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Upregulation of long noncoding RNA FERRE promoted growth and invasion of breast cancer through modulating miR-19a-5p/EZH2 axis

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Abstract. – OBJECTIVE: It has been demonstrated that long non-coding RNA (LncRNA) plays an important regulatory role in a series of diseases. The purpose of this study is to investigate the expression of long non-coding RNA (LncRNA) FERRE and its facilitating effects on proliferation and invasion of breast cancer by regulating oncogene EZH2 through sponging with miR-19a-5p.

PATIENTS AND METHODS: qRT-PCR was performed to detect the expressions of FERRE and EZH2 in human breast cancer tissues and cells. CCK-8 assay was performed to evaluate the MCF-7 cells proliferation and transwell assay was performed to evaluate the MCF-7 cells migration. Correlation analysis between FERRE and miR-19a-5p was detected by statistical analysis. Bioinformatics prediction was made to detect the binding site of FERRE and miR-19a-5p and Luciferase activity was conducted to investigate the interaction between EZH2 and miR-19a-5p. Furthermore, we cloned the mice EZH2 3′-UTR into the Luciferase reporter vector and constructed miR-19a-5p binding mutants to validate the inhibited modulation of miR-19a-5p to the EZH2 expression.

RESULTS: Results showed that expression of FERRE and EZH2 were upregulated in human breast cancer tissues and cells. qRT-PCR and CCK-8 assay showed that FERRE expression is associated with the proliferation of breast cancer cells, upregulated FERRE contributed to cell proliferation of MCF-7. Transwell assay showed that FERRE was associated with the migration ability of tumor cells, increased expression of FERRE promoted the migration and invasion of breast cancer cells. The bioinformatics prediction and Luciferase assay demonstrated that by sponging with miR-19a-5p, FERRE can serve as a molecular sponge to further regulate the expression of EZH2.

CONCLUSIONS: We found that lncRNA-FERRE was upregulated in human breast cancer patients, which could accelerate tumor proliferation, migration and invasion as a molecular sponge by modulating the inhibitory effect of miR-19a-5p on oncogene EZH2.

Key Words:
Breast cancer, FERRE, MiR-19a-5p/EZH2 axis, Cell proliferation and invasion.

Introduction

Breast cancer is the most common cause of cancer-related deaths among females and its burden is increasing all over the world[1]. Particularly in China, breast cancer has an increasing incidence and mortality rates among females[2]. Although new biomarkers for early breast cancer diagnose and multimodal therapy strategies develop quickly, the 5-year survival in breast cancer patients remains poor[3]. In 2017, 10,960 patients among the 28,000 newly diagnosed cases died in the United States[4]. Thus, it is still necessary to explore the mechanisms underlying breast cancer pathogenesis and explore new therapy strategies and targets.

Long noncoding RNAs (lncRNAs) are a series of RNA polymerase II transcriptions with a length of more than 200 nucleotides and without protein-coding capacity[5]. They have been indicated as new regulators of epigenetic networks and transcription[6], which were identified as differentially expressed transcripts between primary and metastatic carcinoma[7,8]. Moreover, lncRNAs, as well as their related signal networks,

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had become new participants in inducting and regulating tumorigenesis[7,9,10]. Some studies[11-13] have revealed the competing endogenous RNAs (ceRNAs) networks participate in the development of tumors. Intriguingly, it has been reported that lncRNAs perform an important role in cancer proliferation, invasion and metastasis through "sponging" microRNAs (miRNAs) and competitively inhibiting their biological functions[14,15]. LncRNA NORAD promoted pancreatic cancer cell invasion, metastasis, and EMT by regulating the expression level of RhoA via sponging hsa-miR-125a-3p[16]. LncRNA CASC2 suppressed invasion, migration and EMT progression by regulating FBXW7 via acting as a ceRNA of miR-367 in hepatocellular carcinoma (HCC)[8]. However, the detailed molecular mechanisms of lncRNA in cell migration and invasion in BC remain poorly understood.

Elevated expression of enhancer of zeste homolog 2 (EZH2) histone methyltransferase, a core member of the polycomb repressive complex 2 (PRC2), results in cancer progression through histone methylation-driven tumor cells dedifferentiation[17]. However, the role of EZH2 in the lncRNA regulation loop has not been well explored. In the present study, we aimed to explore the biological roles of lncRNA- FERRE in breast cancer development and progression, as well as to illustrate the molecular mechanisms. We investigated the function of FERRE in breast cancer and revealed that FERRE, which significantly increased in breast cancer tissues, promoted cell proliferation and invasion of breast cancer cells via affecting miR-19a-5p/EZH2 axis.

Patients and Methods

Cell Culture

Human breast cancer cells MCF-7 cells were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Rockville, MD, USA) supplied with 10% FBS (Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin (Gibco, Rockville, MD, USA) and incubated at 37°C in an atmosphere of 5% CO_2.

Patients and Specimens

16 pairs of breast cancer tissues and adjacent normal tissues were collected from surgically treated and pathologically diagnosed breast cancer cases and then stored at -80°C. All specimens were handled and made anonymous according to the Ethical and legal standards. 16 cases of breast cancer patients based on accepted clinicopathological were enrolled in this study. Patients and their families had been fully informed that their specimens would be used for scientific research, and all participating patients had signed informed consent. The patients and sample information were included in Table I.

Transwell Assay

To test the migration ability of MCF-7 cells, transwell plates with a pore size of 8 μm (Millipore Inc, Billerica, MA, USA) were used to conduct transwell assay. MCF-7 cells were treated differently, and the lower chamber was added with DMEM supplemented with 20% FBS. The upper side of the membrane was wiped with a cotton swab to remove the cells that did not migrate, and cell numbers in five random fields were counted in each sample.

