Long non-coding RNA AFAP1-AS1 promotes tumor progression and invasion by regulating the miR-2110/Sp1 axis in triple negative breast cancer

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Research

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Abstract

Background: LncRNAs have been proved to be involved in the proliferation, apoptosis, invasion, migration and other pathological processes of triple negative breast cancer (TNBC). And the expression level of LncRNA AFAP1-AS1 in TNBC was found to be significantly higher than that in other subtypes and normal tissue samples, but the specific mechanism of LncRNA AFAP1-AS1 affecting the occurrence and development of TNBC needs to be revealed.

Methods: Cell Counting Kit-8 assays, colony formation assays, wound-healing migration, transwell invasion assays and nude mouse xenograft assays were used to confirm the role of LncRNA AFAP1-AS1 in the proliferation, migration of TNBC cells in vitro and in vivo. Bioinformatics analyses, quantitative polymerase chain reaction (qRT-PCR), western blot, and dual-luciferase assays were performed to confirm the interaction between between LncRNA AFAP1-AS1, miR-2110 and Sp1.

Results: In the present study, the silencing of AFAP1-AS1 and Sp1 or the upregulation of miR-2110 would result in the suppression of proliferation, migration and invasion of MDA-MB-231 and MDA-MB-468 cells in vitro as well as tumor growth in vivo. Mechanistically, the dual-luciferase reporter assay highlighted that AFAP1-AS1 functioned as a miR-2110 sponge to increase Sp1 expression. AFAP1-AS1 silencing led to a reduction in Sp1 mRNA and protein levels, which could be reverse by the joint transfection of miR-2110 inhibitor.

Conclusions: Our findings demonstrated that AFAP1-AS1 acts as a miR-2110 sponge in TNBC cells, resulting in the regulation of Sp1 expression. And the AFAP1-AS1/miR-2110/Sp1 axis modulated the proliferation, migration and invasion of breast cancer cells and affected the tumorigenesis in mice.

Background

Breast cancer is one of the most common cancers among women in the world. Data in 2019 showed that breast cancer accounted for 30% of newly diagnosed cancer cases and was the cause of cancer deaths in 15% of women[1]. Clinically, according to the expression differences of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), breast cancer is divided into the following four types: Luminal A (ER+ and/or PR+, HER2−), Luminal B (ER+ and/or PR+, HER2−/+), HER2+ (ER−, PR−, HER2+) and triple negative breast cancer (TNBC). Among them, TNBC subtype accounts for about 15–20% of all breast cancer cases, which is highly malignant and has the characteristics of high recurrence rate, high metastatic potential, poor treatment response and prognosis. In addition to conventional chemotherapy and radiotherapy, there is no effective targeted therapy. Therefore, there is an urgent need to further understand the molecular mechanism of triple-negative breast cancer in tumor progression, and to develop experimental targets with potential clinical application, and it is of great significance for formulating more effective clinical treatment strategies and improving the prognosis of patients[2]. Among them, RNA-based cancer treatment methods have gradually changed from concept to reality. Non-coding RNA (ncRNA) blocked mRNA function by inhibiting its transcription and binding to proteins, which had clinical therapeutic effects on tumors [3, 4].
Long Noncoding RNA (LncRNA), having a length greater than 200 nt, is one type of ncRNA with no protein coding function, and has complicated biological functions. Their abnormal expression or dysfunction has been proved to be closely related to the occurrence and development of human diseases\[5\]. LncRNAs have been proved to be involved in the proliferation, apoptosis, invasion, migration, EMT and other pathological processes of TNBC. Based on the rapid development of high-throughput sequencing and genome analysis technology, a large amount of information about LncRNA has emerged in the past decade with the help of powerful bioinformatics analysis tools. Reiche et al. identified more than 9,500 LncRNA transcripts with significant differences in normal breast tissue and cancer tissue\[6\]. A comprehensive analysis of LncRNAs expression profiles and clinical data from 1097 breast cancer samples from The Cancer Genome Atlas (TCGA) was conducted. 1510 LncRNAs were differentially expressed in TNBC comparing with normal samples, and 672 LncRNAs expression were distinguished between TNBC and non TNBC samples\[7\]. However, the specific mechanism of LncRNAs affecting the occurrence and development of TNBC has not been deeply revealed. Therefore, it is of great significance and urgent desire to explore TNBC related LncRNAs, study the molecular mechanism of TNBC, and provide reference data for the understanding, diagnosis, treatment and prognosis of TNBC.

LncRNA actin fiber associated protein 1-antisense RNA1 (LncRNA-AFAP1-AS1, abbreviated as AFAP1-AS1) was first found in the sequencing of esophageal adenocarcinoma (EAC) and normal tissues, which was located on the antisense chain of the protein coding gene AFAP1 on chr4: 7755817–7780655 (+) of human genome (GRCh37/hg19). AFAP1-AS1 has been reported to be closely related to the proliferation and metastasis of different cancers, and was closely related to the poor prognosis of human malignant tumors\[8\], such as pancreatic ductal adenocarcinoma \[9\], esophageal adenocarcinoma \[10\], lung cancer \[11\] and colorectal cancer \[12\].

In breast cancer, the significantly up-regulated AFAP1-AS1 indicated a poor prognosis \[13,14\], and was identified as the most dysregulated LncRNA from analyzed seven pairs of HER2^+ subtype breast cancer samples \[15\]. Limited knockout of AFAP1-AS1 (about 25–50%) was sufficient to reduce the proliferation and colony formation in MDA-MB-231 cells and HCC1937 cells \[16\], meanwhile repressed BT-549 and MCF-7 cells proliferation, migration by downregulating SEPT2 via sponging miR-497-5p \[17\]. An in silico analysis from cDNA microarray data confirmed that AFAP1-AS1 was potential prognostic factor for TNBC patients \[18\]. And it promoted epithelial-mesenchymal transition by Wnt/β-catenin signaling pathway in TNBC \[19\]. A former study in our lab analyzing AFAP1-AS1 in BC samples of TCGA, and found that the expression level of AFAP1-AS1 in TNBC was significantly higher than that in other subtypes and normal tissue samples \[20\]. These above results suggested that AFAP1-AS1 was involved in the pathogenesis of breast cancer and may become a new biomarker or therapeutic target for TNBC. However, despite a small amount of research, the role of AFAP1-AS1 specific targets and related signaling pathways in the development and progression of TNBC has still been fragmentary and needs further study.

