LncRNA ASMTL-AS1/microRNA-1270 differentiate prognostic groups in gastric cancer and influence cell proliferation, migration and invasion

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ABSTRACT

The objective of this study was to determine the expression levels of ASMTL-AS1 and miR-1270 in gastric cancer, and to explore whether ASMTL-AS1 and miR-1270 is associated with cancer prognosis and progression or not. ASMTL-AS1 and miR-1270 expression were quantified in gastric cancer tissues and adjacent normal tissues (n = 167) and cell lines. The potential of ASMTL-AS1 and miR-1270 as prognostic biomarkers was evaluated by the receiver operating characteristic (ROC) curve, Kaplan-Meier, and multivariate Cox regression analyses. The binding between ASMTL-AS1 and miR-1270 was verified by the Luciferase reporter assay and RNA pull-down assay. Functional roles of ASMTL-AS1/miR-1270 on cells were investigated in HGC-27 and NCI-N87 cells by MTS viability, Transwell migration, and Matrigel invasion assay. ASMTL-AS1 was significantly downregulated while miR-1270 was upregulated in gastric cancer tissues as compared with normal tissue and cell lines. According to the studies, ASMTL-AS1 and miR-1270 were related to unfavorable clinical parameters, such as the advanced TNM stage. Downregulated ASMTL-AS1 and upregulated miR-1270 were associated with reduced 5-year overall survival. Functional studies suggested that ASMTL-AS1 inhibits proliferation, migration, and invasion of HGC-27 and NCI-N87 cells by regulation of miR-1270. In summary, ASMTL-AS1 and miR-1270 are associated with poor prognosis of patients with gastric cancer. ASMTL-AS1 inhibited gastric cancer progression by regulating miR-1270. Therefore, ASMTL-AS1/miR-1270 may be a potential prognostic biomarker and novel strategy for gastric cancer targeted therapy.

Introduction

Gastric cancer is a globally prevalent disease, especially in Asian countries including China [1,2]. According to the data from the World Health Organization (WHO), an expected incidence of 1 million made gastric cancer rank among the fifth largest cancer burden worldwide. As there is late diagnosis at an advanced stage, the mortality from gastric cancer is high, e.g. it was 768,793 in 2020, making it the fifth most common cause of cancer-related deaths [3]. Approximately 11% of total cases and 8% of annual cancer deaths are attributed to gastric cancer worldwide, which translates into a high fatality to case ratio of 70% [4,5]. Nowadays, the treatments have helped to prolong the survival of this disease; however, it is limited to the 5-year survival for stage IA and IB tumors. Patients with stage III or more advanced tumors undergo a dismal 5-year survival rate as low as 18% [6]. These figures indicate the urgency for more effective molecular markers to predict different survival of patients.

Cancer is as much an epigenetic disease as it is a cytogenetic and genetic disease. The epigenetic alterations that cover the aspects of dysregulated non-coding RNA (ncRNA), aberrant DNA methylation or altered post-translational histone modification have emerged as robust classes of biomarkers and are the basis for a growing number of clinical tests for cancer screening and surveillance [7]. Especially, dysregulated ncRNAs, including IncRNAs (long non-coding RNA) and miRNAs (miRNAs), have been widely investigated in recent years. LncRNAs are defined as RNA molecules that are longer than 200 nucleotides while miRNAs indicate RNA molecules comprising 22–25 nucleotides [8]. Although both of them have no canonical protein-coding potential, miRNAs can negatively regulate gene expression while IncRNAs can regulate gene expression by accelerating the deadenylation...
and degradation of target mRNAs or acting as scaffolds for regulatory protein complexes, respectively [9]. Many lncRNAs and miRNAs are linked to the prognosis and progression of gastric cancers, either as tumor suppressors or as oncogenes [10,11]. The lncRNA called Acetylserotonin O-methyltransferase likes antisense RNA 1 (ASMTL-AS1) has already been found to have tumor suppressor functions in triple-negative breast cancer, papillary thyroid carcinoma, hepatocellular carcinoma, and lung adenocarcinoma [12–15]. miR-1270 is with the poor prognosis in human osteosarcoma and bladder cancer [16,17]. However, their roles in gastric cancer prognosis and progression have been unexplored.