RNA Extraction and qRT-PCR

After taking out the culture plates, the cells were washed with PBS. After treatment, total RNA of cells was extracted by using TRIzol reagent (Life Technologies, Waltham, MA, USA) according to the manufacturer's instructions. And samples were stored at room temperature for 30 min. The reverse transcription of cDNA was performed with a PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. And for qRT-PCR,

Table I. Demographic data.

Gender	Female
Patients numbers	16
BMI (kg/m^2) ± SD	20.1 ± 3.2
Age (years)	
< 55	8
≥ 55	8
TNM stage	
I-II	10
III-IV	6
Lymph node metastasis	
Negative	11
Positive	5
Distant metastasis	
Negative	13
Positive	3

All the patients were selected randomly.

PCR primers were synthesized by GenePharma (Shanghai Gene Pharma, Shanghai, China) and sequences were listed in Table II. SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) was used to detect the expression.

CCK8 Assay

The CCK-8 kit (Dojindo, Kumamoto, Japan) was used to measure the cells proliferation according to the manufacturers' instructions. In brief, 5×10^3 cells were seeded in 96-well plates uniformly. After they were treated with regulated medium, the medium was removed, and cells were washed with PBS solution for 3 times. Then CCK8 dilution was added to the 96-well plates and incubated at 37°C in an atmosphere of 5% CO_2 for 2 hours. After incubation, the plates were taken out, and cell proliferation was measured using multi-detection microplate reader. And the absorbance (OD) value at 490 nm of each well was detected.

Construction of Lentivirus and Cell Transfection

Lentiviral Lnc FERRE and lnc FERRE shRNA were synthesized and constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). For miR analysis, the miR-19a-5p mimic, miR-19a-5p inhibitor and the negative control were constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). To knock down EZH2, si-EZH2 plasma and negative control plasma were constructed by Shanghai GenePharma Co., Ltd., (Shanghai, China). For transfection, 1×10^4 cells were seeded in 6-well plates and cultured with RANKL (100 ng/mL) and M-CSF (100 ng/mL). Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) and Opti-MEM® I reduced serum medium were used for transfection. For analysis of Lnc-FERRE, cells were transfected with Lnc-FERRE shRNA (referred as to sh) and negative control shRNA (referred as to nc), respectively. For analysis of miR-19a-5p, cells were transfected with miR-19a-5p inhibitor, and control cells were transfected with empty vector, respectively. The cells without transfection were used as the control (referred as to control). After the cultures were incubated for 30 min, they were replaced with DMEM containing 10% FBS. Then, at indicated time point after transfection, cells were harvested for further study.

Luciferase Assay

After transfection for 48 h, the Luciferase activities were measured by using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's protocol. Renilla Luciferase activities were normalized to the firefly Luciferase activities and the data were expressed as the fold change relative to the corresponding control groups which were defined as 1.0.

Xenograft Mouse Model

The animal study was approved by the Animal research Committee of our hospital. Female BALB/c mice (6-week) were injected with MCF-7 cells transfected with FERRE siRNA/controls, respectively. Four weeks after injection, the tumors were collected and measured.

Statistical Analysis

Unless otherwise indicated, all data are processed by Statistical Product and Service Solutions (SPSS) 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Each assay was applied at least three independent experiments or replicates. All data were presented as mean ± SD. Student's *t*-test, one-way analysis of variance (ANOVA) and multiple comparison between the groups was performed by using SNK method, in which $*p < 0.05$, $**p < 0.01$ were considered as statistically significant.

Table II. Primer sequences for qRT-PCR.

Genes	Forward	Reverse	Tm (°C)
FERRE	5'-ATGGCCTGGGACGGTACCTGA-3'	5'-ATCGGGTTCCAAAAGGTCAC-3'	60
EZH2	5'-GGTCCATGGGTCAGATCAACC-3'	5'-ATGGACCGCCAGACAATTAG-3'	61
miR-19a-5p	5'-ATTTCTCTGGCAGGCCGTA-3'	5'-GGCCTGGGCCGGTACGCCCG-3'	62
GAPDH	5'-TGGATTTGGACGCATTGGTC-3'	5'-TTTGCACTGGTACGTGTTGAT-3'	62
U6	5'-GCTGGCTTCGGCAGCACAGC-3'	5'-AACGCTTCACGAATTGCGGTC-3'	62

Results

FERRE and its Co-expression mRNA EZH2 was Upregulated in Tumor Tissues of Breast Cancer Patients

By bioinformatics analysis of lncRNAs expression data of breast cancer tissues *vs.* adjacent normal tissues, we screened out FERRE and its co-expression gene EZH2, both of which have high specific expression in breast cancer (Figure 1A). For validation, we detected the expressions of FERRE and EZH2 in tumor samples acquired from breast cancer patients. Total RNA of breast cancer tissues and adjacent normal tissues were extracted, and the expressions of FERRE and EZH2 were revealed by qRT-PCR. Results showed that both FERRE and EZH2 were significantly upregulated in breast cancer tissues (Figure 1B, 1C). To further illustrate the biological function of FERRE in breast cancer, qRT-PCR analysis was performed to detect FERRE expression in human breast cancer cell lines MCF-7. Results showed that expressions of FERRE and EZH2 were remarkably increased in MCF-7 cells compared with human epithelia cells HEK293 ($p < 0.01$) (Figure 1D, 1E). From these data, we suggested that FERRE might play a biological role in breast cancer.