In this study, bioinformatics analysis, molecular biology, cell biology and tumorigenesis assay in nude mice were used to study the downstream regulatory relationship of AFAP1-AS1. We found that AFAP1-AS1 and Sp1 knockdown or miR-2110 overexpression would suppress MDA-MB-231, MDA-MB-468 cells proliferation, migration and invasion in vitro as well as tumor growth in vivo. AFAP1-AS1 silencing led to a reduction in Sp1
mRNA and protein levels, which could be reverse by the joint transfection of miR-2110 inhibitor. Mechanistically, the results revealed that AFAP1-AS1 competitively bound to miR-2110, affecting the expression of Sp1, thus regulating the proliferation and migration in TNBC cell and tumor progression in vivo.

**Methods**

**Cell culture**

MDA-MB-231 (ATCC® HTB-26™, The Chinese Academy of Sciences, China) and MDA-MB-468 (BNCC339862, Bnbio, China) cells were cultured in L15 medium containing 20% fetal bovine serum at 37°C and 100% air constant temperature incubator. The original medium was discarded and the cells were digested by 1ml of 0.25% trypsin when the cells grow to 80% confluence. After the adherent cells become round, stop the digestion with 1ml of medium, centrifuge in a low-speed centrifuge at 1000 r/min for 3 min. The supematant was aspirated, an appropriate amount of culture medium was added to the cell pellet. Cells were passaged in the ratio of 1:4 to 1:3 every 2 to 3 days. Observe the morphological changes with an inverted microscope.

**Plasmid construction**

The shLncRNA-AFAP1-AS1 plasmid of LncRNA-AFAP1-AS1 was constructed to the pcDNA3.1-EGFP vector, and the target sequences (shR-AFAP1-AS1-top: 5'-
GATCCGTTCTGGGCTTCAATTTACAAGCAGTCGAGCTCGACTGCTTTGAAATTGAAGCCCAGAACG-3', shR-AFAP1-AS1-bot: 5'-AGCTTCAAAAAGTTCTGGGCTTCAATTTACAAGCAGTCGACTGCTTTGAAATTGAAGCCCAGAACG-3') were inserted between the restriction site NheI (GCTAGC) and AgeI (ACCGGT).

The shSp1 plasmid of Sp1 was also constructed by the pcDNA3.1-EGFP vector, and the target sequences (GCTAGCGCTGGTGGTGATGGAATACATCTCGAGATGTATTCCATCACCACCAGCTTTTGAATTTC) were inserted between the restriction site of NheI (GCTAGC) and EcoRI (GAATTC).

**Cell transfection**

The cells in the logarithmic growth phase were used for the subsequent experiments. The pcDNA3.1-NC plasmid and shLncRNA-AFAP1-AS1 plasmid were transently transfected with Lipofectamine 3000 (L3000015, Thermo Fisher, China) according to the manufacturer's instructions. Taking the 12-well plate transfection as an example. The cells were seeded on the 12-well plate the day before transfection, and cultured overnight. 1μg per well plasmid was transfected when reaching 60-80% density. After transfection, incubate at 37°C for 72 hours for subsequent experiments.

**RT-qPCR**

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) and the RNA quantity and density were verified by a Nanodrop. PrimeScript™ RT Reagent Kit (RR037A, Takara, China) was used to reverse transcription. RT-qPCR was performed using the SYBR Premix Ex Taq™ (RR820A, Takara, China) according to the
manufacturer's instructions. The assays were operated in triplicate and relative gene expression was determined by using the $2^{-ΔΔCt}$ method.

For mRNA RT-qPCR, 2 μg total RNA and random 6 mers were used for mRNA RT reaction. The LncRNA-AFAP1-AS1 primers 5'-AATGGTGGTAGGAGGGAGGA-3' (sense) and 5'-CACACAGGGGAATGAAGAGG-3' (antisense); GAPDH primers 5'-ATGACATCAAGAAGGTGAAGCAGG-3' (sense) and 5'-GGCCTAAAGGGAGGAGTGGG-3' (antisense); and Sp1 primers 5'-TTGCTGCTATGCAAACCTA-3' (sense) and 5'-CCTGAGAGCTGGGAGTCAAG-3' (antisense) were used in the mRNA qPCR reaction.

For miRNA RT-qPCR, 0.5 μg total RNA and miR-2110 RT Primer (or U6 RT Primer, Table S1) were used for miRNA RT reaction. The primers 5'-TGCGGTTGGGGAAACGGCCGCTG-3' (miR-2110, forward), 5'-CCAGTGCAGGGTCCGAGGT-3' (miR-2110, reverse); 5'-GCTCGCTTCGGCAGCACA-3' (U6, forward) and 5'-AACGCTTCACGAATTTCGCTG-3' (U6, reverse) were used in the miRNA qPCR reaction.

**CCK-8 and colony formation**

For CCK-8 assay, 5X10$^3$ cells were inoculated into each well of 96-well plates after 72h transfection. At each time point (0, 24, 48 and 72 h), 10 μL CCK-8 solution (CK04, Dojindo, China) was added into the wells. After 4h incubation, the absorbance of each individual well was determined at 450 nm. The growth curve was drawn with the obtained data.

For colony formation assay, cells were digested into single-cell suspension and 500 cells seeded in each culture dish (6 cm). The appropriate complete medium was added to each dish with the culture medium being refreshed every three days. The cells were then washed twice with PBS, fixed with 4% paraformaldehyde and stained with 1 mL Giemsa stain for 15 min. The number of colonies was counted manually and averaged from the duplicate wells. The clone formation rate (%) = (number of cell clones / total number of cells added) x 100%.