In the present study, it is hypothesized that ASMTL-AS1 and miR-1270 expression are dysregulated in gastric cancer and these dysregulations would carry important clinical significance. With the aim to clarify the role of ASMTL-AS1 and miR-1270 in gastric cancer, their levels were determined and prognostic values were evaluated. The binding between ASMTL-AS1 and miR-1270 would be verified and their functional roles were investigated.

Materials and methods

Gastric cancer patients and tissue specimens

Between 2013 and 2016, 167 patients were diagnosed with gastric cancer who are without previous treatment and underwent total or proximal gastrectomy in Weihai Municipal Hospital. These patients were enrolled in this study and followed for 5 years from the time of surgery. The diagnosis of gastric cancer was established by endoscopic and histopathologic examinations. The amount of samples, both cancerous and para-cancer normal tissues, was confirmed to be sufficient for this study. The ethical approval was granted by the Research Ethics Committee of Weihai Municipal Hospital. Participants also provided the written informed-consent documents for this study.

Gastric cancer cell culture and transfection

The gastric cancer cell lines (HGC-27, NCI-N87, SNU-5 and KATO-III) were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China), along with human gastric mucosal epithelial cell line GES-1 which was used as a control. Cell lines were grown in RPMI 1640 medium (Invitrogen, USA) containing 10% fetal bovine serum (FBS) (Invitrogen, USA), in a 5% CO₂ atmosphere at 37°C. Transfection of the cells was performed using Lipofectamine™ 3000 (ThermoFisher Scientific, USA) by following the manufacturer’s instructions. The sequence used in transfection, including the ASMTL-AS1-expressed pcDNA3.1(+) vectors (ov-ASMTL-AS1), empty pcDNA3.1(+) vector (ov-NC), miR-1270 mimic, and mimic negative control, was from Genebio (Shanghai, China). The effect was examined after 48 hours of transfection by quantitative reverse transcription-polymerase chain reaction (RT-qPCR) assay using the extracted RNA.

Extraction of RNA and RT-qPCR assay

To investigate the expression of ASMTL-AS1 and miR-1270 in the gastric cancer tissues and cell lines, total RNA was obtained using the RNEasy Purification Kit (Qiagen, Germany). The total RNA was quantified by the NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, USA). For determination of ASMTL-AS1, the extracted RNA was reverse transformed into cDNA using the RT2 PreAMP cDNA Synthesis Kit (Qiagen, Germany). The lncRNA-specific RT2 qPCR Primer Assays (Qiagen, Germany) for GAPDH (as reference RNA) and ASMTLA-AS1 were used by adding the RT2 SYBR Green Mastermix on an Applied Biosystems 7500 fast real-time PCR device (Applied Biosystems, USA). For detection of miR-1270, total RNA was reverse transformed into cDNA via microRNA reverse transcription and RT-qPCR kit (GenePharma, China). The primers used are as follows: 5′- TTGTGGAGCTTGT CGTCTGG-3′ (ASMTL-AS1-Forward) and 5′- CTGACGGAGTGATCTGGTTT-3′ (ASMTL-AS1-Reverse); 5′- GGAGGAGATCCCTCCAA AAT-3′ (GAPDH-Forward) and 5′- GGCTTTTC TGATCCTCTCATGG-3′ (GAPDH-Reverse); 5′- CTGGAGATATGGAAAGGCTGT-3′ (miR-1270-Forward) and 5′- TGCAAAAGAGCCACATA GAAGAT-3′ (miR-1270-Reverse); 5′- CTGGAGATATGGAAAGGCTGT-3′ (U6-forward) and 5′- AACGTTACGAATTTGCGT-3′ (U6-reverse). qPCR was carried out on a Bio-Rad CFX system
(Bio-Rad, USA) and initiated by 2 min at 95°C before 40 thermal cycles, 30s each at 95°C and 40s at 60°C with a final extension of 10 min at 72°C. The relative ASMTL-AS1 and miR-1270 levels were referred by the value of $2^{-\Delta\Delta Ct}$.

