**INTRODUCTION**

Globally, oesophageal cancer is the seventh most common malignant cancer type and represents the sixth-highest incidence in cancer mortality with a poor 5-year survival rate.\(^1\) Oesophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of oesophageal cancer\(^2\) and exhibits a striking regional variation in morbidity and mortality in northern China, such as in Hebei, Shanxi and Henan provinces.\(^2\) Although the diagnostic and therapeutic techniques of oesophageal cancer have been largely improved in past decades, the 5-year survival rate of oesophageal cancer remains less than 20%.\(^4\) Moving forward, it is essential for a more thorough understanding of the biological underpinnings of the disease and an urgent need for early detection and effective treatment regimens.

LncRNAs performed diverse functions in gene expression networks depending on their subcellular localization, such as regulating...
chromatin modification and gene transcription in the nucleus, and 
modulating mRNA stability, translation and post-translational mod-
ification in the cytoplasm. LnC RNAs located in the cytoplasm can 
serve as competitive endogenous RNAs (ceRNAs), which could 
sponge miRNAs through competition for shared miRNAs, thereby 
imposing an additional regulation on miRNA targets at post-trans-
scriptional level.6

Epithelial-to-mesenchymal transition (EMT) is a typically funda-
mental transdifferentiation process in development which enables 
cancer cell invasion, contributes to cancer stroma formation, gener-
ates stem-like tumour-initiating cells and increases drug resistance. 
Among a myriad of EMT-regulating factors discovered in the cancer 
microenvironment, transforming growth factor-β (TGF-β) has been 
shown to be a potent signal to initiate and drive EMT.7 To identify and 
characterize novel factors potentially related to TGF-β-induced tu-
mour aggression in oesophageal cancer, we treated human oeso-
ophageal cancer cell line Eca109 with TGF-β and then used microarray 
analysis to compare RNA expression levels between TGF-β-treated 
and untreated cells, observed TGF-β-dependent up-regulation of 
FAM83H-AS1 (FAM83H antisense RNA1, also known as onco-ln-
cRNA-3). In addition, Jiang et al8 identified many super-enhancer 
(SE)-associated and squamous cell carcinoma (SCC)-specific on-
cogenic transcripts profiled by RNA-Seq, including FAM83H-AS1. 
FAM83H-AS1 and its cognate sense strand FAM83H are head-to-
head located on 8q24. Natural antisense transcripts (NATs) are de-
fined as RNA sequences that originate from complements of their 
endogenous sense counterparts in cis or trans.9,10 Notably, some 
natural antisense IncRNAs were reported to exert regulatory effects 
on expression of their sense protein-coding genes.11,12 But the ex-
pression level and correlation between FAM83H-AS1 and FAM83H 
in ESCC were not well characterized. Accumulating evidence was 
illustrating that FAM83H-AS1 was overexpressed in various cancer 
types that promoted cell growth and metastasis by multiple molecu-
lar mechanisms,13-16 while the exact mechanism of FAM83H-AS1 in 
ESCC was largely unclear and its prospect as therapeutic target for 
ESCC was still unexplored.

In the present study, we aimed at providing an integrated analy-
sis on the expression and correlation between FAM83H-AS1 and 
FAM83H, the potential biological function of these antisense-sense 
strands and downstream regulatory mechanism of FAM83H-AS1 in 
the pathogenesis of ESCC, as well as its role in TGF-β-induced EMT.

2 MATERIALS AND METHODS

2.1 Patients and specimens

All the 67 pairs of ESCC tissues and corresponding normal tis-
ues were taken from the surgical specimens of ESCC patients 
from the years of 2015 to 2017 in the Fourth Affiliated Hospital 
of Hebei Medical University. Informed consent was received 
from all patients who were not given any radiotherapy or chemo-
therapy before operation. According to the standard of American

Joint Committee on Cancer system, histological grade was staged. 
Information on clinical data and clinicopathological characteris-
tics was available from hospital recordings and is summarized in 
Table S1. Smokers were defined as former or current individuals 
smoking at least five cigarettes per day for 2 years or longer.17 
Individuals with at least one first-degree relative or at least two 
second-degree relatives having oesophageal/cardia/gastric cancer 
were defined as having family history. Ethical consent was granted 
from the Ethics Committee of the Fourth Affiliated Hospital of 
Hebei Medical University.

