

lncRNA ATXN8OS promotes breast cancer by sequestering miR-204

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Received November 23, 2018; Accepted April 26, 2019

DOI: 10.3892/mmr.2019.10367

Abstract. Breast cancer (BC) is a common malignancy among women and the leading cause of female cancer mortality worldwide. In recent years, increasing evidence has shown that long non-coding RNAs (lncRNAs) can act as competing endogenous RNAs (ceRNAs) in human cancer and that they are involved in many biological processes, including proliferation, migration, apoptosis and invasion. In the present study, the biological function and molecular mechanism of ataxin 8 opposite strand (ATXN8OS) in BC tissue and cell lines were investigated. It was found that ATXN8OS was markedly up-regulated in BC tissue and cell lines, and that its level of overexpression was inversely linked with the overall survival rate of patients with BC. Knockdown of ATXN8OS inhibited proliferation, viability and invasion in the human MCF7 and MDA-MB-231 BC cell lines. In addition, microRNA-204 (miR-204) was negatively associated with the expression of ATXN8OS in BC tissues and cell lines. A luciferase assay demonstrated a direct binding site for miR-204 within ATXN8OS, and inhibition of miR-204 stimulated the tumour-promoting effect of ATXN8OS on BC cells. In conclusion, the present study suggested that ATXN8OS acts as a tumour promoter by sequestering miR-204 during the development of BC, therefore providing a mechanistic insight which may facilitate the diagnosis and treatment of BC.

Introduction

Breast cancer (BC) is one of the most common malignancies worldwide, accounting for 29% of all female cancer cases, and has a high mortality rate (1-3). Although advanced treatments, such as early detection, mastectomy, radiotherapy, chemotherapy, endocrine treatment, targeted therapy and even systemic treatment, have greatly improved, the 5-year overall survival rate for BC is low, especially when the cancer becomes metastatic (4). A high proportion, ~90%, of BC-related mortality is attributed to the formation of metastatic lesions (5). Thus, this malignancy is a threat to the health of women worldwide. Emerging molecular biomarkers that can predict patient outcomes and therapy responses have been identified, and researchers have shown that numerous molecular triggers are important in the development of BC (6-10).

Long non-coding RNAs (lncRNAs), with a length of >200 nucleotides, are a class of RNA molecule that lack the ability to produce functional proteins (11). They have been demonstrated to be involved in complex biological processes, including cell cycle regulation, cell differentiation, transcriptional modification, chromosome remodelling, epigenetic regulation and tumour progression (12-15). lncRNAs, which play an important role in human cancer, are an area of emerging focus for clinical applications (8,16,17).

lncRNAs can function as competing endogenous RNAs (ceRNAs) by sequestering microRNAs (miRNAs/miRs) and regulating downstream targets (18). In BC, previous studies have revealed that the expression of lncRNAs is related to the clinical features and overall survival rate of BC. Dong *et al* (19) found that the lncRNA AGAP2 antisense RNA 1 promoted BC growth and chemoresistance, by regulating the expression of myeloid differentiation primary response protein MyD88 *in vitro* and *in vivo*. ATXN8OS is a lncRNA with a size of 1,236 bp located on chromosome 13q21 (20). Koob *et al* (21) reported that abnormal expression of ATXN8OS occurs in various brain tissues. A previous study revealed that ATXN8OS was up-regulated in BC and was involved in the lncRNA-miRNA-mRNA ceRNA network. However, the specific function of this lncRNA, or its role in the development and progression of BC, was not investigated (22). Therefore,

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Key words: breast cancer, long non-coding RNA, microRNA-204, proliferation, invasion, tumorigenesis

the aim of the present study was to explore the underlying molecular mechanism of ATXN8OS in BC, and to identify its potential role as a putative diagnostic biomarker and therapeutic target.

Materials and methods

Patient samples. Human BC samples (n=120) and matched non-tumour tissues were collected from patients who underwent a radical mastectomy at 900th Hospital of the Joint Logistics Support Force between August 2010 and October 2017. A 60-month follow-up survey was performed. The BC tissues were reviewed blind by two pathologists based on the American Society of Clinical Oncology guidelines (23). Fresh surgical samples were frozen in liquid nitrogen and stored at -80°C. No interventional or other treatments were performed on the patients prior to surgery. The diagnoses of these samples were verified by pathologists in the hospital. Written informed consent was obtained from all of the patients, and the study protocol (no. 20171026) was approved by the Ethics Committee of 900th Hospital of the Joint Logistics Support Force.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. In accordance with the manufacturer's instructions, total RNA was isolated from tissues and cells using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). RT was performed using a Thermo Scientific RT kit (Thermo Fisher Scientific, Inc.). RT was performed by sequential incubations at 50 min at 42°C, 15 min at 70°C and 20 min at 37°C. RT-qPCR was performed using an ABI7500 qPCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.). In total, 5 µl SYBR Premix Ex Taq II (Takara Biotechnology Co., Ltd.), 0.4 µl forward primer (10 µM), 0.4 µl reverse primer (10 µM), 0.2 µl ROX Reference Dye (Takara Biotechnology Co., Ltd.), 1.0 µl cDNA template and 3.0 µl ddH₂O were mixed in the reaction solution. qPCR was performed as follows: Initial denaturation for 10 sec at 95°C, followed by 45 cycles of 5 sec at 95°C and 34 sec at 60°C. Relative ATXN8OS expression was calculated using the comparative quantification cycle (Cq) method. Fold changes were calculated using the $2^{-\Delta\Delta Cq}$ method (24). GAPDH and U6 served as endogenous controls for mRNA or lncRNA/miRNA expression, respectively. The primer sequences used in the present study were as follows: ATXN8OS primer forward, 5'-GCGCGAGAGCCCCGTGTTA-3' and reverse, 5'-TCTCTTGCCCTTCTGCCTTCTACT-3'; tyrosine protein kinase JAK2 primer forward, 5'-GGGAGGTGGTCGCTGTAAAA-3' and reverse, 5'-ACCAGCACTGTAGCAC ACTC-3'; forkhead box A1 (FOXA1) primer forward, 5'-AATCATTGCCATCGTGTG-3' and reverse, 5'-CGCGGCTTAAAATCTGGTAT-3'; miR-204 primer forward, 5'-CTGTCACATCGAGCTGCTGGAATG-3' and reverse, 5'-ACCGTGTCTGGAGTCGGCAATT-3'; GAPDH primer forward, 5'-GTCTCCTCTGACTTCAACAGCG-3 and reverse, 5'-ACCACCCTGTTGCTGTAGCCAA-3; U6 primer forward, 5'-CTCGCTTCGGCAGCACATA-3' and reverse, 5'-AACGATTCCGAATTTGCGT-3'.