Upregulating the FERRE Expression Significantly Promoted the Migration and Invasion of Breast Cancer Cells

To explore the functions of FERRE in breast cancer progression, we constructed FERRE overexpressing lentiviral and transfected it into MCF-7 cells. In addition, we also synthesized small interfering RNA of FERRE to inhibit its expression before it was transfected into MCF-7 cells. After that, the expression of FERRE was detected by qRT-PCR and the results showed that the expression of FERRE in the FERRE overexpressed group was significantly enhanced compared with the control ($p < 0.05$), while the expression levels of FERRE were reduced in the FERRE inhibition group compared with the control group ($p < 0.05$) (Figure 2A, 2B). To investigate whether FERRE influence the migration of tumor cells, we performed transwell assay to detect the migration ability after the expression of FERRE was altered. Results uncovered that

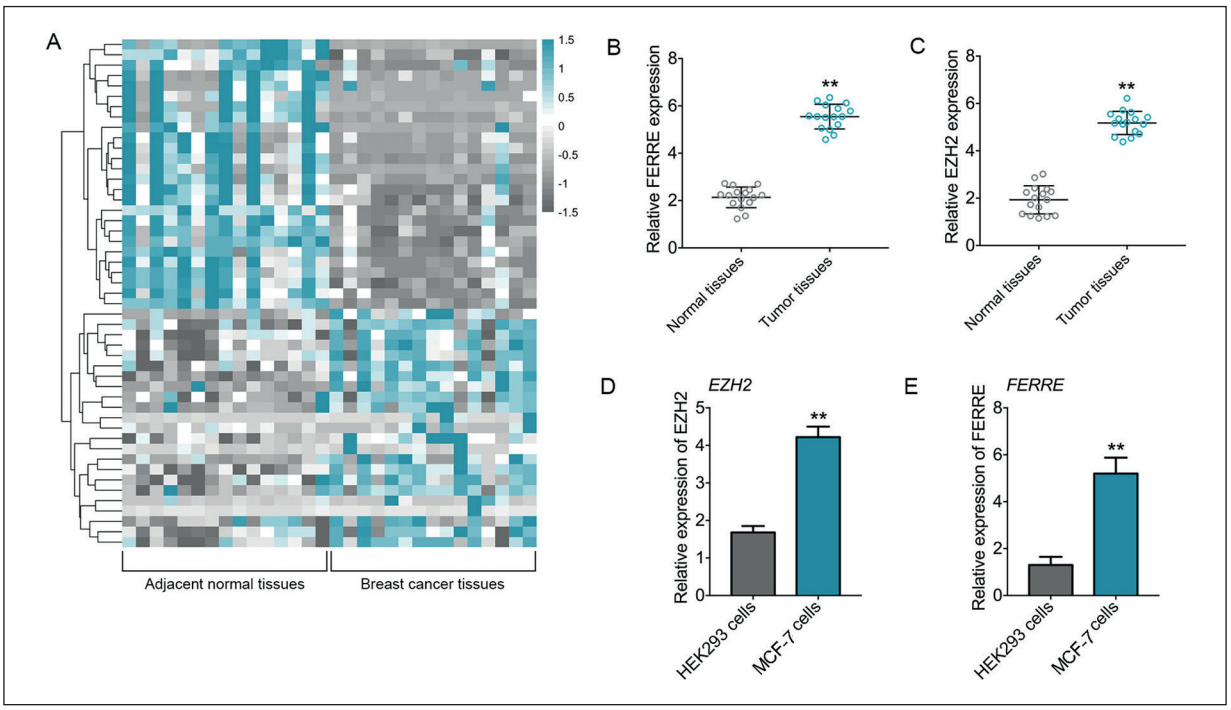

Figure 1. FERRE and its co-expression mRNA EZH2 was upregulated in tumor tissues of breast cancer patients. **A**, Heatmap of differentiated expressed LncRNA in 15 human breast cancer tissues and adjacent non-cancerous normal tissues. Relative mRNA expression levels of **B**, FERRE and **C**, EZH2 in breast cancer tissues and adjacent normal tissues. Relative mRNA expression levels of **D**, FERRE and **E**, EZH2 in human breast cancer cell line MCF-7 and HEK293 cells. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