**Cell proliferation assay**

The HGC-27 and NCI-N87 cells were prepared as single-cell suspensions to seed in a 96-well plate at $2 \times 10^3$ cells/well. GC cells were incubated in a constant temperature incubator at 37°C for 72 hours and added the CellTiter 96 AQueous One MTS Solution (Promega) every 24 hours. Another 2 hours after the addition, the absorbance was recorded at 492 nm on a microplate reader (ThermoFisher, USA) [18].

**Transwell migration assay**

Commerciaally available Chemicon QCM Chemotaxis 5 mum 24-well migration assay system (CHEMICON, Millipore, USA) was used to access the cell migratory ability [19]. Serum starved HGC-27 and NCI-N87 cells were plated at $2 \times 10^4$ cells/well seeding densities on the upper layer of the inserts. Chemo-attractive medium (RPMI 1640 medium with 10% FBS) was placed below the cell-permeable membrane in the lower chambers. After 24 hours of incubation, cells were washed in phosphate buffer saline (PBS) and the cells at the bottom of the chamber were carefully wiped, followed by fixation and staining were followed respectively. The cells were counted using an Eclipse Ti-E Nikon microscope (Nanjing, China).

**Matrigel invasion assay**

The cell invasion potential was evaluated using Chemicon cell invasion assay kit (Millipore, USA). In addition to a culture time of 36 hours, the remaining steps were consistent with the migration experiments mentioned above.

**Target prediction and luciferase reporter assay**

The lincRNASNP2 prediction algorithm (http://bioinfo.life.hust.edu.cn/lincRNASNP#) was used to predict the binding between ASMTL-AS1 and miR-1270. The 3'UTR of ASMTL-AS1 was amplified from the cDNA of GES-1 cells. Then the sequence was cloned into the downstream of Renilla luciferase cDNA, which was then transcribed by phRL-TK Vector to construct WT-ASMTL-AS1 (Promega, USA). The site-directed mutagenesis of ASMTL-AS1 3'UTR disrupted the seed region of the miR-1270 target sites, 5'-UCUGCGAAGCCCCUAGAGGUC-3', and it was cloned into the phRL-TK Vector to construct MUT-ASMTL-AS1. The indicated constructs were mixed with p-GL3-Promoter vector (Promega), for internal normalization. Twenty-four hours for electroporation later, cells were subjected to a dual-luciferase reporter assay which was calculated as the ratios of Renilla luciferase /firefly luciferase activities.

**RNA pull-down assay**

The assay to pull down miR-1270 by ASMTL-AS1 was carried out as previously mentioned [13]. In brief, HGC-27 and NCI-N87 cells were harvested, lysed, and sonicated. The ASMTL-AS1 probe was incubated with streptavidin magnetic beads (Solarbio Life Science, Beijing, China) for 2 hours to generate probe-coated beads. The cell lysates and probe-coated beads were then incubated together, followed by elution using Trizol (Takara, Japan), and finally, the quantification of miR-1270 was done using RT-qPCR.

**Statistical analysis**

Computations of data analysis were performed with GraphPad Prism software version 5.02 (San Diego, California, USA) and SPSS version 22.0. The tests such as Mann Whitney U test, t-test, or analysis of variance (ANOVA) were used for group comparison as indicated. The cutoff values of ASMTL-AS1 and miR-1270 expressions in gastric tumors were determined by ROC curves to distinguish the survivors and non-survivors. Chi-squared was adopted to compare the distribution
of clinical variables between patient groups. Kaplan-Meier analysis along with the log-rank test was utilized to illustrate the relation of survival with ASMTL-AS1 and miR-1270 expressions in patients with gastric cancer. Multivariate survival analysis was carried out to assess the prognostic values of related parameters. Correlations between ASMTL-AS1 and miR-1270 values were assessed after log transformation with the Pearson correlation and linear regression analysis. P values less than 0.05 were statistically significant.