Cell culture. MCF-10A, a normal breast epithelial cell line, and two human BC cell lines, MCF7 and MDA-MB-231, were

obtained from the Type Culture Collection of the Chinese Academy of Sciences. All cells were cultured in DMEM (Biological Industries) supplemented with 50 U/ml penicillin, 0.1 mg/ml streptomycin and 10% FBS (Biological Industries) at 37°C in a 5% CO₂ atmosphere in a humidified incubator.

RNA oligonucleotides and transfection. The ATXN8OS small interfering (si)RNA (si-ATXN8OS; 5'-TAAATGTTTTCTTCCCTCTC-3') and scrambled siRNA (si-ctrl; 5'-CGAGAATTTGGTCTAAAGAGAA-3') were purchased from Shanghai GenePharma Co., Ltd. miR-204 mimic (5'-AAGGGAAACAGTAGGAAGCGGA-3'), miR-204 inhibitor (5'-TCCGCTTCCCTACTGTTTCCCTT-3'), control miRNA mimic (miR-con; 5'-AAGTGACGGTGAGATCCAGGCT-3') and miR-con inhibitor (5'-AGCCTGGATCTCACCGTCACTT-3') were purchased from Shanghai GenePharma Co., Ltd. Briefly, 1x10⁶ cells were seeded into a 6-well plate and transfected until they were 90% confluent. Transfection of the cells with si-ATXN8OS or si-ctrl (10 nM) and the miR-204 or miR-con mimic/inhibitor (10 nM) was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) with Opti-MEM serum-free medium (Gibco; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. The efficiency of transfection was determined in each experiment using RT-qPCR 24 h post-transfection. All functional experiments were carried out 48 h post-transfection.

Cell viability assay. The Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Inc.) was used to evaluate MCF7 and MDA-MB-231 proliferation. Cells were collected 48 h after transfection and seeded at a density of 1,000 cells/well in 96-well plates. At 0, 2 or 4 days, the CCK-8 reagent was added to the 96-well plate and the cells were cultured for a further 2 h at 37°C, according to the manufacturer's protocol. The absorbance was measured at a wavelength of 450 nm using a Benchmark Microplate Reader (Bio-Rad Laboratories, Inc.). Each assay was performed in triplicate and repeated three times.

Flow cytometry. Flow cytometry was used to analyse the cell cycle. MCF7 and MDA-MB-231 cells transfected with si-ATXN8OS and si-ctrl were removed from the culture plates using trypsin and washed three times with cold PBS until they were 90% confluent. The cells were fixed with cold 70% ethanol at 4°C overnight. The cells were then incubated with 20 µg/ml propidium iodide (Sigma-Aldrich; Merck KGaA) for 30 min at room temperature using the BD Cycletest Plus DNA Reagent kit (BD Biosciences) to stain the cells. Cell cycle distribution profiles were generated using a FACSCalibur Flow Cytometry system (BD Biosciences) with FlowJo software version 7.6.1 (Tree Star, Inc.).

Transwell invasion assay. A Transwell invasion assay was carried out to test the invasion ability of cells. The upper Transwell chamber containing DMEM without FBS was precoated with 100 µl of Matrigel and 4x10³ cells in 0.1 ml cell suspension were then added to the coated membrane in the chamber. The lower chamber was filled with 600 µl of 20% FBS DMEM. After 24 h, the cells that did not pass through

the membrane were cleared using cotton swabs and 4% paraformaldehyde was added to fix the cells at room temperature for 20 min, followed by staining the cells with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) for 15 min at room temperature. Images of five random fields were captured using an optical microscope (magnification, x400).

Western blot analysis. Western blot analysis was performed as described previously (25). The primary antibodies were incubated at 4°C overnight and then incubated with the secondary goat antibodies horseradish peroxidase-conjugated (1:2,000; cat. no. ab6112; Abcam) for 2 h at room temperature. The following primary antibodies were used: JAK2 (1:1,000; cat. no. 3230; Cell Signaling Technology, Inc.), FOXA1 (1:1,000; cat. no. 53528; Cell Signaling Technology, Inc.), angiopoietin 1 (ANGPT1; 1:500; cat. no. ab8451; Abcam), TGF- β receptor type 2 (TGF β R2; 1:500; cat. no. ab186838; Abcam) and GAPDH (1:1,000; cat. no. ab181602; Abcam).