**Wound-healing migration, and transwell invasion assays**

For wound-healing migration assay, the cell monolayers were mechanically disrupted using a sterile 10 μL micropipette tip to generate a linear wound, and the cells were washed off by PBS. After that, the culture medium was added. The 24 well plates were taken out at 0h, 24h and 48h for micrograph. The migration distance of cells was observed, the migration rate of cells at different time points was calculated, and the column diagram was drawn. The migration rate (%) = (scratch area of 0h - scratch area of Nh) / (scratch area of 0h) X100%.

For transwell invasion assays, cells were incubated using 24-well transwell plates (8 μm pore size, Corning, NY, USA). 1X10$^5$ cells suspended in serum-free medium were plated in the upper chambers with Matrigel (BD Biosciences, USA), and 600 μL complete medium was added to the lower chamber. After incubation for a suitable amount of time (24h for MDA-MB-231 and 48h for MDA-MB-468), the cells were fixed in 4% iced paraformaldehyde for 30min, stained by Giemsa stain for 15min and counted under a microscope.

**Luciferase reporter assay**
The vectors of luciferase reporters were synthesized to pmirGLO vector to constructed wildtype and mutation plasmids. Cells were inoculated into a 24-well plate and were co-transfected with miRNA mimics or the negative control and the luciferase reporter vector as well as lipofectamine 3000 reagent. After 48h, the dual-luciferase reporter was detected by Dual-Luciferase® Reporter Assay System (E1910, Promega) and relative luciferase activity was normalized to Renilla luciferase activity. The sequence of miRNA-NC mimics was 5'-UCACAACCUCUAGAAAGAGUAGA-3', and the sequence of hsa-miR-2110 mimics was 5'-UUGGGAAACGGCCGCUGAGUG-3'.

**Western blot assays**

For total protein extraction, cell lysates were obtained using RIPA buffer. A total of 10 μL protein was injected into a 10% SDS-PAGE gel and transferred to PVDF membranes at 4°C, 200 mA for 1h. After blocking with blocking solution, the membranes were incubated with primary antibody overnight at 4°C. After PBS washing, membranes were then exposed to the secondary antibody (HRP-Goat anti rabbit / mouse IgG antibody) for 1h. Bands were incubated with an ECL kit and analyzed with an imaging system. The primary antibodies CLDN4 (16195-1-AP), Sp1 (21962-1-AP), RALYL (17179-1-AP), RHBDD1 (20869-1-AP), ZNF703 (21075-1-AP), GAPDH (60004-1-Ig) were all purchased from Proteintech.

**In vivo assays**

72 of 1-5 weeks old BALB/C SPF nude mice were purchased from SPF (Beijing) Biotechnology Co., Ltd., all of them were females (not pregnant), healthy and mature, with an average weight of 18±2g. They were raised in the barrier system of the Laboratory Animal Center of the Institute of Radiation Medicine, Chinese Academy of Medical Sciences. All feed, water, air, bedding and various supplies that enter the barrier system must be sterilized by high temperature and high pressure; all people and animals entering the laboratory must undergo strict microbial control.

After 1 week of adaptive growth, they were randomly divided into 12 groups with 6 mice in each group. 0.1ml of 10^8/ml cells diluted with PBS were inoculated subcutaneously into the armpits. Observing the nude mice's mental state, activity, responsiveness, diet and appearance of the subcutaneous vaccination area every two days. The tumor size was measured every week by a Vernier caliper and the tumor volume (V) was calculated as V (cm^3) = 1/2AB^2 with A and B representing the largest and smallest diameters, respectively. With the tumor volume as the Y axis and growth weeks as the X axis, the tumor growth curves of each group were drawn. Animals were euthanized 30 days after inoculation, and the tumors were taken out, weighed, and then placed in 4% paraformaldehyde for fixation.

**Data analysis**

The data obtained were expressed as mean ± standard deviation (SD) or standard error (SE). ANOVA and t test were performed to analyze the intergroup differences. P < 0.05 indicated significant differences.

**Results**

**AFAP-AS1 promotes the proliferation, migration and invasion of breast cancer cells**
To explore the biological function of AFAP1-AS1, first we designed a shlncRNA-AFAP1-AS1 shRNA. Compared to the negative control group, the expression of AFAP1-AS1 was significantly decreased in MDA-MB-231 and MDA-MB-468 cells confirming by RT-qPCR (Fig.1A). CCK-8 showed that the cell viability was inhibited after the downregulation of AFAP1-AS1 (Fig.1B). The results of colony formation assay indicated that cell proliferation was decreased after the treatment of shlncRNA-AFAP1-AS1 (Fig.1C). Wound-healing migration and transwell invasion assays revealed that AFAP1-AS1 knockdown decreased cell migration and invasion, suggesting that AFAP1-AS1 was positively correlated with cell migration and invasion (Fig.1D and 1E).

AFAP1-AS1 targets miR-2110 in breast cancer cells

Increasing studies have shown that LncRNAs might act as sponges of miRNA, thereby interfering with tumor progression. Therefore, we speculated that AFAP1-AS1 may influence the function of some miRNAs that may play certain roles in breast cancer.

Using bioinformatics prediction assay, 13 miRNAs were predicted by LncBase database (Table.1). MiR-2110 was selected according to its probable cancer-related downstream pathway. Double luciferase reporter assays were carried out via transfecting MDA-MB-231 and MDA-MB-468 cells with luciferase reporter vectors (containing the wild type or mutant sequence of miRNA target). Compared with that of the mutant plasmid (pmirGLO/lncRNA-AFAP1-AS1-MUT), the luciferase reporter activity was significantly decreased by the miR-2110 mimics in cells transfected with the wild type plasmid (pmirGLO/lncRNA-AFAP1-AS1-WT) (Fig.2B). Meanwhile, after silencing AFAP1-AS1, the level of miR-2110 was up-regulated compared with the NC (negative control) group (Fig.2C). Together, our results provide evidence that AFAP1-AS1 could directly bind to miR-2110 in breast cancer cells.