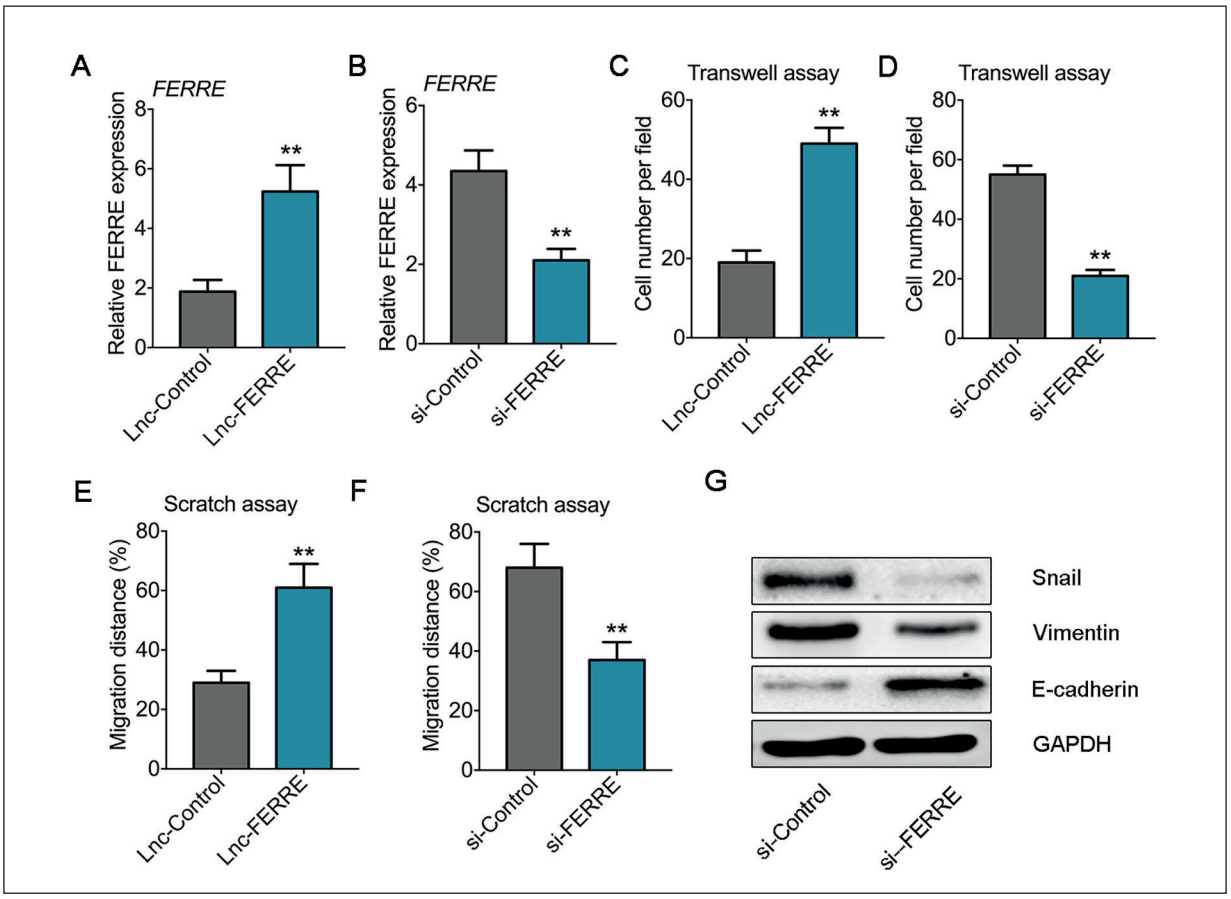

Figure 2. Upregulating the FERRE expression significantly promoted the migration and invasion of breast cancer cells. **A**, Relative mRNA expression levels of FERRE in MCF-7 cells transfected with FERRE overexpressing lentiviral (Lnc-FERRE) and Lnc-Control. **B**, Relative mRNA expression levels of FERRE in MCF-7 cells transfected with si-Control and si-FERRE. **C,** The invasion of cells transfected with Lnc-FERRE was determined by transwell invasion assays. **D**, The invasion of cells transfected with si-FERRE was determined by transwell invasion assays. **E**, Migration distance was measured of cells transfected with Lnc-FERRE. **F**, Migration distance was measured of cells transfected with si-FERRE. **G**, EMT marker E-cadherin, vimentin and snail was detected using Western blotting. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

after upregulating FERRE expression, the number of MCF-7 cells that transferred through transwell chambers was significantly increased compared with control group (Figure 2C), whereas the number of MCF-7 cells transferred through transwell chambers was significantly decreased after inhibition of FERRE expression (Figure 2D). Besides, scratch assay was also performed and found that the migration distance was significantly increased in FERRE overexpression group (Figure 2E), and significantly decreased after inhibition of FERRE expression compared with the control (Figure 2F). In terms of EMT, protein expressions of vimentin and snail were downregulated, whereas E-cadherin expression was upregulated in si-FERRE transfected group compared with si-Control group (Figure 2G). These results demonstrated that FERRE can regulate the migration ability of human breast cancer cells, and that upregulated FERRE can effectively promote the migration ability of breast cancer cells, making FERRE a potential target for therapy of breast cancer. Also, FERRE might regulate EMT markers and promote EMT process in breast cancer cells.

Upregulated Expression of FERRE Could Facilitate the Proliferation and Inhibit the Apoptosis of Breast Cancer Cells

To further investigate the role of FERRE in cell proliferation, CCK8 assay was performed on MCF-7 cells after alteration of FERRE

expression. The results disclosed that overexpressing FERRE significantly increased cell proliferation of MCF-7 compared with the control, whereas inhibition of FERRE expression remarkably reduced the cell proliferation number (Figure 3A, 3B). Besides, qRT-PCR analysis showed that the expression of apoptotic related genes such as Bax and cleaved caspase-3 were significantly decreased whereas the expression of anti-apoptotic gene Bcl-2 was remarkably increased after upregulation of FERRE expression (Figure 3C), and it was reversed after FERRE inhibition (Figure 3D). These results suggested that changing the expression of FERRE can regulate the proliferation ability of breast cancer cells.