Reporter vector construction and luciferase reporter assay. StarBase V3.0 (<http://starbase.sysu.edu.cn/index.php>) was used to search for potential miRNAs that can bind to ATXN8OS. Through this analysis, ATXN8OS fragments containing the proposed binding site of miR-204 were identified. The ATXN8OS fragments were then amplified and integrated into the pGL3-promoter vector (Promega Corporation), between the *NheI* and *BglII* sites, to construct the reporter vector ATXN8OS-wild-type (Wt ATXN8OS). In parallel with Wt ATXN8OS, the related mutant fragments were also cloned to construct the reporter vector ATXN8OS-mutant (Mut ATXN8OS). MCF7 and MDA-MB-231 cells were co-transfected with 100 ng Wt ATXN8OS or Mut ATXN8OS and 10 nM miR-204 or miR-con mimic/inhibitor using Lipofectamine® 2000. Luciferase activity was measured 48 h after transfection using a dual-luciferase reporter assay system (Promega Corporation) according to the manufacturer's protocol. Firefly luciferase activity was normalized to *Renilla* (Promega Corporation) luciferase gene activity.

Statistical analysis. All statistical data were analysed using SPSS 19.0 software (IBM Corp.) and GraphPad Prism 5.0 software (GraphPad Software, Inc.). Clinicopathological characteristics were evaluated using the χ^2 test. The overall survival rate was assessed using the Kaplan-Meier method. The log-rank test was used to compare the survival data. Student's t-test or the Wilcoxon signed-rank test was employed to compare parameters between two groups, and one-way ANOVA and the Dunnett's post hoc test was used to evaluate the differences among three or more groups. Pearson analysis was used to assess the correlation between the expression of the related miR-204 and ATXN8OS sequences. Data are presented as the mean \pm SD. $P < 0.05$ was considered to indicate a statistically significant difference. The experiments were performed >5 times.

Results

Clinical significance of ATXN8OS expression in BC. The expression of ATXN8OS in BC tissues and matched non-tumour tissues was analysed using RT-qPCR. The

expression of ATXN8OS was found to be significantly higher in the human MDA-MB-231 and MCF-7 BC cell lines than in the MCF-10A normal breast cell line (Fig. 1A). The expression of ATXN8OS was also significantly higher in BC tissues than in the matched normal breast tissues (Fig. 1B). Moreover, Kaplan-Meier analysis showed that the overall survival rate of patients with BC was lower in the group with high ATXN8OS expression and higher in the group with low ATXN8OS expression ($P = 0.0439$; Fig. 1C). The correlations between the expression of ATXN8OS and the clinicopathological characteristics were assessed with the χ^2 test. High ATXN8OS was correlated with the size of the carcinoma ($P = 0.0017$), tumour-node-metastasis (TNM) stage ($P = 0.0038$) and lymphatic metastasis ($P = 0.0004$). However, no significant differences were found between the expression of ATXN8OS and sex, age, oestrogen receptor status, progesterone receptor status or human EGF receptor 2 (Her2)/neu status (Table I).

Down-regulation of ATXN8OS inhibits BC cell proliferation and invasion. To investigate the function of ATXN8OS in BC cells, MDA-MB-231 and MCF7 BC cells were transfected with si-ATXN8OS. RT-qPCR showed an efficient knockdown of ATXN8OS in the cell lines (Fig. 2A). The CCK-8 assay was used to measure the effect of ATXN8OS on the proliferation of human BC cells. Cell viability was reduced significantly in ATXN8OS-knockdown BC cells compared with the control knockdown cells (Fig. 2B and C). Flow cytometry analysis showed that transfection with si-ATXN8OS induced G₀/G₁ arrest and decreased the S phase population compared with the control knockdown (Fig. 2D and E). A Transwell assay was performed to determine whether ATXN8OS regulated BC cell invasion. Knockdown of ATXN8OS in cells reduced the number of invasive cells compared with the control knockdown (Fig. 2F and G).

ATXN8OS directly inhibits miR-204 expression by targeting its 3' untranslated region (UTR). To explore the molecular mechanism of ATXN8OS in BC, StarBase V3.0 was used to predict the potential targets of ATXN8OS. The analysis revealed that ATXN8OS contains complementary binding sites to the 3'UTR of miR-204. RT-qPCR showed that the expression level of miR-204 was negatively correlated with the expression of ATXN8OS ($P = 0.0017$; Fig. 3A). To identify the relationship between ATXN8OS and miR-204, the expression levels of miR-204 and its downstream genes in si-ATXN8OS BC cells were determined. The results demonstrated that the expression of miR-204 was significantly upregulated after knockdown of ATXN8OS in human MDA-MB-231 and MCF-7 BC cells (Fig. 3B). The expression of downstream targets of miR-204, including JAK2, FOXA1, ANGPT1 and TGF β R2, was significantly decreased after the inhibition of ATXN8OS in both of the human BC cell lines tested (Fig. 3C-E). To determine whether ATXN8OS suppresses miR-204 by directly binding miR-204 in BC cells, Wt ATXN8OS and Mut ATXN8OS sequences containing the target sites of miR-204 were cloned into a luciferase reporter system (Fig. 3F). The luciferase activity of Wt ATXN8OS was reduced in the BC cells containing the miR-204 mimic; however, the luciferase activity of Wt ATXN8OS was increased in the BC cells containing the miR-204 inhibitor (Fig. 3G).

Table I. Correlation between ATXN8OS expression in breast cancer and clinical characteristics.

Characteristic	No.	Relative ATXN8OS expression		P-value
		Low	High	
Sex				
Male	10	6	4	0.7445
Female	110	56	54	
Age, years				
>50	74	41	33	0.2602
≤50	46	20	26	
Size, cm				
>2	65	20	45	0.0017
≤2	55	33	22	
Tumour-node-metastasis stage				
I-II	58	38	20	0.0038
III-IV	62	24	38	
Lymphatic metastasis				
Negative	72	47	25	0.0004
Positive	48	15	33	
Oestrogen receptor status				
Negative	36	12	24	0.3151
Positive	84	37	47	
Progesterone receptor status				
Negative	42	26	16	0.3367
Positive	78	40	38	
Human EGF receptor 2/neu status				
Negative	84	44	40	0.6915
Positive	36	17	19	

ATXN8OS, ataxin 8 opposite strand.