**Results**

With the hypothesis that ASMTL-AS1 and miR-1270 expression are dysregulated in gastric cancer, their level was determined and compared with nonmalignant samples. With the aim to clarify the role of ASMTL-AS1 and miR-1270 in gastric cancer, the receiver operating characteristic (ROC) curves based on survivors and non-survivors were used to determine the cutoff value. Kaplan-Meier and multivariate Cox regression analyses were used to access the prognostic value of ASMTL-AS1 and miR-1270 among clinicopathologic characteristics. The binding between ASMTL-AS1 and miR-1270 was verified by Luciferase reporter assay and RNA pull-down assay. Functional roles of ASMTL-AS1/miR-1270 were investigated in HGC-27 and NCI-N87 cells by MTS viability, Transwell migration, and Matrigel invasion assay.

**Expression of ASMTL-AS1 and miR-1270 in gastric cancer**

The RT-qPCR analysis of the gastric cancer tissues showed that ASMTL-AS1 expression was significantly lower in gastric cancer tissues (n = 167) than in adjacent normal gastric tissue samples (P < 0.001 (Figure 1(a)). Similarly, the expression of ASMTL-AS1 in cancerous cells HGC-27, NCI-N87, SNU-5, KATO-III) was lower than that in the normal gastric mucosal epithelial cell line (GES-1) (P < 0.001) (Figure 1(b)). The HGC-27 and NCI-N87 cell lines with particularly low ASMTL-AS1 expression were used for further assays. In contrast, the investigation of miR-1270 relative expression in gastric tissues and cell lines found that miR-1270 was considerably higher in gastric tissues and all gastric cancer cell lines as compared with in noncancerous tissues and cells.

*Figure 1. ASMTL-AS1 was downregulated in gastric cancer while miR-1270 was upregulated in gastric cancer. (a) (b) The expression of ASMTL-AS1 was upregulated in gastric cancer tissues and cell lines measured by RT-qPCR assay. (c) (d) The expression of miR-1270 in gastric cancer tissues and cell lines was measured by RT-qPCR assay. ***P < 0.001.*
(P < 0.001) (Figure 1(c,d)). These results suggest that ASMTL-AS1 is downregulated in gastric cancer but miR-1270 is upregulated in gastric cancer.

**Predictive value of ASMTL-AS1 in gastric cancer survival**

The prognostic power of ASMTL-AS1 was evaluated using its correlation with the clinical parameters and discriminatory power between the survivors and non-survivors. The cutoff ROC value of ASMTL-AS1, which distinguishes the survivors and non-survivors, was determined by the maximum Youden index to be 0.4061 with an area under the curve (AUC) of 0.900 (Figure 2(a)). Based on this cutoff, the patients were divided into two groups: the ASMTL-AS1 low-expression group and the high-expression group. Table 1 shows the clinicopathological characteristics of all 167 patients and correlative characters with ASMTL-AS1 using the Chi-squared test. There was a significant correlation among ASMTL-AS1 levels and the TNM stage (P = 0.011), pN classification (P < 0.001), and pM classification (P < 0.001). By using Kaplan-Meier analysis, low and high ASMTL-AS1 stratified patients into poor and good overall survival groups with significantly different survival rates (Log rank P < 0.001) (Figure 2(b)). Table 3 shows the results of an analysis of the correlation with the overall survival (OS). As a result, ASMTL-AS1 was an independent poor prognostic factor in multivariate analyses (hazard ratio [HR] = 3.549, 95% confidence interval [CI] = 1.780–7.074, P < 0.001).

