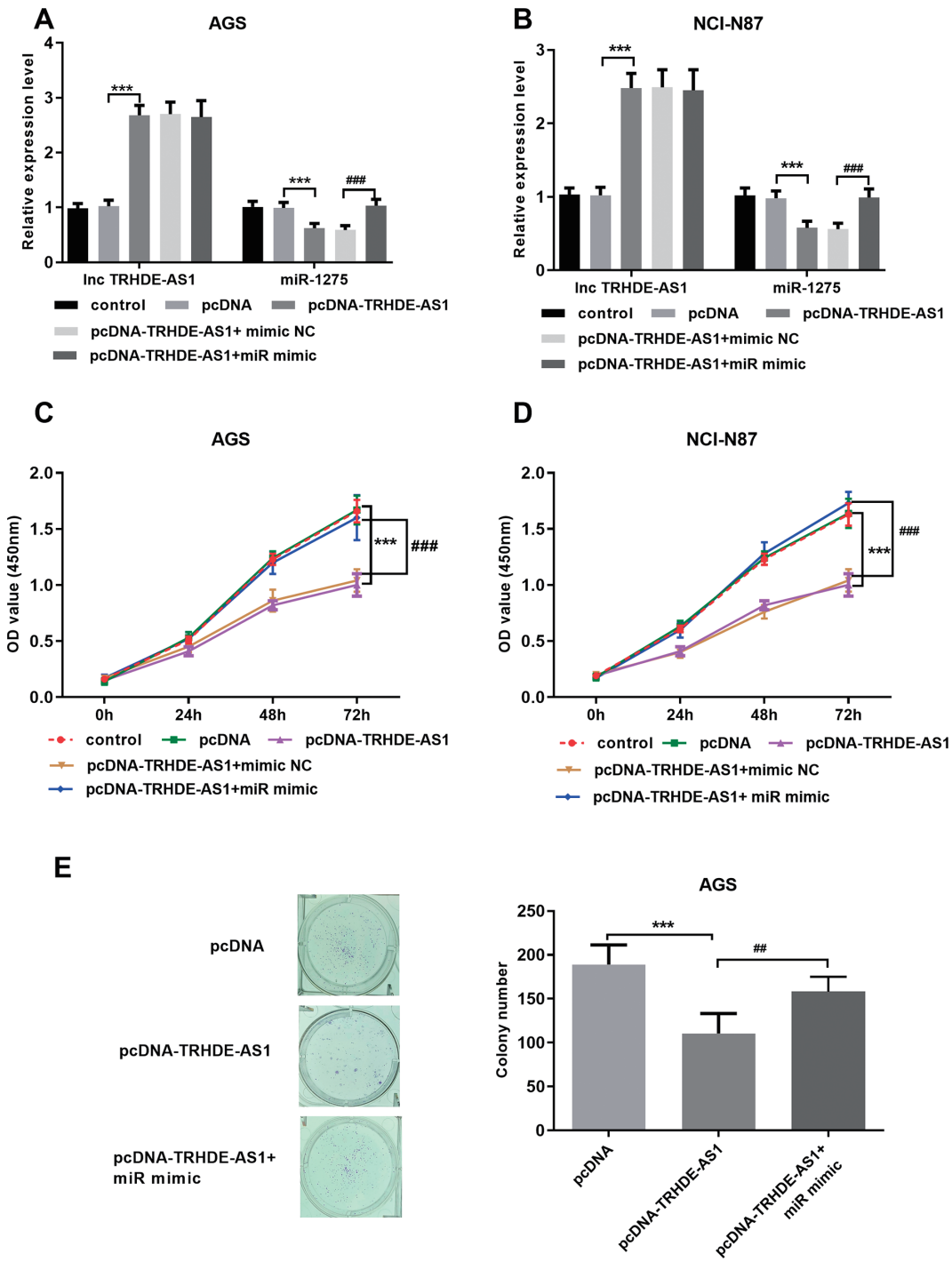

Fig. 4. Upregulation of lnc-TRHDE-AS1 inhibited cell proliferation of gastric cancer.
A and B. The expression of lnc-TRHDE-AS1 in AGS and NCI-N87 cells after transfection (***$P < 0.001$ ratio to pcDNA transfected group; $^{###}P < 0.001$ ratio to pcDNA-TRHDE-AS1 + mimic NC co-transfected group). C and D. Inhibitory effect of lnc-TRHDE-AS1 overexpression on the proliferation of AGS and NCI-N87 cells can be resumed by miR-1275 mimic. E. lnc-TRHDE-AS1 overexpression suppressed the colony formation of AGS cells, but miR-1275 mimic can offset that. (***$P < 0.001$ ratio to pcDNA transfected group; $^{##}P < 0.01$ ratio to pcDNA-TRHDE-AS1 + mimic NC co-transfected group). Data were shown as the mean of triple samples with the error bar (SD).

score model with five long non-coding RNAs including lnc-TRHDE-AS1 for predicting prognosis in gastric cancer (Wu et al. 2021). So, combined with all these, lnc-TRHDE-AS1 can act as a prognosis biomarker, and may be a new and effective therapy target for gastric cancer.

During the past few years, many attempts have been made to elucidate the functional role as well as the clinical significance of miRNAs in gastric cancer (Zhang et al.