AFAP1-AS1 upregulates Sp1 level via sponging miR-2110

According to previous studies, miR-2110 was found to have differentiation-inducing and oncosuppressive function in neuroblastoma, and was significantly related to the survival rate of patients[21]. Thus, we hypothesized that miR-2110 may functioned as suppressor in TNBC cells and AFAP1-AS1 promoted tumor progression by protecting downstream oncogenes from downregulation by miR-2110.

In order to verify our hypothesis, firstly, 509 miR-2110 potential target genes were found after the prediction and overlapping of three miRNA interaction database: TargetScan, miRDB and miRTarBase. Subsequently, 462 of them were analyzed by R cluster profiler package. Combined with the subsequent KEGG analysis and the reported pro-oncogenic and up-regulated genes, five downstream genes with the highest correlation with miR-2110 were screened (Table 2, Fig.3A and 3B) and miR-2110 possessed binding sites in 3' UTR of predicted gene (Fig.3C). Firstly, miR-2110 mimics were transfected into MDA-MB-231 and MDA-MB-468 cells to observe the changes of protein expression levels of candidate target genes (Fig.3D). The results showed that compared with CLDN4, RALYL, RHBDD1 and ZNF703, the Sp1 protein expression decreased significantly after transfection with miR-2110 mimics, while the expression of Sp1 increased after transfection with miR-2110 inhibitor (Fig.3E and Fig.S1). It is speculated that miR-2110 has the strongest targeting ability to Sp1. Subsequently, dual-luciferase assay was conducted to verify the target relationship between miR-2110 and Sp1. The results in MDA-MB-231 and MDA-MB-468 cells showed that the relative luciferase reporter intensity was 50% lower after the transfection of miR-2110 mimics comparing with the wild type plasmid alone
(pmirGLO/Sp1-WT), while miR-2110 mimics had no effect on the mutation plasmid (pmirGLO/Sp1-MUT), which demonstrated that Sp1 was a critical target of miR-2110 (Fig.3F).

To further demonstrate the link among AFAP1-AS1, miR-2110, and Sp1 expressions, cells were subjected to different transfections. First, RT-qPCR assay showed that Sp1 mRNA level was significantly decreased after transfected with miR-2110 mimics in MDA-MB-231 and MDA-MB-468 cells, meanwhile the miR-2110 inhibitor caused an up-regulated mRNA level of Sp1 (Fig.3G). Moreover, cells were then transfected with NC or shLncRNA-AFAP1-AS1, and AFAP1-AS1 silencing led to a reduction in Sp1 mRNA and protein levels, which could be rescued by the co-transfection of miR-2110 inhibitor (Fig.3H and 3I). Together, our results show that miR-2110 levels were negatively correlated, whereas AFAP1-AS1 levels were positively correlated to the Sp1 expression, suggesting that AFAP1-AS1 upregulated Sp1 expression via sponging miR-2110.

**AFAP1-AS1/miR-2110/Sp1 axis affect the proliferation, migration and invasion of breast cancer cells**

To further verify whether AFAP1-AS1 induced breast cancer progression through the miR-2110 sponge, rescue experiments were carried out. CCK-8, colony formation, wound healing and transwell invasion assays were performed. The results showed that miR-2110 inhibition promoted cell proliferation, migration, and invasion (Fig. 4B-4E, group 1 vs group 3), meanwhile these phenotype were all dismissed by AFAP1-AS1 silencing via the co-transfection of shLncRNA-AFAP1-AS1 (Fig. 4B-4E, group 3 vs group 4). These results showed that AFAP1-AS1 silencing reversed the miR-2110 downregulation-induced phenotype of breast cancer cells.

We then evaluated the roles of Sp1 in breast cancer cells by Sp1 knockdown. After the transfection, the expression of Sp1 was verified by western blot, which was significantly decreased in shSp1 group than that in NC group (Fig. 4A). CCK-8 and colony formation assays showed that the cell viability was inhibited after Sp1 knockdown, and wound healing, transwell invasion assays revealed that Sp1 knockdown decreased cell migration, invasion of breast cancer cells (Fig. 4B-4E, group 1 vs group 5 and Fig.S2). In a similar way, all these effects are offset by the joint addition of miR-2110 inhibitor (Fig. 4B-4E, group 5 vs group 6 and Fig.S2). These results indicated that downregulated Sp1 inhibited tumor progression, and miR-2110 downregulation reversed the Sp1 silencing-induced phenotype of breast cancer cells.

**AFAP1-AS1/miR-2110/Sp1 axis affect the tumorigenesis in mice**

The mice were subcutaneously injected with MDA-MB-231 or MDA-MB-468 cells as the following groups: 1.NC+inhibitor control; 2.shLncRNA-AFAP1-AS1+inhibitor control; 3.NC+miR-2110-inhibitor; 4.shLncRNA-AFAP1-AS1+miR-2110-inhibitor; 5.shSp1+inhibitor control; 6.shSp1+miR-2110-inhibitor, and they were used for investigating the role of AFAP1-AS1/miR-2110/Sp1 axis in tumor formation.

The findings showed that tumors in the AFAP1-AS1-silencing group had a slower growth rate and particularly less average volume and weight (Fig. 5, group 1 vs group 2). And the tumor volume and weight of the miR-2110-silencing group increased significantly (Fig. 5, group 1 vs group 3), while these phenotype were all balanced by AFAP1-AS1 silencing in the shLncRNA-AFAP1-AS1+ miR-2110-inhibitor group (Fig. 5, group 3 vs group 4), in which the tumor size and growth rate were not significantly different from the negative control group (Fig. 5, group 1 vs group 4). At the same time, the silence of Sp1 also caused a significant decrease in tumor volume and growth rate (Fig. 5, group 1 vs group 5). After the joint addition of miR-2110 inhibitor
(shSp1+miR-2110-inhibitor group), related parameters such as tumor volume and growth rate increased (Fig. 5, group 5 vs group 6). The results showed that the AFAP1-AS1/miR-2110/Sp1 axis affected the growth rate, average size and weight of tumor.