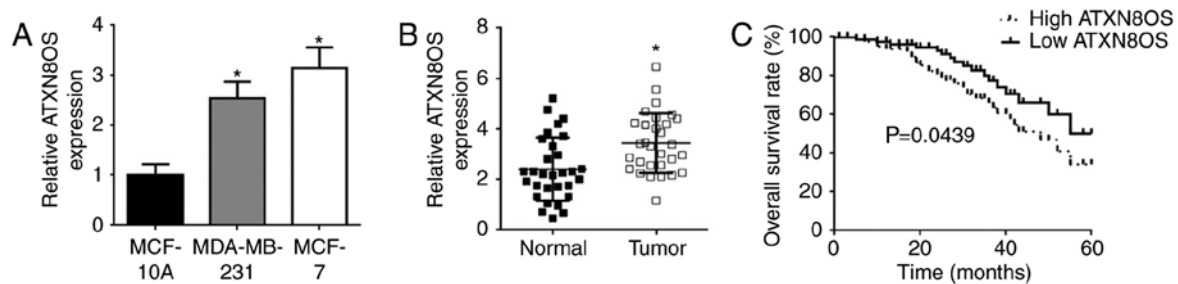


Figure 1. Prognostic value and expression of ATXN8OS in BC specimens. (A) The expression of ATXN8OS was examined in BC cell lines (n=5) and (B) specimens (n=120) by reverse transcription-quantitative PCR. (C) The correlation between ATXN8OS expression and overall survival rates determined by Kaplan-Meier analyses. *P<0.05 vs. respective control. ATXN8OS, ataxin 8 opposite strand; BC, breast cancer.

Inhibition of miR-204 reverses ATXN8OS-induced effects on BC. To confirm whether ATXN8OS exerts its biological function by targeting miR-204, BC cells were co-transfected with si-ATXN8O and the miR-204 inhibitor. RT-qPCR showed a reduced expression of miR-204 in BC cells after transfection with the miR-204 inhibitor (Fig. 4A). Proliferation and cell cycle distribution were analysed using the CCK-8 assay and flow cytometry, respectively. The results showed

that cell growth was increased in the si-ATXN8OS cells co-transfected with the miR-204 inhibitor (Fig. 4B). The inhibition of miR-204 resulted in fewer BC cells in the G₀/G₁ phase and an increased percentage of cells in the S phase (Fig. 4C). Invasion was increased in cells co-transfected with si-ATXN8OS and the miR-204 inhibitor compared with cells co-transfected with si-ATXN8OS and the miR-con inhibitor (Fig. 4D).

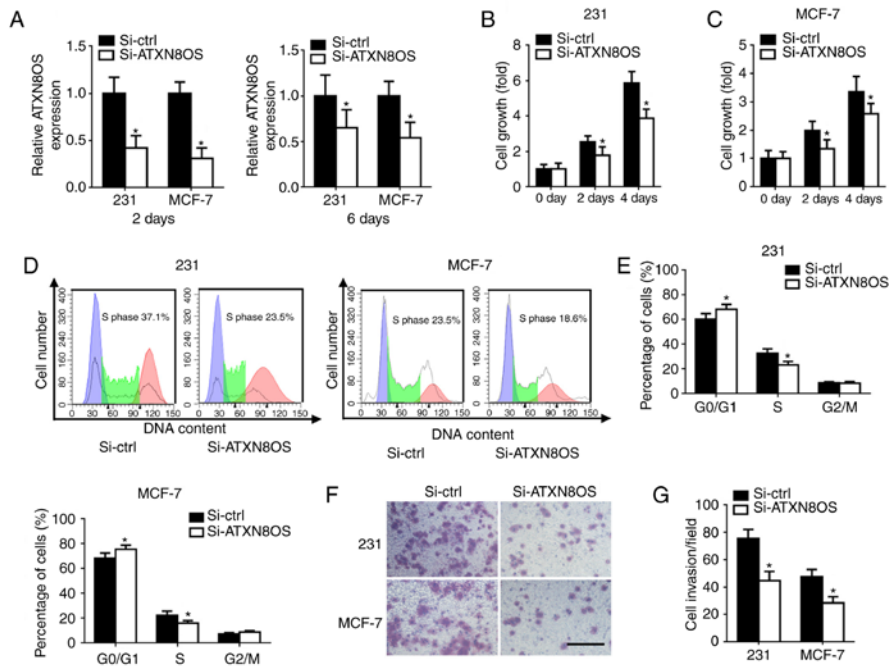


Figure 2. Function of ATXN8OS in BC proliferation and invasion. MCF-7 and 231 human BC cell lines were transfected with si-ATXN8OS or si-ctrl for 2 and 6 days. (A) Expression of ATXN8OS in 231 and MCF-7 cells determined by reverse transcription-quantitative PCR analysis. The Cell Counting Kit-8 assay was used to analyse cell viability at the indicated time points in (B) 231 and (C) MCF-7 cells. (D) Flow cytometry analysis was performed to examine the cell cycle distribution of MDA-MB-231 and MCF-7 cells following transfection with si-ATXN8OS. Blue, G₀/G₁ phase; green, S phase; red, G₂/M phase. (E) Quantification of cell cycle distribution. (F) The number of invasive cells was assessed using a Transwell assay. Scale bar, 50 μ m. (G) Quantification of invasive cells. *P<0.05 vs. respective si-ctrl. n=5. ATXN8OS, ataxin 8 opposite strand; BC, breast cancer; si-ATXN8OS, small interfering RNA targeting ATXN8OS; si-ctrl, control small interfering RNA; 231, MDA-MB-231.