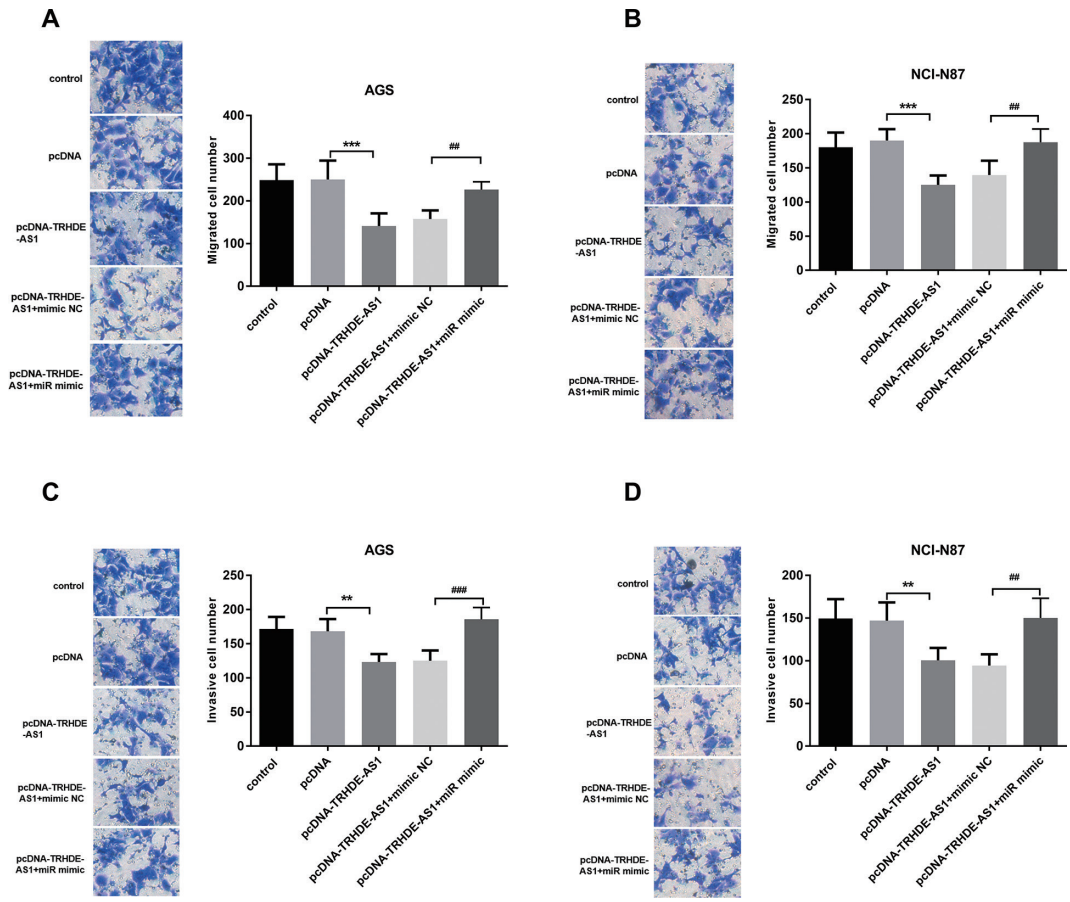

Fig. 5. Upregulation of TRHDE-AS1 repressed cell migration and invasion of gastric cancer.
A and B. Migration-suppressive effect of lnc-TRHDE-AS1 overexpression in AGS and NCI-N87 cells can rescue by miR-1275 overexpression (***$P < 0.001$ ratios to pcDNA transfected group. $^{\#\#}P < 0.01$ ratio to pcDNA-TRHDE-AS1 + mimic NC co-transfected group). C and D. Migration-suppressive effect of lnc-TRHDE-AS1 overexpression in AGS and NCI-N87 cells can restore by miR-1275 overexpression (**$P < 0.01$ ratio to pcDNA transfected group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ ratios to pcDNA-TRHDE-AS1 + mimic NC co-transfected group). Data were shown as the mean of triple samples with the error bar (SD).

2018; Ishikawa et al. 2020). For instance, a previous study suggested that aberrant miR-1307-3p expression in tumor tissues may be used as a prognostic indicator for patients with gastric cancer (Ma et al. 2021). MiR-1275 were reported to be upregulated in drug-sensitive cells following Y-Box protein by microarray hybridizations. Our group focused on the miR-1275 expression in gastric cancer and tried to explore the clinical significance of miR-1275 in gastric cancer prognosis and progression. Based on the results we obtained, miR-1275 was with an upregulated expression in the gastric tissues and cells when compared with normal ones. This dysregulation in miR-1275 expression inspired us that it may contribute to the prediction of gastric cancer prognosis. So, by Kaplan-Meier curves and Multi-variate Cox proportional regression analysis, we identified the prediction value of miR-1275 in gastric cancer prognosis. Recently, other reports indicated that hsa-miR-1275 is a tumor-suppressor miRNA with the potential as a prognostic biomarker in gastric cancer (Mei et al. 2019; Qi and Zhang 2021). All these suggest that miR-1275 has prognostic potential in gastric cancer, which is pivotal for treatment targets in gastric cancer.