**Discussion**

To date, a large number of LncRNAs have been recently discovered through functional genomics studies. Although many studies have shown that LncRNAs can participate in the pathological process of breast cancer, there are relatively few studies on TNBC-related LncRNAs, especially the specific mechanism that affects the occurrence and development of TNBC has not yet been deeply revealed.

AFAP1-AS1 was found on the antisense chain of the protein coding gene AFAP1, and involved in the development of a variety of cancers, such as, relating to the poor prognosis of tongue squamous cell carcinoma (TSCC), played a role of oncogene in TSCC by activating Wnt / β-catenin signaling pathway and inhibiting the expression of EMT related genes[22]. In gastric cancer tissues and cells, AFAP1-AS1 was significantly up-regulated and regulated the proliferation and apoptosis of gastric cancer cells through PTEN / p-Akt pathway[23]. The expression of AFAP1-AS1 was also up-regulated in esophageal squamous cell carcinoma, and was significantly correlated with TNM stage and tumor size[24]. Hypo-methylation and high expression of AFAP1-AS1 was found in Barrett’s esophagus and EAC. Its expression interference could also inhibit the proliferation and colony forming ability of EAC cells[25]. In breast cancer, AFAP1-AS1 also showed a significantly up-regulated expression compared with adjacent tissues[13]. Here, after analyzing the expression of AFAP1-AS1 in the BC samples of the TCGA database, our previous study found that the expression of AFAP1-AS1 in TNBC was significantly higher than other subtypes and normal tissue sample[20], suggesting that AFAP1-AS1 may be involved in the pathogenesis of breast cancer and may become a new biomarker or therapeutic target for TNBC. After discovering the potential importance of AFAP1-AS1, we deeply studied its function on breast cancer cells, and found that AFAP1-AS1 could promote cell proliferation, clone formation and was positively correlated with cell migration and invasion. Our research will undoubtedly enrich the research field of LncRNA in TNBC.

Studies have shown that LncRNAs participate in multiple regulatory mechanisms, such as ceRNAs, regulation of transcription, translation, protein modification, and the formation of RNA-protein or protein-protein complexes. The ceRNA networks link the function of mRNAs with non-coding RNAs such as miRNAs and LncRNAs, which assumes that LncRNAs serve as miRNA sponge to eliminate miRNA inhibition on its target genes. In this study, dual-luciferase reporter assay confirmed that AFAP1-AS1 acted as a sponge for miR-2110. There are few studies on miR-2110, but it was considered to be a tumor suppressor in neuroblastoma[21], which may be functioned by directly targeting Tsukushi[26]. Meanwhile, our research also proved that decreased miR-2110 promoted cell proliferation, migration, and invasion in TNBC cells. MiRNAs are capable of regulating physiological processes by inhibiting target mRNA translation or promoting mRNA degradation. The bioinformatics results of miRNA target prediction showed five potential miR-2110 target genes with the highest correlation, including CLDN4, RALYL, RHBDD1, ZNF703 and Sp1. Compared with CLDN4, RALYL, RHBDD1 and ZNF703, the Sp1 expression had more significantly distinction after miR-2110 mimics and
inhibitor transfection. And it was a well-known transcription factor with pro-oncogenic function in multiple tumors, therefore Sp1 was selected for the following research.

Sp1 is a transcription factor that binds to GC-rich motifs of many promoters and is involved in many cellular processes, including cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, and chromatin remodeling. It is overexpressed in multiple tumors and is a negative prognostic factor for patient survival. For example, Kim et al. studied the expression profile of 203 TNBC patients during adjuvant chemotherapy and found that the increase in Sp1 expression was associated with poor prognosis. The Sp1 expression in the multivariate Cox regression model was an effective indicator for predicting the long-term prognosis of TNBC patients treated with doxorubicin. In addition, a large number of studies have shown that Sp1-regulated genes were related to the pro-oncogenic activity. Such as, ERK/Sp1 signaling pathways mediated the TGF-β-induced EGFR upregulation, resulting in the promotion of migration and invasion in breast cancer cells. STAT3 and Sp1 cooperated to induce high expression of the small GTPase Ras Homolog Family Member U RhoU and enhanced breast cancer cells migration. In the present study, the down-regulated Sp1 suppressed the proliferation, migration and invasion of MDA-MB-231 and MDA-MB-468 cells as well as the tumor growth in vivo. And LncRNA-AFAP1-AS1 was shown to competitively bind to miR-2110 to reduce the inhibition effect of Sp1 by miR-2110, resulting in the promoted breast cancer progression. All these above findings indicated that AFAP1-AS1 served as a ceRNA to contribute to breast cancer progression through the miR-2110/Sp1 axis.

On the other hand, some studies have shown that Sp1, as a sequence-specific DNA binding protein, could initiate the transcription of many cellular genes (including LncRNAs) and participate in various biological processes such as cell proliferation, differentiation, and tumor formation. For example, in retinal malformation cells, Sp1 directly bound to the LncRNA PADAR promoter region and promoted its transcription to regulate apoptosis caused by the Bcl-2/caspase-3 pathway. Sp1 could bind LncRNA SPRY4-IT1 promoter and activate its transcription, playing a carcinogenic role in cholangiocarcinoma. It was noteworthy that, JASPAR platform predicted that there were 18 Sp1 binding sites in the AFAP1-AS1 promoter region, indicating that Sp1 may bind to the AFAP1-AS1 promoter region and regulate the expression of AFAP1-AS1. So, we presumes that AFAP1-AS1 acts as miR-2110 sponge reducing its inhibition effect on Sp1, and in turn, the elevated Sp1 binds to the AFAP1-AS1 promoter region and activates its transcription, forming a positive feedback system. Certainly, these hypothesis also need further exploration and discovery in follow-up research.