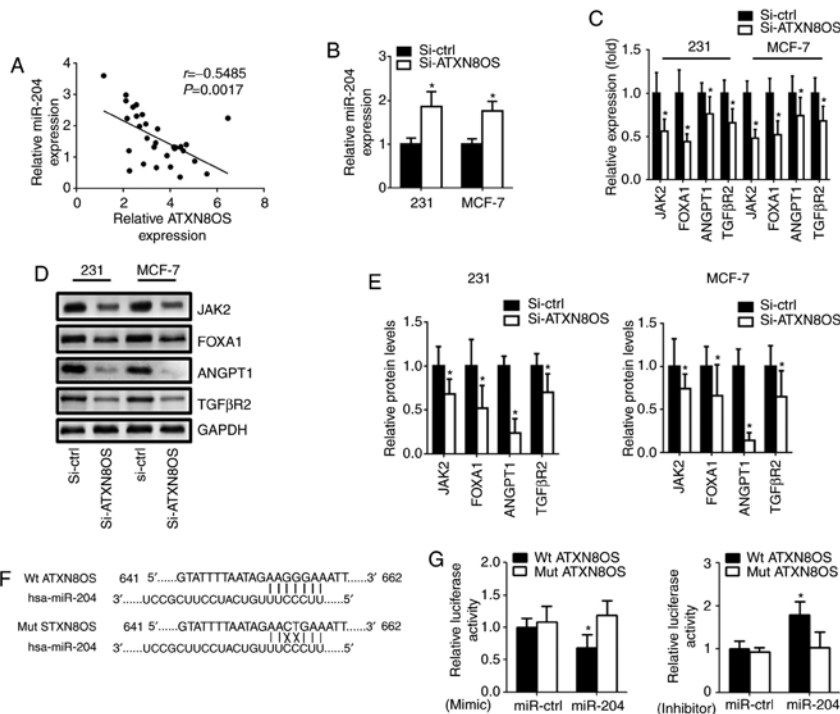


Figure 3. ATXN8OS targets miR-204. (A) An inverse relationship was found between ATXN8OS and miR-204 in BC tissues. (B) Up-regulation of miR-204 was found in BC cells subjected to knockdown of ATXN8OS. (C) Reverse transcription-quantitative PCR showed reduced expression of JAK2, FOXA1, ANGPT1 and TGF β R2 in 231 and MCF-7 cells 48 h post-transfection. (D) The protein levels of JAK2, FOXA1, ANGPT1 and TGF β R2 were determined by western blot analysis. GAPDH was used as an internal control. (E) Relative protein expression was calculated based on the densitometric analysis of band intensities. (F) Putative ATXN8OS target sequences in miR-204 are displayed. (G) Dual-luciferase reporter system analysis was conducted in 231 cells co-transfected with Wt- or Mut-ATXN8OS and miR-204 or miR-con mimics/inhibitors. *P<0.05 vs. respective control. n=5. ATXN8OS, ataxin 8 opposite strand; BC, breast cancer; miR-204, microRNA-204; miR-con, microRNA control; si-ATXN8OS, small interfering RNA targeting ATXN8OS; si-ctrl, control small interfering RNA; 231, MDA-MB-231; JAK2, tyrosine protein kinase JAK2; FOXA1, forkhead box A1; ANGPT1, angiopoietin-1; TGF β R2, TGF- β receptor type 2; Wt, wild type; Mut, mutant.