2.2 Cell culture and treatment

Human oesophageal cancer cell lines Kyse150, Kyse170, TE1 and 
Eca109 were purchased from American Type Culture Collection and 
were cultured in RPMI 1640 (Invitrogen) medium containing 10% 
foetal bovine serum (Invitrogen) at 37°C in an atmosphere containing 
5% CO2. The cells were treated with 10 ng/mL of recombinant TGF-
β1 (R&D Systems) for 7 days with the medium replenishment every 
2 days.

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

Total RNA from the tissues and cells was isolated using TRizol rea-
gent (Invitrogen) in accordance with the manufacturer’s instruc-
tions. Transcriptor First Strand cDNA Synthesis Kit (Roche) was 
used to generate cDNA according to the manufacturer’s protocol.

qRT-PCR was performed in the StepOne Real-Time PCR System 
(Applied Biosystems) using GoTaq® qPCR Master Mix (Promega).

GAPDH and U6 snRNA were employed as endogenous controls 
for mRNA/IncRNA and miRNA, respectively. The relative expres-
sion level of RNAs was calculated using the 2^-ΔΔCT method.18 Each 
specimen was tested in triplicate. Primer sequences are displayed 
in Table S2.

2.4 Subcellular fractionation

The nuclear and cytoplasmic fractions of oesophageal cancer cell 
lines were isolated by PARIS™ Kit Protein and RNA Isolation System 
(Invitrogen) according to the manufacturer’s protocol.

2.5 Cell transfection

The shRNAs targeting FAM83H-AS1 and the pcDNA3.1-FAM83H-
AS1 were designed and synthesized by GenePharma and Sangon 
Biotech, respectively. The miR-10a-5p mimics, inhibitor and negative 
control were purchased from GenePharma. The FAM83H siRNAs and 
si-NC were synthesized by General Biosystems. Transfections were
performed using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer’s protocol. The sequences of four shRNAs, three siRNAs and miR-10a-5p mimics and inhibitor are listed in Table S3.

2.6 | Cell proliferation assay

The ability for cellular proliferation was detected by MTS assay and clone formation assay. The MTS assay was measured using CellTiter96®AQueous One Solution Cell Proliferation Assay kit (Promega). For MTS assay, the transfected cells were seeded into 96-well plate with 1 × 10^3 per well. After incubation at 0, 24, 48, 72 and 96 hours, cells of each well were added with 20 μL (500 μg/mL) of MTS reagent and incubated at CO₂ incubator for 2 hours. The optical density was measured with a microplate reader at a wavelength of 490 nm. For clone formation assay, 3 × 10^3 cells per well following transfection for 24 hours were inoculated into a six-well plate and regularly cultured for 1 week. More than 50 cells were considered to be one clone, and the numbers of clone were counted under a microscope.

2.7 | Transwell migration and invasion assays

Cell migration assay was conducted using non-Matrigel-coated chambers (Corning) with 8-μm pore membranes. A total of 1 × 10^5 cells per well were seeded into the upper compartment of chamber. After 24 hours of incubation at 37°C, the invasive cells located on the lower surface of the membrane were counted in five randomly sequenced visual fields using a Leica DMI4000B microscope. For invasion assay, the upper surface of the membrane was pre-coated with 50 μL 1× Matrigel® Basement Membrane Matrix (Corning) to form a matrix assay, the upper surface of the membrane was pre-coated with 50 μL 1× Matrigel® Basement Membrane Matrix (Corning) to form a matrix barrier; the remaining steps were used for invasion assay as described above.

2.8 | Western blot analysis

Total proteins were extracted from transfected cells using RIPA lysis buffer containing PMSF (Solarbio) and protease inhibitor cocktail (Promega) according to the instructions of the manufacturer. The protein concentration was determined by BCA Protein Assay Kit (Multi Sciences). After mixed with loading buffer and heated at 99°C for 5 minutes, the protein lysates were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Millipore). The transferred membranes were blocked with 5% skim milk for 1 hour at room temperature and incubated overnight with the specific primary antibodies at 4°C. Subsequently, the membranes were incubated at room temperature with horseradish peroxidase–conjugated goat anti-rabbit IgG (KPL) for 1 hour. The bands were visualized with enhanced chemiluminescence (ECL) detection reagent (Multi Sciences by Chemi XT 4 (Syngene). The primary antibodies were used: anti-β-actin (AC026, ABclonal), anti-E-cadherin (E-AB-64011, Elabscience) and anti-N-cadherin (E-AB-64011, Elabscience) and anti-FAM83H (ab121816, Abcam).