FERRE can Sponge with miR-19a-5p in Breast Cancer Cells

To investigate the detailed mechanism of FERRE that promoted growth and invasion of breast cancer cells, we used StarBase 2.0 database to predict the target miRNA of FERRE and found that miR-19a-5p was a target miRNA of FERRE. Then, we used qRT-PCR analysis to detect the miR-19a-5p expressions of human breast cancer tissues and MCF-7 cells. Results showed that miR-19a-5p was low expressed in breast cancer tissues compared with adjacent normal tissues and was also downregulated in MCF-7 cells compared with HEK293 cells (Figure 4A and 4B). Correlation analysis was performed to investigate the expression relationship between FERRE and miR-19a-5p. Results showed that miR-19a-5p was negatively correlated with FERRE, which suggested that miR-19a-5p might be sponged by FERRE (Figure 4C). Previous studies reported that LncRNAs can act as a competing sponge in regulating miRNAs to further influence gene expression. Hence, we want to know if there is a direct binding relationship between FERRE and miR-19a-5p. We constructed FERRE-wt Luciferase reporter vector and FERRE-mut 3'UTR Luciferase reporter vector and performed Luciferase reporter assay (Fig-

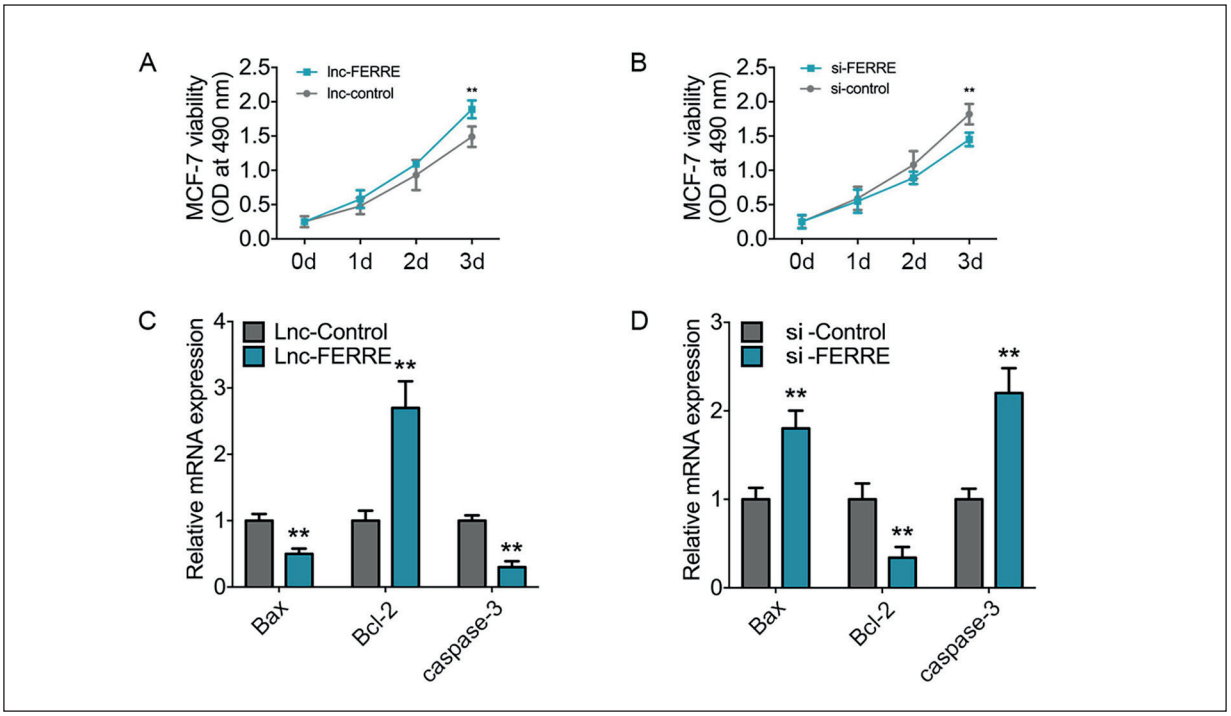

Figure 3. Upregulated expression of FERRE could facilitate the proliferation and inhibit the apoptosis of breast cancer cells. **A**, Absorption at 490 nm of MCF-7 cells treated with Lnc-FERRE and Lnc-Control detected by CCK-8 assay at 1 d, 2 d and 3 d. **B**, Absorption at 490 nm of MCF-7 cells treated with si-FERRE and si-Control detected by CCK-8 assay at 1 d, 2 d and 3 d. **C**, Relative mRNA expression in cells transfected with Lnc-FERRE. **D**, Relative mRNA expression in cells transfected with si-FERRE. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