Conclusion

In summary, we detected an LncRNA (AFAP1-AS1) that was overexpressed in TNBC tissues, upregulating Sp1 level via sponging miR-2110. Meanwhile the AFAP1-AS1/miR-2110/Sp1 axis modulated the proliferation, migration and invasion of breast cancer cells and affected the tumorigenesis in mice. Our results not only elucidated the potential mechanism by which LncRNAs regulate the TNBC progression, but also suggested that the AFAP1-AS1/miR-2110/Sp1 axis could be a potential target for triple negative breast cancer.

Abbreviations
BC: Breast cancer; TNBC: Triple negative breast cancer; NC: Negative control; AFAP1-AS1: Abbreviation of LncRNA-AFAP1-AS1, LncRNA actin fiber associated protein 1-antisense RNA1; Sp1: Transcription factor specificity protein 1; CLDN4: Claudin-4; RALY: RALY heterogeneous nuclear ribonucleoprotein; RHBDD1: Rhomboid domain containing 1; ZNF703: Zinc finger protein 703;

**Declarations**

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**Authors’ contributions**

XH.Z and FY.L conceived and designed the experiments; XH.Z, FY.L and YD.Z prepared the manuscript; XH.Z, F.M and Y.L performed the experiments; XH.Z, FY.L, YT.L, S.Z and SJ.S analyzed the data; Q.S made the final revisions.

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**Availability of data and materials**

All the data reported by the manuscript are publicly available and the materials are also freely available.

**Ethics approval and consent to participate**

All animal experiments were conducted in accordance with the Guidelines for Animal Health and Use (Ministry of Science and Technology of China, 2006).

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.

**References**

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA-Cancer J Clin. 2019; 69: 7-34.

[2] Soo-Yeon, Hwang, Seojeong, Park, Youngjoo, Kwon. Recent therapeutic trends and promising targets in triple negative breast cancer. Pharmacol Ther. 2019; 199: 30-57.
[3] Burnett JC, Rossi JJ. RNA-Based Therapeutics: Current Progress and Future Prospects. Chem Biol. 2012; 19: 60-71.

[4] Sullenger BA, Nair S. From the RNA world to the clinic. Science. 2016; 352: 1417-20.

[5] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009; 10: 155-9.

[6] Reiche K, Kasack K, Schreiber S, Lüders T, Due EU, Naume B, Riis M, Kristensen VN, Horn F, Børresen-Dale AL. Long Non-Coding RNAs Differentially Expressed between Normal versus Primary Breast Tumor Tissues Disclose Converse Changes to Breast Cancer-Related Protein-Coding Genes. Plos One. 2014; 9: e106076.

[7] Chun-Ni F, Lei M, Ning L. Comprehensive analysis of novel three-long noncoding RNA signatures as a diagnostic and prognostic biomarkers of human triple-negative breast cancer. J Cell Biochem. 2018; 120: 3185-96.

[8] Ji D, Zhong X, Jiang X, Leng K, Xu Y, Li Z, Huang L, Li J, Cui Y. The role of long non-coding RNA AFAP1-AS1 in human malignant tumors. Pathol Res Pract. 2018; 214: 1524-31.

[9] Ye Y, Chen J, Zhou Y, Fu Z, Zhou Q, Wang Y, Gao W, Zheng S, Zhao X, Chen T, Chen R. High expression of AFAP1-AS1 is associated with poor survival and short-term recurrence in pancreatic ductal adenocarcinoma. J Transl Med. 2015; 13: 137.

[10] Wu W, Bhagat TD, Yang X, Song JH, Cheng Y, Agarwal R, Abraham JM, Ibrahim S, Bartenstein M, Hussain Z, Suzuki M, Yu Y, Chen W, Eng C, Greally J, Verma A, Meltzer SJ. Hypomethylation of noncoding DNA regions and overexpression of the long noncoding RNA, AFAP1-AS1, in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology. 2013; 144: 956-66.e4.

[11] Leng X, Ding X, Wang S, Fang T, Shen W, Xia W, You R, Xu K, Yin R. Long noncoding RNA AFAP1-AS1 is upregulated in NSCLC and associated with lymph node metastasis and poor prognosis. Oncol Lett. 2018; 16: 727-32.

[12] Wang F, Ni H, Sun F, Li M, Chen L. Overexpression of IncRNA AFAP1-AS1 correlates with poor prognosis and promotes tumorigenesis in colorectal cancer. Biomed Pharmacother. 2016; 81: 152-9.

[13] Dianatpour A, Faramarzi S, Geranpayeh L, Mirfakhraie R, Motevaseli E, Ghafouri-Fard S. Expression analysis of AFAP1-AS1 and AFAP1 in breast cancer. Cancer Biomark. 2018; 22: 49-54.

[14] Ma D, Chen C, Wu J, Wang H, Wu D. Up-regulated IncRNA AFAP1-AS1 indicates a poor prognosis and promotes carcinogenesis of breast cancer. Breast cancer-Tokyo. 2019; 26: 74-83.

[15] Fan Y, Shixu L, Siyang D, Yehuan L, Ouchen W, Xiaohua Z. Expression profile analysis of long noncoding RNA in HER-2-enriched subtype breast cancer by next-generation sequencing and bioinformatics. OncoTargets Ther. 2016; 9: 761-72.
[16] Xi Y, Shi J, Li W, Tanaka K, Dent SYR. Histone modification profiling in breast cancer cell lines highlights commonalities and differences among subtypes. BMC Genomics. 2018; 19: 150.

[17] Cai B, Wang X, Bu Qa, Li P, Xue Q, Zhang J, Ding P, Sun D. LncRNA AFAP1-AS1 Knockdown Represses Cell Proliferation, Migration, and Induced Apoptosis in Breast Cancer by Downregulating SEPT2 Via Sponging miR-497-5p. Cancer Biotherapy and Radiopharmaceuticals. 2020: Epub.