2.9 | Vectors construction

The restriction sites of pcDNA3.1-FAM83H-AS1 were Hind III/EcoRI I. By double digested with EcoRI I and Xho I (Takara) of pSL-MS2-12X (Addgene), we subcloned MS2-12X fragment into pcDNA3.1, named pcDNA3.1-MS2, pcDNA3.1-FAM83H-AS1, pcDNA3.1-MS2-FAM83H-AS1-MUT, and pcDNA3.1-MS2-FAM83H-AS1-MS2 and pcDNA3.1-FAM83H-AS1-MS2-MUT, respectively. The sequences of FAM83H-AS1 or Girdin 3’ UTR containing the miR-10a-5p recognition sites were PCR-amplified and subcloned into pmirGLO vector (Promega) with the restriction sites of Nhe I/Xho I or Xho I/Xba I, named pmirGLO-FAM83H-AS1-1 (WT), pmirGLO-FAM83H-AS1-2 (WT) and pmirGLO-Girdin 3’ UTR (WT). The point mutations of FAM83H-AS1 or Girdin 3’ UTR binding to miR-10a-5p were promoted using a Q5® Site-Directed Mutagenesis Kit (New England Biolabs), named pmirGLO-FAM83H-AS1-1 (MUT), pmirGLO-FAM83H-AS1-2 (MUT) and pmirGLO-Girdin 3’ UTR (MUT). Mutation primers were designed in NEBase changer (http://nebasechanger.neb.com/) and synthesized by Generay Biotech. All constructed plasmids were sequenced correctly. The primers used for vectors construction are listed in Table S4.

2.10 | RNA immunoprecipitation (RIP) assay

Eca109 cells were cotransfected with pcDNA3.1-MS2, pcDNA3.1-MS2-FAM83H-AS1, pcDNA3.1-MS2-FAM83H-AS1-MUT and pMS2-GFP (Addgene). After 48 hours, cells were used to perform RNA immunoprecipitation (RIP) experiments using GFP antibody (Roche) and Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacturer’s instructions. IgG and SNRNP70 were respectively used as negative control and positive control.

2.11 | Luciferase reporter assay

PmirsGLO-FAM83H-AS1 (WT), pmirGLO-FAM83H-AS1 (MUT), pmirGLO-Girdin 3’ UTR (WT) and pmirGLO-Girdin 3’ UTR (MUT) were cotransfected with miR-10a-5p mimics or miR-negative control into Eca109 cells using Lipofectamine 2000. Luciferase activity was measured with Dual-Luciferase Reporter Assay System (Promega) at 48 hours after transfection and normalized to Renilla luciferase activity.

2.12 | Statistical analysis

All data were expressed as mean ± SD. The significance of differences between two groups or among multiple groups was
3 | RESULTS

3.1 | FAM83H-AS1 emerges as a potential oncogenic lncRNA and is associated with clinicopathological characteristics in ESCC

Based on scanning the NCBI and GEPIA data set, the relative expression levels of FAM83H-AS1 in different normal tissues and in most of the tumour types were detected (Figure S1A,B). By evaluating FAM83H-AS1 expression in 67 pairs of ESCC tissues and corresponding normal tissues, it was confirmed that FAM83H-AS1 expression level was significantly elevated in ESCC tissues (Figure 1A). Additionally, the expression level of FAM83H-AS1 in a panel of human oesophageal cancer cell lines was performed, which was remarkably higher in all oesophageal cancer cell lines, especially in Kyse150 and TE1 cells (Figure 1B). It was identified that high expression level of FAM83H-AS1 was closely associated with lymph node metastasis, TNM stage and pathological differentiation (Figure 1C). LncRNAs have been shown to play functional roles in both the nucleus and cytoplasmic compartments. FAM83H-AS1 was found to be predominantly located in the cytoplasm of oesophageal cancer cells by subcellular fractionation assay (Figure 1D). Coding Potential Calculator and Coding Potential Assessment Tool were further used to analyse the coding potential of FAM83H-AS1, and no protein-coding potential of FAM83H-AS1 was found (Figure S1C,D).