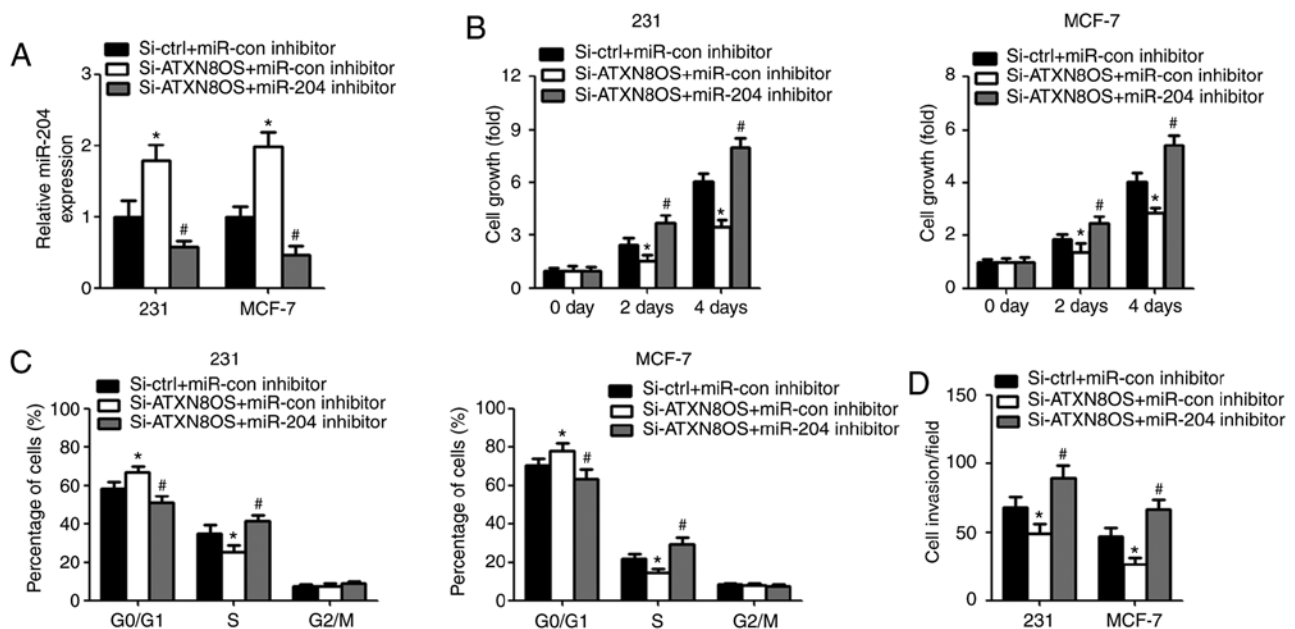


Figure 4. Inhibition of miR-204 reverses the effect of ATXN8OS knockdown. MCF-7 and 231 cells were co-transfected with si-ATXN8OS or si-ctrl and the miR-204 inhibitor or miR-con inhibitor for 48 h. (A) The inhibition of miR-204 was analysed by reverse transcription-quantitative PCR. (B) Cell growth was determined using the Cell Counting Kit-8 assay. (C) The cell cycle distribution was examined by flow cytometry analysis. (D) The invasive ability of cells was determined using a Transwell assay. * $P < 0.05$ vs. si-ctrl+miR-con inhibitor; # $P < 0.05$ vs. si-ATXN8OS+miR-con inhibitor. $n = 5$. ATXN8OS, ataxin 8 opposite strand; miR-204, microRNA-204; miR-con, microRNA control; si-ATXN8OS, small interfering RNA targeting ATXN8OS; si-ctrl, control small interfering RNA; 231, MDA-MB-231.

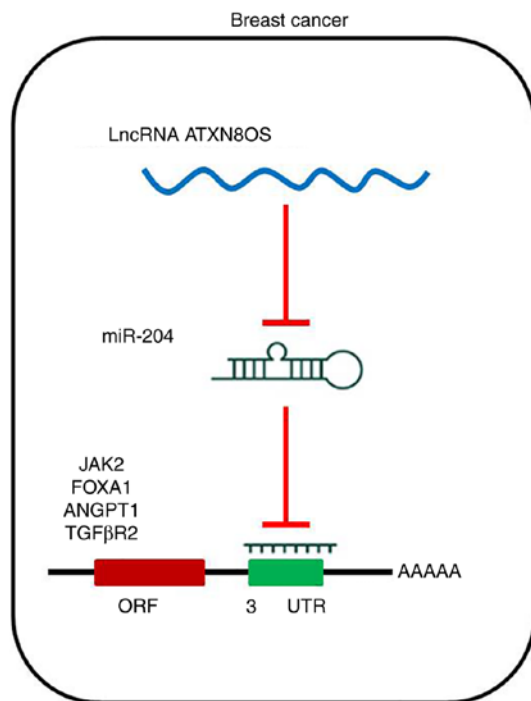


Figure 5. A schematic diagram illustrating the involvement of ATXN8OS and miR-204 in breast cancer. lncRNA, long non-coding RNA; ATXN8OS, ataxin 8 opposite strand; miR-204, microRNA-204; JAK2, tyrosine protein kinase JAK2; FOXA1, forkhead box A1; ANGPT1, angiopoietin-1; TGFβR2, TGF-β receptor type 2; ORF, open reading frame; UTR, untranslated region.

Discussion

The identification of novel molecular targets for BC is becoming increasingly important, with the aim of improving

the diagnosis, therapeutic strategies and clinical follow-up of BC (26-28). Accumulating evidence supports the role of lncRNAs functioning as ceRNAs in the occurrence and progression of a number of human cancer types (29,30). Despite rapid improvements in the detection, diagnosis, treatment and prediction of the prognosis of BC, the recurrence and mortality rates of BC remain some of the biggest challenges for patients with BC. Therefore, it is important to determine the exact molecular mechanism of action involved in the initiation and progression of BC, and to explore possible prognostic markers. Increasing experimental evidence indicates that lncRNAs can exert important functions in many biological processes and are closely associated with the development and prognosis of cancer (31). Consequently, elucidation of the relationship between lncRNAs and their downstream targets would shed light on the diagnosis and treatment of patients with BC.

The results of the present study found that ATXN8OS was up-regulated in human BC tissues compared with normal human tissues; an increased expression of ATXN8OS was also found in the BC cell lines tested. These data indicated that ATXN8OS might promote the occurrence of carcinomas. In addition, the expression of ATXN8OS was found to be related to tumour size, TNM stage and lymphatic metastasis, suggesting that ATXN8OS may be involved in the development of BC. Conversely, it was found that the knockdown of ATXN8OS suppressed proliferation, increased the percentage of cells in the G₀/G₁ phase of the cell cycle and decreased cell invasion in BC cell lines. Therefore, ATXN8OS may be a novel factor involved in the progression of BC, by arresting the cell cycle in the G₀/G₁ phase. In addition, the overall survival rate was low in patients with ATXN8OS overexpression according

to the 60-month follow-up survival survey, indicating a strong correlation between ATXN8OS overexpression and poor prognostic outcomes of BC. This result suggested that ATXN8OS may be an important prognostic marker in forecasting the prognosis of BC. There are three sub-types of BC cell lines, two of which were used in the present study. The luminal-like MCF7 cell line and the basal-like MDA-MB-231 cell line were used; in addition, the normal breast epithelium cell line MCF10A was used. The other sub-type of BC cell lines, the Her-2 elevated type, including SKBR3, will be investigated in future studies.