[18] Rodrigues de Bastos D, Nagai MA. In silico analyses identify IncRNAs: WDFY3-AS2, BDNF-AS and AFAP1-AS1 as potential prognostic factors for patients with triple-negative breast tumors. Plos One. 2020; 15: e0232284.

[19] Zhang K, Liu P, Tang H, Xie X, Kong Y, Song C, Qiu X, Xiao X. AFAP1-AS1 Promotes Epithelial-Mesenchymal Transition and Tumorigenesis Through Wnt/β-Catenin Signaling Pathway in Triple-Negative Breast Cancer. Front Pharmacol. 2018; 9: 1248.

[20] Zhang X, Zhou Y, Mao F, Lin Y, Shen S, Sun Q. IncRNA AFAP1-AS1 promotes triple negative breast cancer cell proliferation and invasion via targeting miR-145 to regulate MTH1 expression. Sci Rep. 2020; 10: 7662.

[21] Zhenze, Zhao, Veronica, Partridge, Michaela, Sousares, Spencer, Shelton, Cory. microRNA-2110 functions as an onco-suppressor in neuroblastoma by directly targeting Tsukushi. Plos One. 2018; 13: e0208777.

[22] Wang ZY, Hu M, Dai M-h, Xiong J, Zhang S, Wu H-j, Zhang S-s, Gong Z-j. Upregulation of the long non-coding RNA AFAP1-AS1 affects the proliferation, invasion and survival of tongue squamous cell carcinoma via the Wnt/β-catenin signaling pathway. Mol Cancer. 2018; 17: 3.

[23] Guo JQ, Li S-j, Guo G-x. Long Noncoding RNA AFAP1-AS1 Promotes Cell Proliferation and Apoptosis of Gastric Cancer Cells via PTEN/p-AKT Pathway. Dig Dis Sci. 2017; 62: 2004-10.

[24] Hong-lei, Luo, Ming-de, Huang, Jia-ni, Guo, Rui-hua, Fan, Xiao-tian, Xia. AFAP1-AS1 is upregulated and promotes esophageal squamous cell carcinoma cell proliferation and inhibits cell apoptosis. Cancer Med. 2016; 5: 2879-85.

[25] Wu W, Bhagat TD, Yang X, Song JH, Cheng Y, Agarwal R, Abraham JM, Ibrahim S, Bartenstein M, Hussain Z. Hypomethylation of Noncoding DNA Regions and Overexpression of the Long Noncoding RNA, AFAP1-AS1, in Barrett's Esophagus and Esophageal Adenocarcinoma. Gastroenterology. 2013; 144: 956-66.e4.

[26] Zhao Z, Partridge V, Sousares M, Shelton SD, Holland CL, Pertsemlidis A, Du L. microRNA-2110 functions as an onco-suppressor in neuroblastoma by directly targeting Tsukushi. Plos One. 2018; 13: e0208777.

[27] Kim JY, Jung HH, Ahn S, Bae S, Lee SK, Kim SW, Lee JE, Nam SJ, Ahn JS, Im Y-H. The relationship between nuclear factor (NF)-κB family gene expression and prognosis in triple-negative breast cancer (TNBC) patients receiving adjuvant doxorubicin treatment. Sci Rep. 2016; 6: 31804.

[28] Safe S, Imanirad P, Sreevalsan S, Nair V, Jutooru I. Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target. Expert Opin Ther Targets. 2014; 18: 759-69.
[29] Zhao Y, Ma J, Fan Y, Wang Z, Tian R, Ji W, Zhang F, Niu R. TGF-β transactivates EGFR and facilitates breast cancer migration and invasion through canonical Smad3 and ERK/Sp1 signaling pathways. Mol Oncol. 2018; 12: 305-21.

[30] Monteleone E, Orecchia V, Corrieri P, Schiavone D, Avalle L, Moiso E, Savino A, Molineris I, Provero P, Poli V. SP1 and STAT3 Functionally Synergize to Induce the RhoU Small GTPase and a Subclass of Non-canonical WNT Responsive Genes Correlating with Poor Prognosis in Breast Cancer. Cancers. 2019; 11: 101.

[31] Sheng L, Wu J, Gong X, Dong D, Sun X. SP1-induced upregulation of IncRNA PANDAR predicts adverse phenotypes in retinoblastoma and regulates cell growth and apoptosis in vitro and in vivo. Gene. 2018; 668: 140-5.

[32] Xu Y, Yao Y, Jiang X, Zhong X, Wang Z, Li C, Kang P, Leng K, Ji D, Li Z. SP1-induced upregulation of IncRNA SPRY4-IT1 exerts oncogenic properties by scaffolding EZH2/LSD1/DNMT1 and sponging miR-101-3p in cholangiocarcinoma. J Exp Clin Cancer Res. 2018; 37: 81.

[33] Ma X, Miao H, Jing B, Pan Q, Zhang H, Chen Y, Zhang D, Liang Z, Wen Z, Li M. Claudin-4 controls the proliferation, apoptosis, migration and in vivo growth of MCF-7 breast cancer cells. Oncol Rep. 2015; 34: 681-90.

[34] Lanigan F, McKiernan E, Brennan DJ, Hegarty S, Millikan RC, McBryan J, Jirstrom K, Landberg G, Martin F, Duffy MJ, Gallagher WM. Increased claudin-4 expression is associated with poor prognosis and high tumour grade in breast cancer. Int J Cancer. 2009; 124: 2088-97.

[35] Bondy-Chorney E, Baldwin RM, Didillon A, Chabot B, Jasmin BJ, Côté J. RNA binding protein RALY promotes Protein Arginine Methyltransferase 1 alternatively spliced isoform v2 relative expression and metastatic potential in breast cancer cells. Int J Biochem Cell Biol. 2017; 91: 124-35.