**Predictive value of miR-1270 in gastric cancer survival**

The prognostic impact of miR-1270 in gastric cancer was also assessed by adopting the Chi-square test, Kaplan-Meier analysis, and multivariate analysis. The cutoff value of miR-1270 in gastric cancer patients was 1.7777 according to ROC curves with an AUC of 0.881 (Figure 3(a)). miR-1270 expression was related to the TNM stage (P = 0.009), pN classification (P = 0.028) and pM classification (P < 0.001) (Table 2). There was a marked difference in the 5-year survival rates for the miR-1270 low expression group and high expression group (Log rank P < 0.001) (Figure 3(b)). As a result of multivariate analysis (Table 3), miR-1270 would be an independent poor prognostic factor (HR = 2.483, 95% CI = 1.412–4.366, P = 0.02).

**miR-1270 is a potential target of ASMTL-AS1**

First, the bioinformatics database LncRNASNP was used to predict that miR-1270 might be a target of ASMTL-AS1 (Figure 4(a)). The RT-qPCR assay results validate the opposite trend between the expression levels of ASMTL-AS1 and

![Figure 2](image-url)

Figure 2. The prognostic power of ASMTL-AS1 in gastric cancer overall survival. (a) The power of ASMTL-AS1 in distinguishing survivors from non-survivors by ROC curves. (b) Kaplan-Meier survival analysis of patients based on the high and low ASMTL-AS1 expression. Log-rank test P < 0.001.
miR-1270 (P < 0.001) (Figure 4(b)). In addition, miR-1270 levels were reduced by overexpression of ASMTL-AS1 in HGC-27 and NCI-N87 cells (P < 0.01) (Figure 4(c,d)). Luciferase assays were conducted to verify whether the disruption of the binding site altered the ASMTL-AS1-miR-1270 interaction. As shown in Figure 4(e,f), overexpression of miR-1270 decreased luciferase activity of wild-type ASMTL-AS1 (P < 0.05) (Figure 4(e,f)). But if the binding sites were mutated, miR-1270 no longer influenced the luciferase activity of mutant-type ASMTL-AS1 (P < 0.05) (Figure 4(e,f)). Finally, to examine if miR-1270 could bind directly with ASMTL-AS1,

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### Table 1. Correlation between ASMTL-AS1 expression and clinical characteristics in patients with gastric cancer.

| Parameters          | Cases (n = 167) | ASMTL-AS1 expression | P values |
|---------------------|-----------------|----------------------|----------|
|                     | Low (n = 100)   | High (n = 67)        |          |
| Age (years)         |                 |                      | 0.874    |
| < 60                | 86              | 52                   | 34       |
| ≥ 60                | 81              | 48                   | 33       |
| Gender              |                 |                      | 0.592    |
| Female              | 79              | 49                   | 30       |
| Male                | 88              | 51                   | 37       |
| Tumor size (cm)     |                 |                      | 0.090    |
| < 5                 | 99              | 54                   | 45       |
| ≥ 5                 | 68              | 46                   | 22       |
| TNM stage           |                 |                      | 0.011    |
| I–II                | 87              | 44                   | 43       |
| III–IV              | 80              | 56                   | 24       |
| pN classification   |                 |                      | <0.001   |
| pN0-pN1             | 79              | 32                   | 47       |
| pN2-pN3             | 88              | 68                   | 20       |
| pM classification   |                 |                      | <0.001   |
| pM0                 | 137             | 73                   | 64       |
| pM1                 | 30              | 27                   | 3        |
| Lauren classification|                |                      | 0.394    |
| Intestinal type     | 88              | 50                   | 38       |
| Diffuse type        | 79              | 50                   | 29       |
| CA125 (U/ml)        |                 |                      | 0.138    |
| < 70                | 83              | 45                   | 38       |
| ≥ 70                | 84              | 55                   | 29       |

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### Table 2. Correlation between miR-1270 expression and clinical characteristics in patients with gastric cancer.