3.2 | FAM83H is significantly up-regulated in ESCC patients

A NCBI search identified that FAM83H-AS1 was in a head-to-head orientation relative to FAM83H (Figure 1E). As indicated by NCBI and GEPIA data set, the relative expression levels of FAM83H in normal tissues and in various tumour types were similar to FAM83H-AS1 (Figure S1E,F). Subsequently, qRT-PCR analysis detected increased mRNA expression level of FAM83H in ESCC tissues and FAM83H exhibited concordant co-regulation with FAM83H-AS1 (Figure 1F,G). Meanwhile, the expression level of FAM83H was significantly higher in Kyse150 and TE1 cells than other tested cell lines, Kyse150 and TE1 cells were selected for subsequent experiments (Figure 1H). In addition, analysis of the correlation between FAM83H expression and clinicopathological characteristics showed that FAM83H expression level was intimately associated with pathological differentiation (Figure 1I).

3.3 | The effect of FAM83H-AS1 and FAM83H on oesophageal cancer cell proliferation, migration and invasion

To determine the biological function of FAM83H-AS1 and FAM83H on oesophageal cancer cells, the gain- and loss-of-function assays were performed in oesophageal cancer cells. First of all, we transfected sh-FAM83H-AS1 specific for FAM83H-AS1 in Kyse150 and TE1 cells, respectively, and qRT-PCR analysis demonstrated that the shRNAs could significantly decrease the expression level of FAM83H-AS1 with successful transfection efficiency (Figure 2A). The sh-FAM83H-AS1-1, which exhibited the most evident knockdown efficacy, was selected for follow-up experiments. By performing MTS assay, we observed that FAM83H-AS1 knockdown significantly inhibited cell proliferation in Kyse150 and TE1 cells compared with parallel cells transfected with sh-NC (Figure 2B). Similar growth inhibiting effect was also validated by clone formation assay (Figure 2C). By performing transwell migration and invasion assays, we then found that the migration and invasion ability of Kyse150 and TE1 cells were markedly attenuated after FAM83H-AS1 shRNA transfection (Figure 2D,E).

As shRNA knockdown studies may be affected by off-target effects, the impacts of FAM83H-AS1 overexpression on oesophageal cancer cells were subsequently performed. The pCDNA3.1-FAM83H-AS1 was transfected into Eca109 cells which could dramatically promote the expression level of FAM83H-AS1 in comparison with the empty vector control (Figure S2A). A series of functional experiments were performed, which validated the oncogenic role of FAM83H-AS1 in reinforcing the proliferation, migration and invasion capacities of Eca109 cells (Figure S2B-E).
If FAM83H in regulating biological processes was in accordance with the oncogenic role of FAM83H-AS1, the functional experiments of FAM83H were conducted in Kyse150 and TE1 cells. We screened si-FAM83H-1 with high interference efficiency compared with the non-targeting control si-NC group (Figure 3A). As displayed in Figure 3B-D, siRNA-mediated FAM83H knockdown notably inhibited cell proliferation, migration and invasion, which largely phenocopied sh-FAM83H-AS1 inhibition in oesophageal cancer cells.

3.4 | FAM83H-AS1 regulates FAM83H at mRNA and protein levels

To identify the correlation between the levels of FAM83H-AS1 and FAM83H, we assessed expression changes in FAM83H level following the knockdown or overexpression of FAM83H-AS1. As expected, down-regulation of FAM83H-AS1 in Kyse150 cells decreased mRNA and protein expression levels of FAM83H. Conversely,
FIGURE 3  FAM83H implicates in ESCC progression and is regulated by FAM83H-AS1 at mRNA and protein level. A, The interfering efficiency against FAM83H analysed by qRT-PCR method. B, Cell proliferation was assessed using MTS assay with silenced FAM83H in Kyse150 and TE1 cells. C, Transwell migration and D, invasion assays were carried out with silenced FAM83H in Kyse150 and TE1 cells (magnification, ×200). E, and F, The influence of FAM83H-AS1 on FAM83H mRNA and protein level detected by qRT-PCR and Western blot assays. G, The influence of FAM83H on FAM83H-AS1 expression analysed by qRT-PCR method. Data are shown as mean ± SD; *P < .05 and **P < .01.
overexpression of FAM83H-AS1 in Eca109 cells resulted in significantly increased expression of FAM83H at both mRNA and protein levels (Figure 3E-F). However, we failed to detect any significant expression changes of FAM83H-AS1 upon FAM83H knockdown (Figure 3G).