miR-204 was identified as a predicted target gene of ATXN8OS, and their expression was found to be inversely correlated in BC tissues and cell lines. miR-204 has been identified as an anti-oncogene and is reported to be down-regulated in diverse human malignancies, including intrahepatic cholangiocarcinoma, glioma, non-small cell lung cancer, endometrial cancer, gastric cancer, head and neck squamous cell carcinoma, and thyroid cancer (32-35). Previous studies revealed that JAK2 and FOXA1 are direct targets of miR-204 through binding sites in their 3'UTRs in BC (2,36). Another previous study showed that miR-204 functions by down-regulating the expression of ANGPT1 and TGF β 2 by targeting binding sites in their 3'UTRs in BC (37). Based on these previous studies, it is proposed that the expression of JAK2, FOXA1, ANGPT1 and TGF β 2 may be inhibited in si-ATXN8OS cells if miR-204 is a direct downstream target of ATXN8OS. The reduced expression of transcriptional control genes downstream of miR-204 observed in the present study suggested that miR-204 is a direct downstream target of ATXN8OS in BC cell lines (Fig. 5). More experiments need to be performed to understand the binding interaction between miR-204 and the lncRNA ATXN8OS. The inhibition of miR-204 expression reversed the enhancing effects of ATXN8OS on proliferation, cell cycle and invasion of BC cells *in vitro*.

In conclusion, the results of the present study showed that ATXN8OS was up-regulated in the tissues of patients with BC and in BC cell lines, and that its aberrant overexpression was significantly correlated with poor prognostic outcomes and lower overall survival rates. The results of the present study revealed the potential molecular mechanism by which ATXN8OS exerts its stimulating functions on proliferation and invasion in BC cells, by sequestering miR-204. The findings of the present study indicated that ATXN8OS may be an oncogenic factor that promotes the development and progression of BC, and might be a potential biomarker for the clinical diagnosis and treatment of patients with BC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Fujian Province (grant no. 2016J05196), Military Medical Scientific Youth Cultivation Project (grant no. 15QNP205) and Fujian Provincial Science and Technology Major Project (grant no. 2012YZ0001-1).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JT contributed to the conception and design of the study. ZD, LL, HC, WW and LZ performed the experiments. SY and JC analysed the data and JT contributed to manuscript drafting.

Ethics approval and consent to participate

Written informed consent was obtained from all patients, and the study protocol (no. 20171026) was approved by the Ethics Committee of 900th Hospital of the Joint Logistics Support Force.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Tian T, Wang M, Lin S, Guo Y, Dai Z, Liu K, Yang P, Dai C, Zhu Y, Zheng Y, *et al*: The impact of lncRNA dysregulation on clinicopathology and survival of breast cancer: A systematic review and meta-analysis. *Mol Ther Nucleic Acids* 12: 359-369, 2018.
2. Wang X, Qiu W, Zhang G, Xu S, Gao Q and Yang Z: MicroRNA-204 targets JAK2 in breast cancer and induces cell apoptosis through the STAT3/BCI-2/survivin pathway. *Int J Clin Exp Pathol* 8: 5017-5025, 2015.
3. DeSantis C, Ma J, Bryan L and Jemal A: Breast cancer statistics, 2013. *CA Cancer J Clin* 64: 52-62, 2014.
4. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. *CA Cancer J Clin* 63: 11-30, 2013.
5. Feng W, Wang C, Liang C, Yang H, Chen D, Yu X, Zhao W, Geng D, Li S, Chen Z and Sun M: The dysregulated expression of KCNQ10T1 and its interaction with downstream factors miR-145/CCNE2 in breast cancer cells. *Cell Physiol Biochem* 49: 432-446, 2018.
6. Bertero T, Cottrill KA, Lu Y, Haeger CM, Dieffenbach P, Annis S, Hale A, Bhat B, Kaimal V, Zhang YY, *et al*: Matrix remodeling promotes pulmonary hypertension through feedback mechanoactivation of the YAP/TAZ-miR-130/301 circuit. *Cell Rep* 13: 1016-1032, 2015.
7. Cai H, Xu J, Han Y, Lu Z, Han T, Ding Y and Ma L: Integrated miRNA-risk gene-pathway pair network analysis provides prognostic biomarkers for gastric cancer. *Onco Targets Ther* 9: 2975-2986, 2016.
8. Batista PJ and Chang HY: Long noncoding RNAs: Cellular address codes in development and disease. *Cell* 152: 1298-1307, 2013.
9. Hsieh IS, Chang KC, Tsai YT, Ke JY, Lu PJ, Lee KH, Yeh SD, Hong TM and Chen YL: MicroRNA-320 suppresses the stem cell-like characteristics of prostate cancer cells by downregulating the Wnt/beta-catenin signaling pathway. *Carcinogenesis* 34: 530-538, 2013.
10. Zhang T, Zou P, Wang T, Xiang J, Cheng J, Chen D and Zhou J: Down-regulation of miR-320 associated with cancer progression and cell apoptosis via targeting Mcl-1 in cervical cancer. *Tumour Biol* 37: 8931-8940, 2016.
11. Gooding AJ, Zhang B, Jahanbani FK, Gilmore HL, Chang JC, Valadkhan S and Schiemann WP: The lncRNA BORG Drives Breast Cancer Metastasis and Disease Recurrence. *Sci Rep* 7: 12698, 2017.