| Parameters          | Cases (n = 167) | miR-1270 expression | P values |
|---------------------|-----------------|---------------------|----------|
|                     | Low (n = 76)    | High (n = 91)       |          |
| Age (years)         |                 |                     | 0.329    |
| < 60                | 86              | 36                  | 50       |
| ≥ 60                | 81              | 40                  | 41       |
| Gender              |                 |                     | 0.767    |
| Female              | 79              | 35                  | 44       |
| Male                | 88              | 41                  | 47       |
| Tumor size (cm)     |                 |                     | 0.118    |
| < 5                 | 99              | 50                  | 49       |
| ≥ 5                 | 68              | 26                  | 42       |
| TNM stage           |                 |                     | 0.009    |
| I–II                | 87              | 48                  | 39       |
| III–IV              | 80              | 28                  | 52       |
| pN classification   |                 |                     | 0.028    |
| pN0-pN1             | 79              | 43                  | 36       |
| pN2-pN3             | 88              | 33                  | 55       |
| pM classification   |                 |                     | P < 0.001|
| pM0                 | 137             | 71                  | 66       |
| pM1                 | 30              | 5                   | 25       |
| Lauren classification|                |                     | 0.543    |
| Intestinal type     | 88              | 42                  | 46       |
| Diffuse type        | 79              | 34                  | 45       |
| CA125 (U/ml)        |                 |                     | 0.189    |
| < 70                | 83              | 42                  | 41       |
| ≥ 70                | 84              | 34                  | 50       |

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**Figure 3.** The prognostic power of miR-1270 in gastric cancer overall survival. (a) The power of miR-1270 in distinguishing survivors from non-survivors by ROC curves. (b) Kaplan-Meier survival analysis of patients based on the high and low miR-1270 expression. Log-rank test P < 0.001.
a biotinylated ASMTL-AS1 probe was used to pull down miRNA and the miR-1270 was measured in the ASMTL-AS1-containing pellet using qRT-PCR. As shown in Figure 4(g), the results indicate that miR-1270 could bind directly to ASMTL-AS1. These results indicated that miR-1270 is a potential target of ASMTL-AS1 in gastric cancer.

**ASMTL-AS1 inhibited gastric cancer cell proliferative, migratory, and invasive ability while miR-1270 restored these abilities**

The transfected HGC-27 and NCI-N87 cell lines were examined to identify the effect of ASMTL-AS1 and miR-1270 on gastric cancer cells. The ASMTL-AS1 pcDNA3.1(+) elevated the expression of ASMTL-AS1 compared with empty pcDNA3.1 (+) vector, whereas ASMTL-AS1 pcDNA3.1(+) and miR-1270 mimic co-transfected cells showed downregulated ASMTL-AS1 level when compared with ASMTL-AS1 pcDNA3.1(+) and – mimic NC co-transfected ones (P < 0.01) (Figure 5(a,b)). The measurements of cell proliferation over time suggested that the cells with upregulated ASMTL-AS1 proliferated slowly than the negative control, while cells with upregulated both ASMTL-AS1 and miR-1270 proliferated rapidly than cells with upregulated ASMTL-AS1 only (P < 0.05) (Figure 5(c,d)). Moreover, increased ASMTL-AS1 levels obstructed the migration of the cells versus the negative control, but the simultaneous increase of ASMTL-AS1 and miR-1270 promoted migration than the single increase of ASMTL-AS1 (P < 0.05) (Figure 5(e)). The same trend was also found in cell invasion (P < 0.05) (Figure 5(f)). Hence, these results showed ASMTL-AS1 could inhibit gastric cancer cell proliferation, migration, and invasion by targeting miR-1270.

**Discussion**

Gastric cancer is currently a worldwide burden, as it has high mortality, and it is especially prevalent in Asia [6]. Previous studies have hypothesized that gastric cancer is a genetic-dysregulated disease involving multi-step changes in the genome [20].