3.5 | FAM83H-AS1 is up-regulated in TGF-β-induced Eca109 cells and contributes to the process of EMT

Because EMT is a crucial step of metastasis, it is of great interest to examine whether FAM83H-AS1 regulates the migration and invasion of oesophageal cancer cells via EMT. We first measured the cell phenotype after incubation with TGF-β and found that TGF-β-treated Eca109 cells underwent morphological changes to a spindle-shaped appearance (Figure 4A). Moreover, the cells displayed decreased expression of E-cadherin, as well as up-regulated expression of N-cadherin, vimentin, Snail and Twist1 (Figure 4B). These results suggested that the cells displayed EMT-associated signatures and exhibited a proper biological response to TGF-β treatment. Additionally, the expression level of FAM83H-AS1 was assessed and up-regulation of FAM83H-AS1 was detected in TGF-β-treated cells compared with untreated cells (Figure 4C). Subsequently, knockdown of FAM83H-AS1 was found to promote the expression of E-cadherin and inhibit the expression of N-cadherin, vimentin, Snail
3.6 | FAM83H-AS1 sponges miR-10a-5p through direct binding in oesophageal cancer cells

FAM83H-AS1 was mainly located in the cytoplasm of oesophageal cancer cells; therefore, we hypothesized that FAM83H-AS1 might also function as a molecular sponge to competitively bind certain miRNAs. According to the prediction in online databases (RAID v2.0), miR-10a-5p was found to contain two potential binding sites to FAM83H-AS1 (Figure 5A) and chosen for subsequent experiments for its tumour-suppressive role in ESCC.

The expression level of miR-10a-5p was down-regulated in ESCC tissues and oesophageal cancer cells, as well as closely associated with TNM stage and lymph node metastasis (Figure 5B-D). In addition, a significant inverse correlation between FAM83H-AS1 and miR-10a-5p expression in ESCC tissues was found (Figure 5E). The efficiency of miR-10a-5p mimics and inhibitor was validated prior to further analysis (Figure 5F). As shown in Figure 5G-J, the overexpression of miR-10a-5p by transfection with miR-10a-5p mimics hindered the proliferation, migration and invasion of Kyse150 and Eca109 cells, whereas transfection of miR-10a-5p inhibitor in Kyse150 and Eca109 cells displayed opposite effects.

Furthermore, we explored the relationship between FAM83H-AS1 and miR-10a-5p in Kyse150 and Eca109 cells. FAM83H-AS1 knockdown resulted in the increase of miR-10a-5p expression, and FAM83H-AS1 overexpression caused visible reductions in miR-10a-5p expression (Figure 6A). However, no significant difference in FAM83H-AS1 expression was found after knockdown or overexpression of miR-10a-5p (Figure 6B). The RIP assay was performed to pull down endogenous miRNAs associated with FAM83H-AS1 and demonstrated that FAM83H-AS1 significantly enriched with miR-10a-5p compared to the empty vector (Figure 6C). Consistently, the relative luciferase activity of FAM83H-AS1 wild-type was obviously decreased after cotransfection with miR-10a-5p mimics, but did not affect the activity of mutant type, which further verified that miR-10a-5p is a direct target of FAM83H-AS1 (Figure 6D).

3.7 | miR-10a-5p directly targets Girdin in oesophageal cancer cells

By using four independent miRNA target-predicting algorithms (DIANA, TargetScan, Starbase and miRDB), potential downstream target genes of miR-10a-5p were predicted (Figure 6E). Among these 50 predicted target genes, Girdin (also named CCDC88A) attracted our attention because of its critical role in the migration and invasion of cancer cells. Girdin regulates actin reconstruction and Akt-dependent cell motility, and involves in remodelling actin cytoskeleton which is essential for cell migration.19 The conserved binding site of Girdin 3’ UTR for miR-10a-5p is illustrated in Figure 6F. Girdin expression was found to be higher in ESCC tissues than that in corresponding normal tissues (Figure 6G) and was negatively correlated with miR-10a-5p (Figure 6H). Meanwhile, Girdin expression was correlated with TNM stage, lymph node metastasis and pathological differentiation in ESCC tissues (Figure 6I). Subsequently, overexpression of miR-10a-5p dramatically decreased the expression level of Girdin, while down-regulation of miR-10a-5p markedly exhibited the opposite effect in oesophageal cancer cells (Figure 6J).