12. Ransohoff JD, Wei Y and Khavari PA: The functions and unique features of long intergenic non-coding RNA. *Nat Rev Mol Cell Biol* 19: 143-157, 2018.
13. Beermann J, Piccoli MT, Viereck J and Thum T: Non-coding RNAs in development and disease: Background, mechanisms, and therapeutic approaches. *Physiol Rev* 96: 1297-1325, 2016.
14. Fang Y, Wang J, Wu F, Song Y, Zhao S and Zhang Q: Long non-coding RNA HOXA-AS2 promotes proliferation and invasion of breast cancer by acting as a miR-520c-3p sponge. *Oncotarget* 8: 46090-46103, 2017.
15. Wang Y, Zhou J, Wang Z, Wang P and Li S: Upregulation of SOX2 activated LncRNA PVT1 expression promotes breast cancer cell growth and invasion. *Biochem Biophys Res Commun* 493: 429-436, 2017.
16. Cho JY: Molecular diagnosis for personalized target therapy in gastric cancer. *J Gastric Cancer* 13: 129-135, 2013.
17. Esteller M: Non-coding RNAs in human disease. *Nat Rev Genet* 12: 861-874, 2011.
18. Wang S, Lan F and Xia Y: lncRA ANCR inhibits non-small cell lung cancer Cell migration and invasion by inactivating TGF- β pathway. *Med Sci Monit* 24: 6002-6009, 2018.
19. Dong H, Wang W, Mo S, Chen R, Zou K, Han J, Zhang F and Hu J: SP1-induced lncRNA AGAP2-AS1 expression promotes chemoresistance of breast cancer by epigenetic regulation of MyD88. *J Exp Clin Cancer Res* 37: 202, 2018.
20. Chen IC, Lin HY, Hsiao YC, Chen CM, Wu YR, Shiao HC, Shen YF, Huang KS, Su MT, Hsieh-Li HM and Lee-Chen GJ: Internal ribosome entry segment activity of ATXN8 opposite strand RNA. *PLoS One* 8: e73885, 2013.
21. Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW and Ranum LP: An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat Genet* 21: 379-384, 1999.
22. Fan CN, Ma L and Liu N: Systematic analysis of lncRNA-miRNA-mRNA competing endogenous RNA network identifies four-lncRNA signature as a prognostic biomarker for breast cancer. *J Transl Med* 16: 264, 2018.
23. Schnipper LE, Davidson NE, Wollins DS, Tyne C, Blayney DW, Blum D, Dicker AP, Ganz PA, Hoverman JR, Langdon R, *et al*: American Society of clinical oncology statement: A conceptual framework to assess the value of cancer treatment options. *J Clin Oncol* 33: 2563-2577, 2015.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
25. Wang D, Zhu C, Zhang Y, Zheng Y, Ma F, Su L and Shao G: MicroRNA-30e-3p inhibits cell invasion and migration in clear cell renal cell carcinoma by targeting Snail1. *Oncol Lett* 13: 2053-2058, 2017.
26. Campos-Parra AD, López-Urrutia E, Orozco Moreno LT, López-Camarillo C, Meza-Menchaca T, Figueroa González G, Bustamante Montes LP and Pérez-Plasencia C: Long non-coding RNAs as new master regulators of resistance to systemic treatments in breast cancer. *Int J Mol Sci* 19: E2711, 2018.
27. Huang XJ, Xia Y, He GF, Zheng LL, Cai YP, Yin Y and Wu Q: MALAT1 promotes angiogenesis of breast cancer. *Oncol Rep* 40: 2683-2689, 2018.
28. Wang G, Chen X, Liang Y, Wang W, Fang Y and Shen K: Long noncoding RNA signature and disease outcome in estrogen receptor-positive breast cancer patients treated with tamoxifen. *J Breast Cancer* 21: 277-287, 2018.
29. Wang KC and Chang HY: Molecular mechanisms of long noncoding RNAs. *Mol Cell* 43: 904-914, 2011.
30. Zhang J, Yuan L, Zhang X, Hamblin MH, Zhu T, Meng F, Li Y, Chen YE and Yin KJ: Altered long non-coding RNA transcriptomic profiles in brain microvascular endothelium after cerebral ischemia. *Exp Neurol* 277: 162-170, 2016.
31. Troy A and Sharpless NE: Genetic 'Inc'-age of noncoding RNAs to human disease. *J Clin Invest* 122: 3837-3840, 2012.
32. Courboulin A, Paulin R, Giguère NJ, Saksouk N, Perreault T, Meloche J, Paquet ER, Biardel S, Provencher S, Côté J, *et al*: Role for miR-204 in human pulmonary arterial hypertension. *J Exp Med* 208: 535-548, 2011.
33. Sacconi A, Biagioni F, Canu V, Mori F, Di Benedetto A, Lorenzon L, Ercolani C, Di Agostino S, Cambria AM, Germoni S, *et al*: miR-204 targets Bcl-2 expression and enhances responsiveness of gastric cancer. *Cell Death Dis* 3: e423, 2012.
34. Ying Z, Li Y, Wu J, Zhu X, Yang Y, Tian H, Li W, Hu B, Cheng SY and Li M: Loss of miR-204 expression enhances glioma migration and stem cell-like phenotype. *Cancer Res* 73: 990-999, 2013.
35. Chung TK, Lau TS, Cheung TH, Yim SF, Lo KW, Siu NS, Chan LK, Yu MY, Kwong J, Doran G, *et al*: Dysregulation of microRNA-204 mediates migration and invasion of endometrial cancer by regulating FOXCl. *Int J Cancer* 130: 1036-1045, 2012.
36. Shen SQ, Huang LS, Xiao XL, Zhu XF, Xiong DD, Cao XM, Wei KL, Chen G and Feng ZB: miR-204 regulates the biological behavior of breast cancer MCF-7 cells by directly targeting FOXA1. *Oncol Rep* 38: 368-376, 2017.
37. Flores-Pérez A, Marchat LA, Rodríguez-Cuevas S, Bautista-Piña V, Hidalgo-Miranda A, Ocampo EA, Martínez MS, Palma-Flores C, Fonseca-Sánchez MA, Astudillo-de la Vega H, *et al*: Dual targeting of ANGPT1 and TGFBR2 genes by miR-204 controls angiogenesis in breast cancer. *Sci Rep* 6: 34504, 2016.



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