**Table 3.** Cox regression analysis of ASMTL-AS1 and miR-1270 expression and the clinicopathological features of patients.

|                | HR    | 95% CI   | P Value |
|----------------|-------|----------|---------|
| ASMTL-AS1      | 3.549 | 1.780–7.074 | <0.001  |
| miR-1270       | 2.483 | 1.412–4.366 | 0.002   |
| Age            | 1.035 | 0.689–1.554 | 0.868   |
| Gender         | 1.197 | 0.782–1.830 | 0.408   |
| Tumor size     | 1.158 | 0.747–1.796 | 0.512   |
| TNM stage      | 1.580 | 1.008–2.478 | 0.046   |
| pN classification | 1.268 | 0.808–1.992 | 0.302   |
| pM classification | 1.363 | 0.845–2.199 | 0.204   |
| Lauren classification | 1.243 | 0.620–1.885 | 0.304   |
| CA125          | 1.053 | 0.688–1.610 | 0.813   |

**Figure 4.** ASMTL-AS1 could bind with miR-1270. (a) The putative miR-1270 binding sites on ASMTL-AS1. (b) The negative correlation between ASMTL-AS1 and miR-1270 expression. (c) Relative expression of miR-1270 after overexpression of ASMTL-AS1 in HGC-27 cells. (d) Relative expression of miR-1270 after overexpression of ASMTL-AS1 in NCI-N87 cells. (e) Luciferase reporter assay in HGC-27 cells. (f) RNA pulled down assay by biotinylated ASMTL-AS1 with RT-qPCR detection of miR-1270 expression. *P < 0.05, **P < 0.01, ***P < 0.001.
In addition, 70% of the human genome is transcribed into RNAs, yielding thousands of ncRNAs [9,21]. In the last few decades, increasing studies have convincingly revealed that ncRNAs, as regulators of epigenetics, participate in regulating various cell functions and cell physiology [22].

Figure 5. Functions of ASMTL-AS1/miR-1270 in gastric cancer cell lines. (a) (b) qRT-PCR detection of the relative expression of ASMTL-AS1 and miR-1270 in HGC-27 and NCI-N87 cells after transfection. (c) (d) The proliferation assay of HGC-27 and NCI-N87 cells by using the MTS method. (e) Transwell migration assay of HGC-27 and NCI-N87 cells after transfection. (f) Matrigel invasion assay of transfected HGC-27 and NCI-N87 cells. *P < 0.05, **P < 0.01, ***P < 0.001.
Accordingly, the abnormal level of ncRNAs strongly contributes to the development of neoplasia and may be promising candidate biomarkers for prognosis and potential therapeutic targets of neoplasm [23]. For instance, IncRNA HCP5 has been verified to be a novel and promising target for gastric cancer treatment [24]. In this study, we focus on ASMTL-AS1 and miR-1270 expression levels in gastric cancer, with a particular emphasis on their prognostic significance and multifaceted roles in cell function.

ASMTL-AS1 has come to light as a novel tumor suppressor over the past two years. Here, the level of ASMTL-AS1 in gastric cancer tissues and cells was quantitatively assessed by RT-qPCR. Previously, ASMTL-AS1 was reported to be significantly downregulated in triple-negative breast cancer and bladder cancer tissues [13,25]. Similarly, ASMTL-AS1 was significantly decreased in gastric tissues and cells when compared with the normal ones. Due to the role of ASMTL-AS1 in mediating cell functions and predicting overall survival [14,15,26], we also hypothesized that this IncRNA may play a role as a tumor suppressor in gastric cancer and may have the potential as a prognostic marker. Therefore, the distinguishing ability of ASMTL-AS1 between survivors and non-survivors of patients also was analyzed by ROC curves, and the cutoff value was thus determined. Further, the Kaplan-Meier curves presented the potential power of ASMTL-AS1 in the prediction of gastric cancer over survival. The association of ASMTL-AS1 level with advanced TNM stage, pN, and pM classification also revealed that downregulation of ASMTL-AS1 was related to unfavorable prognosis in gastric cancer. Multivariate survival analysis directly showed the excellent prognostic value of this IncRNA in gastric cancer. Therefore, downregulation of ASMTL-AS1 indicated an unfavorable prognosis of patients with gastric cancer.