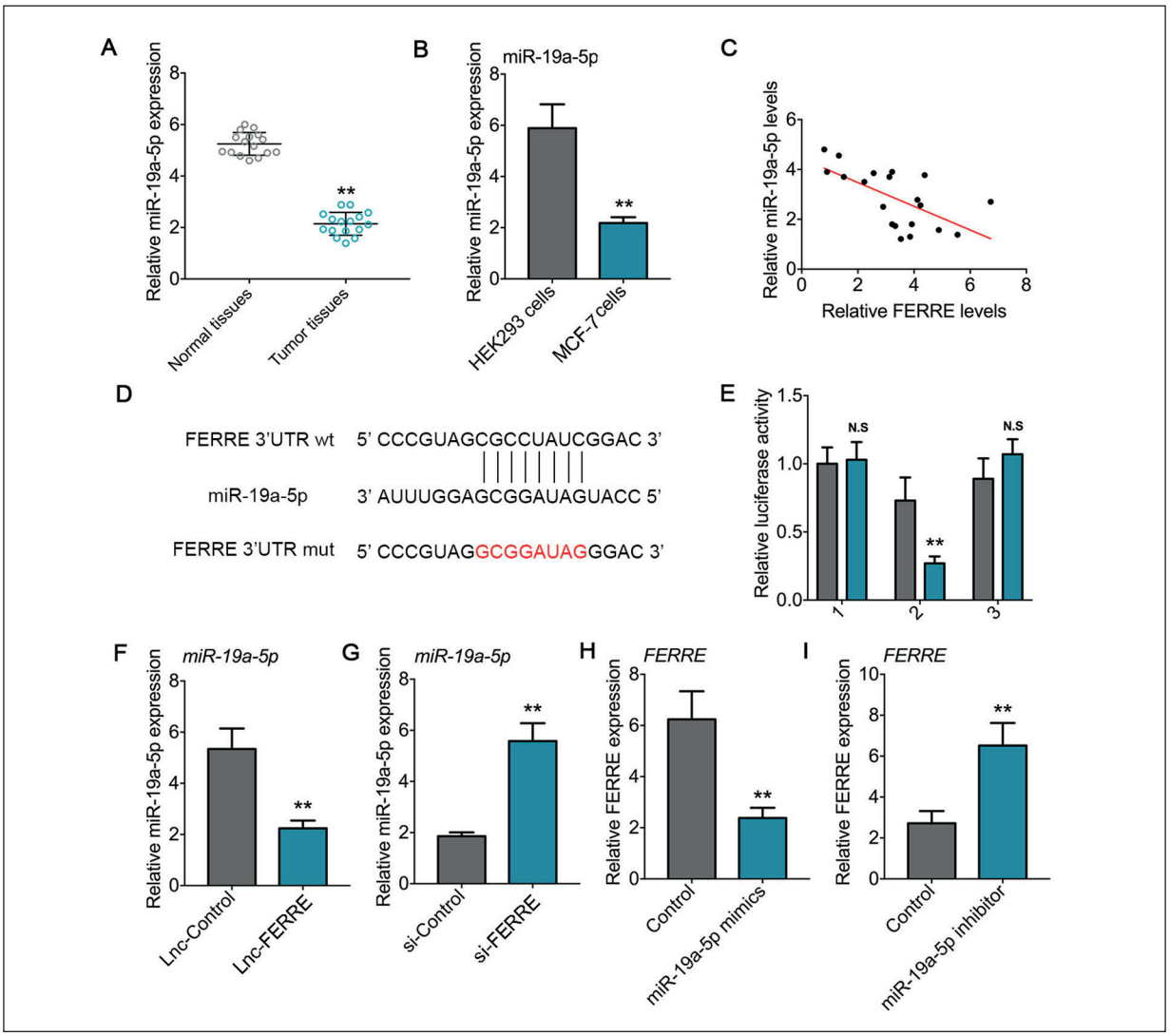

Figure 4. FERRE can sponge with miR-19a-5p in breast cancer cells. **A**, Relative expression in breast cancer tissues and adjacent normal tissues detected by qRT-PCR. **B**, Relative miR-19a-5p expression in MCF-7 cells and HEK293 cells detected by qRT-PCR. **C**, Correlation analysis was performed to evaluate the relationship between miR-19a-5p and FERRE. **D**, Schematic illustration of the predicted miR-19a-5p binding sites and mutant sites in FERRE. **E**, Relative Luciferase activity of MCF-7 cells. **F-G**, qRT-PCR analysis of miR-19a-5p expression level in MCF-7 cells transfected with lentiviral FERRE and si-FERRE. **H-I**, Relative FERRE expression was detected in MCF-7 cells after treated with miR-19a-5p mimics and miR-19a-5p inhibitor by RT-PCR. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

ure 4D). The results showed that compared with the control, the Luciferase activity of MCF-7 cells that co-transfected with wide type FERRE (FERRE-wt) and miR-19a-5p mimic was significantly decreased ($p < 0.01$), and it was reversely increased after mutation at the binding site of FERRE (FERRE-mut) compared with FERRE-wt ($p < 0.01$) (Figure 4E). These results identified that FERRE could directly bind to miR-19a-5p. In addition, overexpression of FERRE significantly inhibited miR-19a-5p expression and FERRE downregulation reversely supported miR-19a-5p expression in MCF-7 cells (Figure 4F, 4G). We also transfected miR-19a-5p mimic and miR-19a-5p inhibitor into MCF-7 cells; the results revealed that miR-19a-5p mimic inhibited FERRE expression and miR-19a-5p inhibitor increased FERRE expression (Figure 4H, 4I). Taken together, these findings demonstrated that FERRE can directly sponge with miR-19a-5p.

FERRE Modulated the Expression of EZH2 by Serving as a Molecular Sponge of MiR-19a-5p

EZH2 plays an important role in tumor progression and invasion. To explore whether miR-19a-5p can interact with EZH2, we performed qRT-PCR analysis to detect EZH2 expression in the presence of miR-19a-5p mimics. Results showed that EZH2 expression was decreased in miR-19a-5p mimics compared with control, which suggested that miR-19a-5p could inhibit EZH2 expression (Figure 5A). To validate this mechanism, mice EZH2 3′-UTR were cloned into the Luciferase reporter vector and miR-19a-5p binding mutants were constructed in which the putative miR-19a-5p binding sites GUACU in the EZH2 3′-UTR were mutated into CAUGA (Figure 5B). As expected, Dual-Luciferase report results showed that miR-19a-5p mimics significantly decreased the EZH2 expression whereas point mutations in the EZH2 3′-UTR alleviate the inhibited effect of miR-19a-5p (Figure 5C). Furthermore, we investigate whether FERRE can regulate EZH2 expression via sponging with miR-19a-5p. The results showed that FERRE could significantly increase EZH2 expression; nevertheless, mutation of the binding site with FERRE of miR-19a-5p eliminated the function effectively (Figure 5D). Conversely, inhibition of miR-19a-5p overcame the suppression of EZH2