Luciferase reporter assay manifested that enforced expression of miR-10a-5p reduced the luciferase activity of pmirGLO-Girdin 3’ UTR wild-type vector while showed no obviously effect on the luciferase activity of pmirGLO-Girdin 3’ UTR mutant type in Eca109 cells (Figure 6K), indicating the indeed regulatory role of miR-10a-5p on Girdin mRNA expression through direct binding to its 3’ UTR.

3.8 | FAM83H-AS1 positively regulates Girdin in a miR-10a-5p-dependent manner

Due to the fact that FAM83H-AS1 shared common binding sites with Girdin, we wondered whether FAM83H-AS1 could modulate Girdin dependent on miR-10a-5p. In oesophageal cancer cells, down-regulation of FAM83H-AS1 significantly decreased Girdin expression, whereas miR-10a-5p inhibitor overcame such a decrease. Similarly, miR-10a-5p mimics could abrogate the increased effect of FAM83H-AS1 overexpression on Girdin expression (Figure 7A). Besides, a dramatically positive correlation...
between FAM83H-AS1 and Girdin expression was identified in 67 pairs of ESCC tissues (Figure 7B). In gain- and loss-of-function experiments, miR-10a-5p inhibitor could partially rescue the inhibitory effect of FAM83H-AS1 knockdown on cell proliferation, migration and invasion capacity. Reciprocally, miR-10a-5p mimics could abolish biological functions caused by FAM83H-AS1 overexpression (Figure 7C-F). Overall, these results revealed a vital role of FAM83H-AS1 in modulating Girdin expression by competitively binding with miR-10a-5p.

4 | DISCUSSION

There is obvious evidence that the proverbial lncRNAs take up a significant portion operating as either oncogene or tumour suppressor in the pathological development of ESCC. In our study, we verified that FAM83H-AS1 and its sense transcript FAM83H were consistently up-regulated, and concordant co-regulation was detected between these sense-antisense pairs which possessed as oncogenes in facilitating cell proliferation, migration and invasion. Furthermore, FAM83H-AS1 promoted the TGF-β-induced EMT and functioned as a ceRNA to regulate Girdin expression by competitively binding miR-10a-5p in tumorigenesis and progression of ESCC.

NATs are RNA sequences that originate from the opposite DNA strands and partly overlap with sense RNA, promoter or regulatory region. NATs have garnered increased attention on their various regulatory functions ranging from transcriptional regulation to post-transcriptional regulation. For post-transcriptional regulation, NATs can regulate gene expression mainly at post-transcriptional level. Therefore, endogenous NATs have been confirmed to regulate APC expression and the Wnt/β-catenin pathway. Huang et al. reported that TGF-β-induced lncRNAs implicating in the malignant biological behaviour of cancer by regulating EMT.27-29 Yuan et al.27 reported that lncRNA ATB (lncRNA activated by TGF-β), a mediator of TGF-β signalling, was crucial for the invasion-metastasis cascade in hepatocellular carcinoma. Lu et al.28 observed that TBILA (TGFβ-induced lncRNA) promoted HGAL expression and bound with S100A7 to enhance its carcinogenic effects in non-small cell lung cancer. In the present study, FAM83H-AS1 expression level was higher in TGF-β-treated Eca109 cells than control cells, and high level of FAM83H-AS1 resulted in the up-regulation of EMT-initiating transcriptional factors and mesenchymal markers, and the down-regulation of epithelial markers at mRNA and protein levels, suggesting that FAM83H-AS1 expression was induced by TGF-β and promoted EMT in oesophageal cancer metastasis.