LncRNAs have been reported to play regulatory roles in a variety of cancers caused by dysregulation (Gutschner et al. 2013). Though the potential mechanisms of how lncRNAs alter in gastric cancer remain largely undefined, emerging evidence suggests that lncRNAs may crosstalk with other RNAs by sharing miRNAs and participate in a competitive endogenous RNA (ceRNA) network (Guo et al. 2015). For instance, lncRNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p to confer malignant phenotype to tumor cells in gastric cancer (Liu et al. 2014). In the present study, we found lnc-TRHDE-AS1 can slow down the proliferation, migration, and invasion, which could be rescued by the overexpression of miR-1275. This also implied that lnc-TRHDE-AS1 may act as a carcinostatic lncRNA.

Recent studies demonstrated that lnc-TRHDE-AS1 could directly bind to miR-103 to suppress lung cell proliferation and invasion, or bind to miR-181a-5p to inhibit scar fibroblasts proliferation (Zhuan et al. 2019; Wei et al. 2021). In this study, lnc-TRHDE-AS1 may suppress the progres-

sion of gastric cancer partly by binding to miR-1275. It is reported that JAZF1 is a direct target of miR-1275 in gastric cancer cells and regulates vimentin and E-cadherin expression (Mei et al. 2019). So, we speculate that lnc-TRHDE-AS1/miR-1275 axis played its role in gastric cancer cell metastasis via targeting JAZF1 and regulating vimentin and E-cadherin expression. However, the results of this study lack evidence of *in vivo* experiments, and clinical validation are needed in the following study.

In conclusion, lnc-TRHDE-AS1 was found to be significantly downregulated and miR-1275 was upregulated in gastric cancer tissues and cell lines. The dysregulation of TRHDE-AS1 and miR-1275 expression showed great significance in the poor prognosis of gastric cancer. From the results of cell experiments, overexpression of lnc-TRHDE-AS1 significantly suppressed gastric cancer cell proliferation, migration, and invasion by targeting miR-1275.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

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