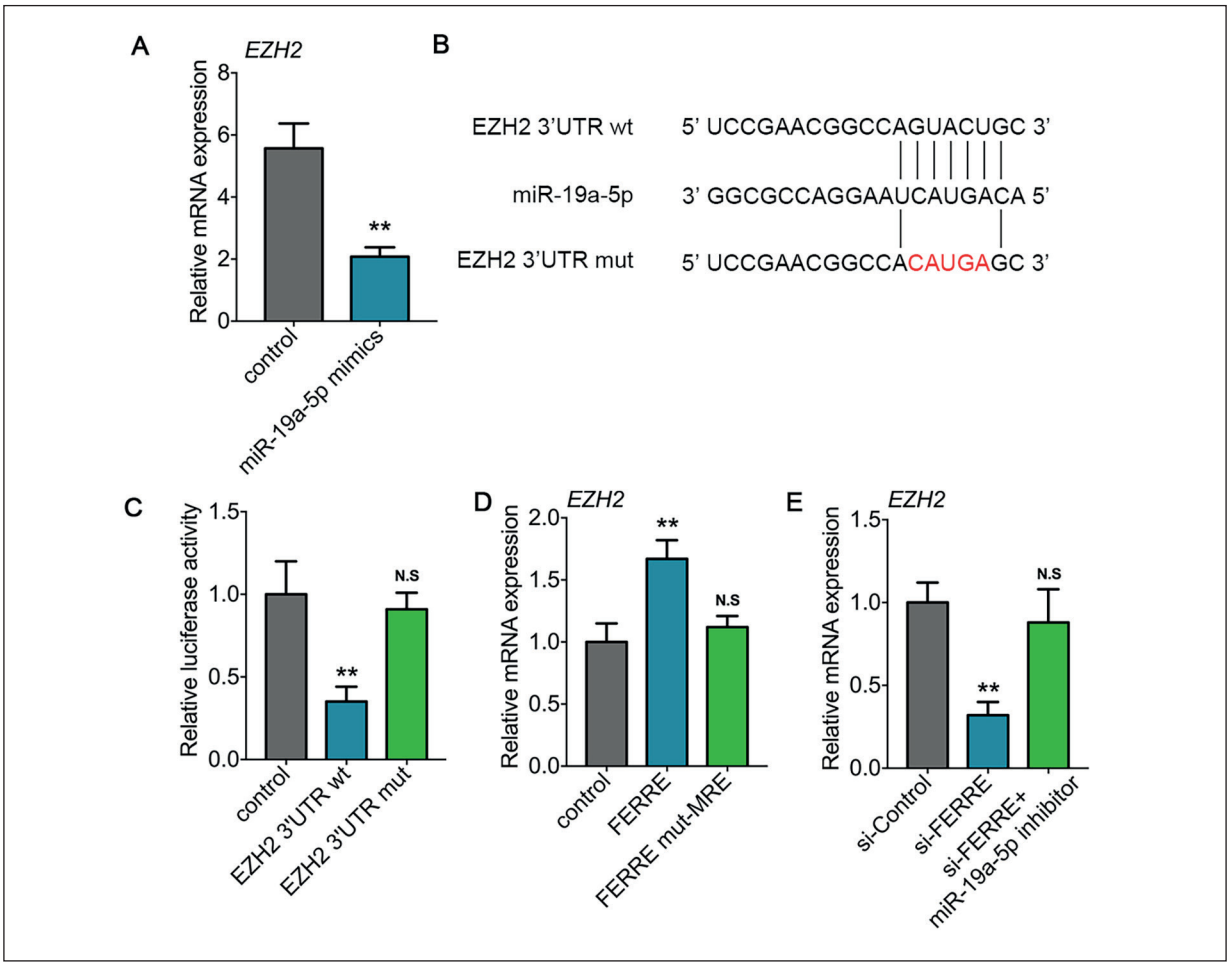

Figure 5. FERRE served as a molecular sponge for miR-19a-5p to further modulate the expression of EZH2. **A**, qRT-PCR analysis of EZH2 mRNA expression level in MCF-7 cells treated with the miR-19a-5p mimics. **B**, Schematic illustration of the predicted EZH2 binding sites and mutant sites in miR-19a-5p. **C**, Relative Luciferase activity of MCF-7 cells. **D**, Relative mRNA expression levels of EZH2 in MCF-7 cells transfected with FERRE and FERRE mut-MRE. **E**, Relative mRNA expression levels of EZH2 in MCF-7 cells transfected with si-FERRE, si-FERRE and miR-19a-5p inhibitor by qRT-PCR analysis. The data in the figures represent the averages ± SD. Statistically significant differences between the treatment and control groups are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

by FERRE knockdown (Figure 5E). Taken together, these findings suggested that FERRE could serve as a molecular sponge for the miR-19a-5p to further alter the expression EZH2.

FERRE Knockdown Suppressed Tumor Growth and EMT In Vivo

FERRE knockdown significantly suppressed tumor growth and weight in xenograft mouse models. EMT marker genes in mice tumors were examined (Figure 6A). The results showed that FERRE knockdown significantly promoted E-cadherin expression but inhibited vimentin and Snail expressions than si-control. Conversely, inhibition of miR-19a-5p overcame the suppression of vimentin and Snail by FERRE knockdown. Besides, the immunostaining cells of vimentin and Snail were decreased, and E-cadherin was increased in mice primary tumors injected with si- FERRE compared with si-Control MCF-7 cells injected group (Figure 6B). Taken together, LncRNA FERRE mediates miR-19a-5p/EZH2 signaling to promote cell progression, migration, invasion and EMT process in breast cancer.