MiR-1270 has been found with aberrant upregulation in osteosarcoma, papillary thyroid cancer, renal cell carcinoma, and bladder cancer [16,17,27,28]. In this study, miR-1270 was also shown to be upregulated in gastric cancer. Based on the analysis results of ROC curves, Chi-square test, Kaplan-Meier survival analysis, and multivariate survival analysis, miR-1270 was associated with poor clinical parameters and shorter overall survival of gastric cancer patients. Previously, the dysregulation of miR-1270 was correlated to an advanced stage and shorter overall survival in bladder cancer patients, and it was identified as a predictive marker of the prognosis of bladder cancer [17]. In this study, miR-1270 has shown the potential to be a biomarker of gastric cancer prognosis.

When it comes to mechanism in cell function, IncRNAs can function as the main kind of competing endogenous RNAs (ceRNAs), lead to a regulatory effect on miRNA level, and therefore play an important role in the molecular functions [29,30]. Interestingly, miR-1270 was found and verified to be a target of ASMTL-AS1 in gastric cancer cells in this study. Because ASMTL-AS1 and miR-1270 were negatively correlated in expression level, miR-1270 mimic can reduce the fluorescence intensity of the wide-type ASMTL-AS1, and ASMTL-AS1 could pull down miR-1270. The ceRNA role of ASMTL-AS1 was also found in osteosarcoma with miR-342-3p, hepatocellular carcinoma with miR-1343-3p, and breast cancer by sponging miR-1228-3p [12,13,31,32]. Given the dysregulation of ASMTL-AS1 and miR-1270 in gastric cancer, whether ASMTL-AS1/miR-1270 regulatory loop affects the cell biological behaviors of gastric cells was further explored. Firstly, ASMTL-AS1 and miR-1270 overexpression vectors were constructed and verified to be effective. A series of cell experiments were designed to explore the role of the ASMTL-AS1/miR-1270 regulatory loop in gastric cancer. Compared with the negative control, ASMTL-AS1 overexpression markedly hindered the proliferative and migratory abilities of the cells. Additionally, the upregulation of miR-1270 could partially reverse the regulatory effects of ASMTL-AS1 on gastric cells. There are cases where miR-1270 functions as tumor-promoting miRNA in several cancers, such as bladder cancer and pancreatic cancer [33,34]. It has been reported previously that miR-1270 could suppress the production of TAP2 by binding to this single nucleotide polymorphisms in the 3’-UTR of this gene [35]. And TAP2 was verified to be decreased in gastric cancer [36]. Thus, ASMTL-AS1/miR-1270 may bring about their effect on proliferation and migration via TAP2. In
Summary, ASMTL-AS1/miR-1270 exerted a vital function in gastric cancer cell biological behaviors, and this regulatory loop may be a new therapeutic target of gastric cancer.

Conclusion
In conclusion, ASMTL-AS1 expression was down-regulated while miR-1270 expression was upregulated in gastric cancer, and both of them were associated with poor survival outcomes. ASMTL-AS1 overexpression might affect the expression of miR-1270 and thereby inhibiting the tumor cell growth and metastasis. Hence, it can be suggested that the regulatory loop of ASMTL-AS1 and miR-1270 may be potential prognostic biomarkers and novel therapeutic strategy for gastric cancer.

Highlights
ASMTL-AS1 was downregulated and may be a prognostic marker in gastric cancer.

- miR-1270 was upregulated and may be a prognostic marker in gastric cancer.
- ASMTL-AS1 inhibited gastric cancer progression by regulating miR-1270.
- ASMTL-AS1/miR-1270 may be a novel strategy for gastric cancer therapy.

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