[36] Zhang X, Zhao Y, Wang C, Ju H, Liu W, Zhang X, Miao S, Wang L, Sun Q, Song W. Rhomboid domain-containing protein 1 promotes breast cancer progression by regulating the p-Akt and CDK2 levels. Cell Commun Signal. 2018; 16: 65.

[37] Huang C, Ji X, Peng Y, Wu M, Wu W, Luo Y, Cheng G, Zhu Y. Silencing of rhomboid domain containing 1 to inhibit the metastasis of human breast cancer cells in vitro. Iran J Basic Med Sci. 2018; 21: 1161-6.

[38] Holland DG, Burleigh A, Git A, Goldgraben MA, Perez-Mancera PA, Chin SF, Hurtado A, Bruna A, Ali HR, Greenwood W, Dunning MJ, Samarajiwa S, Menon S, Rueda OM, Lynch AG, McKinney S, Ellis IO, Eaves CJ, Carroll JS, Curtis C, Aparicio S, Caldas C. ZNF703 is a common Luminal B breast cancer oncogene that differentially regulates luminal and basal progenitors in human mammary epithelium. EMBO Mol Med. 2011; 3: 167-80.

Tables

Table 1 Searching of AFAP1-AS1 (ENSG00000272620) potential interacted miRNAs via LncBase database.
Table 2. The predicted target genes of miR-2110.

| miRNA     | Potential targets | Function in BC                                                                                                                                 |
|-----------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| hsa-miR-2110 | Sp1               | Associated with poor prognosis[27]; promotion of migration and invasion in BC cells [29, 30].                                                   |
|           | CLDN4             | Promoting BC cell proliferation, migration [33]; correlated positively with tumor grade and Her2, and negatively with ER [34].                  |
|           | RALY              | Increasing expression in BC cells and tumors, and correlated with decreased patient survival [35].                                                |
|           | RHBDD1            | Promoting BC progression [36] and metastasis [37].                                                                                           |
|           | ZNF703            | BC oncogene, overexpressed in BC [38].                                                                                                        |

**Supplementary Information**

**Additional file Figure S1.** Relative protein expression in breast cancer cells with different treatments of miR-2110 mimics or inhibitor. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.
Additional file Figure S2. (A) Wound healing and (B) transwell invasion assays performed in MDA-MB-231 and MDA-MB-468 cells.

Additional file Table S1. Primers or sequences used in the study.

Figures

Figure 1
AFAP1-AS1 promoted the proliferation, migration and invasion of breast cancer cells. (A) Relative expression of AFAP1-AS1 after the treatment of shlncRNA-AFAP1-AS1. (B) CCK-8 assay showed the effect of shlncRNA-AFAP1-AS1 on the proliferation of MDA-MB-231 and MDA-MB-468 cells at 0, 24, 48, and 72 hours after transfection. (C) Colony formation, (D) wound healing, and (E) transwell invasion performed in MDA-MB-231 and MDA-MB-468 cells. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.

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**Figure 2**

AFAP1-AS1 targeted miR-2110 in breast cancer cells. (A) Putative binding site of miR-2110 with AFAP1-AS1 3’UTR. (B) The luciferase activity of pmirGLO/lncRNA-AFAP1-AS1-WT/MUT plasmid in MDA-MB-231 and MDA-MB-468 cells after co-transfection with miR-2110. (C) Relative miR-2110 expression with shlncRNA-AFAP1-AS1 or NC. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.
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Figure 3

AFAP1-AS1 upregulated Sp1 level via sponging miR-2110. (A) Schematic illustration exhibiting overlapping of miR-2110 target mRNAs in three database. (B) KEGG analysis of 462 predicted target genes. (C) Putative binding site of miR-2110 with Sp1 3’UTR. (D) The miR-2110 expression in MDA-MB-231 and MDA-MB-468 cells were examined through RT-qPCR. (E) Relative protein level of Sp1 after miR-2110 mimics or inhibitor treatment. (F) The luciferase activity of pmir-GLO/Sp1-WT/MUT plasmid after co-transfection with miR-2110. (G) The relative mRNA level of Sp1 after miR-2110 mimics or inhibitor treatment. (H-I) Relative protein and mRNA level of Sp1 after shlncRNA-AFAP1-AS1, miR-2110 mimics and inhibitor treatment. Unpaired student’s t-
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Figure 4
AFAP1-AS1/miR-2110/Sp1 affected the proliferation, migration and invasion of breast cancer cells. (A) The Sp1 expression in MDA-MB-231 and MDA-MB-468 cells were examined through western blot. (B)CCK-8 assay performed. * represented the significant analysis between group 1 vs group 2 (*), group 1 vs group 3 (*), group 3 vs group 4 (*), group 1 vs group 5 (#), group 5 vs group 6 (*), respectively. (C) Colony formation, (D) wound-healing and (E) Transwell assays performed after different treatment. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.

Figure 4
AFAP1-AS1/miR-2110/Sp1 affected the proliferation, migration and invasion of breast cancer cells. (A) The Sp1 expression in MDA-MB-231 and MDA-MB-468 cells were examined through western blot. (B) CCK-8 assay performed. * represented the significant analysis between group 1 vs group 2 (*), group 1 vs group 3 (*), group 3 vs group 4 (*), group 1 vs group 5 (#), group 5 vs group 6 (*), respectively. (C) Colony formation, (D) wound-healing and (E) Transwell assays performed after different treatment. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.
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Figure 5
AFAP1-AS1/miR-2110/Sp1 affected the tumorigenesis in mice. (A) Tumor tissues, (B) weight and (C) growth curve in each group are shown. * represented the significant analysis between group 1 vs group 2 (*), group 1 vs group 3 (*), group 3 vs group 4 (*), group 1 vs group 5 (#), group 5 vs group 6 (*), respectively. Unpaired student’s t-test and one-way ANOVA test were used for the statistical analyses. *p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001; ns, no significant.

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