Discussion

RNA plays a vital role in the entire gene expression process, and acts as a bridge between DNA and protein[18,19]. While, this regulation has been neglected in the past few decades. In recently years, several studies have revealed the important role of lncRNA in cellular process, including cell proliferation, differentiation and other biological processes[20-23]. LncRNAs have an effect on cell proliferation and migration of many cancer cells or stem cells[10]. Yang et al[24] reported that lnc-CCAT1 promoted cell proliferation, migration and invasion of thyroid cancer cells. As an important member of non-coding RNA, lncRNAs have been reported to play a vital role in breast cancer[25]. However, less is known about the mechanism of lncRNA in breast cancer development and progression.

Breast cancer is the most common cancer affecting women worldwide, and its incidence and mortality rates are expected to increase significantly in the next years[26,27]. Despite significant advances in cancer research setting, breast cancer remains a major health problem and currently represents a top biomedical research priority[28]. LncRNAs in breast cancer may be involved in cell growth, apoptosis, cell migration and invasion, cell adhesion, epithelial-to-mesenchymal transition (EMT), as well as cancer cell stemness. In recent years, a growing number of researches had suggested that lncRNA is involved in various biological cell processes as well as multiple cancers. In particular, lncRNA TUG1 could promote the migration and invasion of pancreatic cancer cells by sponging miR-29c[29]. NR2F2-AS1 promotes tumor invasion and proliferation of non-small-cell lung carcinoma cells via competitively binding to the miR-320b to regulate BMI1 expression[30]. Meanwhile,

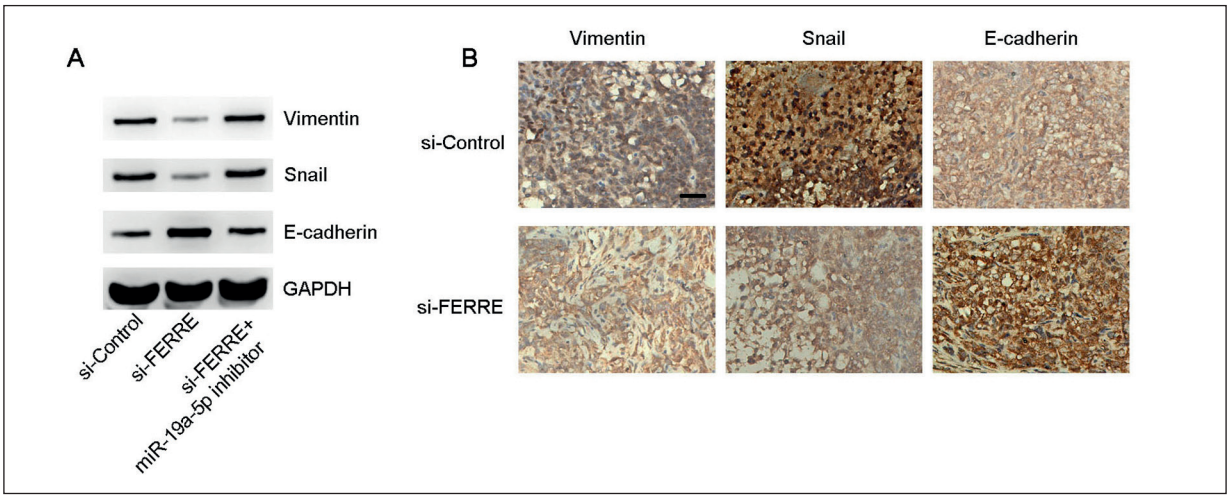

Figure 6. FERRE knockdown suppressed tumor growth and EMT *in vivo.* **A**, EMT marker E-cadherin, vimentin and snail were detected using Western blot assay in MCF-7 transfected with si- FERRE and si- FERRE+miR-19a-5p inhibitor. **B**, Immunohistochemistry of E-cadherin, vimentin and snail were detected in mice tumors. Scale bar represents 100 μm and the magnification is 200×.

some lncRNAs may play vital roles in breast cancer development and prognosis by serving as tumor oncogenes or suppressors, which may become potential therapeutic targets[31-33]. AFAP1-AS1 plays a role in the regulation of breast cancer cell proliferation, apoptosis, and metastasis, and it is correlated with poor prognosis of breast cancer[34-36]. Also, Qiao et al[37] revealed that LINC00673 activated by YY1 can promote the proliferation of breast cancer cells via the miR-515-5p/MARK4/Hippo signaling pathway. However, the role of FERRE in breast cancer progression has not been identified.

In our study, a miRNA-mRNA-lncRNA network including differently expressed mRNAs, lncRNAs, and miRNAs in breast cancer was revealed. We observed that FERRE expression was significantly enhanced in breast cancer tissues and cell lines when compared with adjacent normal tissues and HEK293 cells, respectively. Then, we further verified the effect of FERRE on biological process of human breast cancer cells MCF-7 including proliferation, invasion and migration. The results revealed that FERRE was associated with the proliferation, migration and invasion abilities of MCF-7. From bioinformatics prediction, we found miR-19a-5p was a target miRNA of FERRE and validated the combination relationship of FERRE and miR-19a-5p using Luciferase reporter assay. Besides, we found that miR-19a-5p can interact with FERRE co-expression gene EZH2 and downregulate the expression of EZH2. Mechanism analysis revealed that FERRE functions in breast cancer as a competing endogenous RNA (ceRNA) that regulate EZH2 expression by acting as a sponge for the miRNA-19a-5p. In summary, we figured out that FERRE can serve as a sponge of miR-19a-5p to elevate EZH2 expression, thus promoting cell proliferation and invasion.

Conclusions

Summarily, the above data demonstrated that FERRE/miR-19a-5p/EZH2 axis plays a vital role in the cell growth, migration, invasion, tumorigenesis, and EMT in breast cancer cells, which indicated that FERRE could act as a potential therapeutic target for human breast cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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