LncRNAs involved in versatile aspects of gene regulation and biological processes depending on their subcellular localization. FAM83H-AS1 was mainly exported to the cytoplasm, where lncRNA could regulate gene expression mainly at post-transcriptional level. However, Barr et al.23 reported that FAM83H-AS1 expression was significant enrichment in the nuclear fractions in comparison with the cytoplasmic fractions, which seemed contradictory with our results. This may be explained by the distinct mechanism of gene in different cancer types. For example, HOXD-AS1 enriched in the cytoplasm and acted as a ceRNA that sponged up miRNAs to regulate gene expression in ovarian cancer, liver cancer, bladder cancer, non-small cell lung cancer and glioma in previous studies.30-35 But in prostate cancer and colorectal cancer, HOXD-AS1 was determined to be enriched in the nucleus by interacting with critical epigenetic regulators.36,37 In the cytoplasm, lncRNAs can regulate mRNA stability by associating miRNAs in a ceRNA manner.5 Currently, growing studies revealed that lncRNAs fulfilled their roles as sponging miRNAs to modulate their target mRNA expression and biological functions. For example, Yang et al.28 found that LINCO1133 inhibited gastric cancer progression and metastasis by acting as a ceRNA for miR-106a-3p to regulate APC expression and the Wnt/β-catenin pathway. Huang et al. report that lncRNA binding with miR-10a-5p was able to modulate gene expression in cancer cells.
et al. demonstrated that TRPM2-AS took important regulatory parts in gastric carcinoma development by functioning as a ceRNA to regulate HMGA1 via sponging miR-195. However, there are no reports concerning that FAM83H-AS1 acted as a ceRNA of miRNAs in ESCC, and then we investigated this potential regulatory mechanism of FAM83H-AS1 in the cytoplasm. In the present study, miR-10a-5p was selected as the potential miRNA due to possessing the complementary binding sites with FAM83H-AS1. MiR-10a has been previously reported to be a tumour suppressor by analysing the miRNA microarray in ESCC. Combined with RIP assay and luciferase reporter assay, it confirmed the direct binding between FAM83H-AS1 and miR-10a-5p, implying that FAM83H-AS1 acted as a molecular sponge of miR-10a-5p.

Generally, miRNAs are ubiquitous post-transcriptional regulators that impact RNA stability and translation rate by binding to miRNAs in a sequence-specific manner. Therefore, this regulation for target mRNA becomes an important part of the ceRNA network. In this study, Girdin was finally screened out as the specifically target mRNA of miR-10a-5p proved by luciferase reporter experiment. Girdin is a novel component of the PI3K/Akt signalling pathway that is a core-signalling transduction pathway in cancer progression. Previous study has confirmed that Girdin exhibited an enhanced expression in ESCC and presented a positive role in oesophageal cancer cell proliferation, migration and invasion. Meanwhile, we detected that FAM83H-AS1 positively regulated Girdin expression abrogated by ectopic expression of miR-10a-5p. Taken together, these results supported that FAM83H-AS1, miR-10a-5p and Girdin formed a ceRNA regulatory network in the progression of ESCC.

In conclusion, the current study demonstrated that TGF-β induced FAM83H-AS1 served as a novel oncogene in ESCC and marked concordant expression with its cognate sense counterpart FAM83H. Additionally, FAM83H-AS1 was proved to regulate EMT process, and acted as a ceRNA in competitively sponging miR-10a-5p to enhance Girdin expression. Furthermore, these findings provided novel insights into the underlying mechanism of the aggressive biological behaviour of ESCC, which highlighted a potential target for ESCC therapy.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Wei Guo designed the study and revised the manuscript; Bo Feng performed the experiments, analysed the data and drafted the paper; Gaoyan Wang and Xiaoliang Liang performed the experiments; Zheng Wu and Xinchen Wang prepared the figures and tables; Yanli Guo and Zhiming Dong performed the statistical analysis; and Supeng Shen and Jia Liang recruited the patients and collected the data. All authors read and approved the final manuscript. Bo Feng: Investigation (lead); Software (lead); Writing-original draft (lead). Gaoyan Wang: Investigation (equal). Xiaoliang Liang: Investigation (equal). Zheng Wu: Software (equal). Xinchen Wang: Software (equal). Zhiming Dong: Software (supporting). Yanli Guo: Software (supporting). Supeng Shen: Methodology (equal). Jia Liang: Methodology (equal). Wei Guo: Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Wei Guo https://orcid.org/0000-0001-9690-161X

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Arnold M, Soerjomataram I, Ferlay J, et al. Global incidence of oesophageal cancer by histological subtype in 2012. Gut. 2015;64:381-387.
3. Guohong Z, Min S, Duemwi E, et al. Genetic heterogeneity of oesophageal cancer in high-incidence areas of southern and northern China. PLoS One. 2010;5:e9668.
4. Rustgi AK, El-Serag HB. Esophageal carcinoma. N Engl J Med. 2014;371:2499-2509.
5. Yao RW, Wang Y, Chen L. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019;21:542-551.
6. Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011;146:353-358.
7. Morikawa M, Derynck R, Miyazono K. TGF-β family: A stone of a hidden RNA language? Cell. 2011;146:353-358.
8. Katayama S, Tomaru Y, Kasukawa T, et al. Antisense transcription in the mammalian transcriptome. Science. 2005;309:1564-1566.
9. Wight M, Werner A. The functions of natural antisense transcripts. Essays Biochem. 2013;54:91-101.
10. Michael DR, Phillips AO, Krupa A, et al. The human hyaluronan synthase 2 (HAS2) gene and its natural antisense RNA exhibit coordinated expression in the renal proximal tubular epithelial cell. J Biol Chem. 2011;286:19523-19532.
11. Carrieri C, Cimatti L, Biagioli M, et al. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature. 2012;491:454-457.
12. Lu S, Dong W, Zhao P, et al. IncRNA FAM83H-AS1 is associated with the prognosis of colorectal carcinoma and promotes cell proliferation by targeting the Notch signaling pathway. Oncol Lett. 2018;15:1861-1868.
13. Yang F, Lv SX, Lv L, et al. Identification of IncRNA FAM83H-AS1 as a novel prognostic marker in luminal subtype breast cancer. Oncol Targets Ther. 2016;9:7039-7045.
14. Zhang J, Feng S, Su W, et al. Overexpression of FAM83H-AS1 indicates poor patient survival and knockdown impairs cell proliferation and invasion via MET/EGFR signaling in lung cancer. Sci Rep. 2017;7:42819.
15. Bi Y, Shen G, Quan Y, et al. Long noncoding RNA FAM83H-AS1 exerts an oncogenic role in glioma through epigenetically silencing CDKN1A (p21). J Cell Physiol. 2018;233:8896-8907.
17. Wang Y, Guo W, He Y, et al. Association of MTHFR C677T and SHMT(1) C1420T with susceptibility to ESCC and GCA in a high incident region of Northern China. Cancer Causes Control. 2007;18:143-152.

18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^(-Delta Delta C(T)) Method. Methods. 2001;25:402-408.

19. Enomoto A, Murakami H, Asai N, et al. Akt/PKB regulates actin organization and cell motility via Girdin/APE. Dev Cell. 2005;9:389-402.

20. Rosikiewicz W, Makalowska I. Biological functions of natural antisense transcripts. Acta Biochim Pol. 2016;63:665-673.

21. Chen C, Li HF, Hu YJ, et al. Family with sequence similarity 83 member H promotes the viability and metastasis of cervical cancer cells and indicates a poor prognosis. Yonsei Med J. 2019;60:611-618.

22. Kim KM, Hussein UK, Park SH, et al. FAM83H is involved in stabilization of β-catenin and progression of osteosarcomas. J Exp Clin Cancer Res. 2019;38:267.

23. Barr JA, Hayes KE, Brownmiller T, et al. Long non-coding RNA FAM83H-AS1 is regulated by human papillomavirus 16 E6 independently of p53 in cervical cancer cells. Sci Rep. 2019;9:3662.

24. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006;7:131-142.

25. Javaid S, Zhang J, Anderssen E, et al. Dynamic chromatin modification sustains epithelial-mesenchymal transition following inducible expression of Snail-1. Cell Rep. 2013;5:1679-1689.

26. Chang H, Liu Y, Xue M, et al. Synergistic action of master transcription factors controls epithelial-to-mesenchymal transition. Nucleic Acids Res. 2016;44:2514-2527.

27. Yuan JH, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-β promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell. 2014;25:665-671.

28. Lu Z, Li Y, Che Y, et al. The TGFβ-induced IncRNA TBILA promotes non-small cell lung cancer progression in vitro and in vivo via cis-regulating HGal and activating S100A7/JAB1 signaling. Cancer Lett. 2018;432:156-168.

29. Guo H, Ingolia NT, Weissman JS, et al. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466:835-840.

30. Friedman RC, Farh KK, Burge CB, et al. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19:92-105.

31. Hawkin RS, Draper SB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2009;136:227-241.

32. Wang Y, Zhang W, Wang Y, et al. HOXD-AS1 promotes cell proliferation, migration and invasion through miR-10a-5p/Girdin axis. J Cell Mol Med. 2020;24:8962-8976. https://doi.org/10.1111/jcmm.15530

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.