Upregulation and hypomethylation of lncRNA AFAP1-AS1 predicts a poor prognosis and promotes the migration and invasion of cervical cancer

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Abstract. Although lncRNA AFAP1 antisense RNA1 (AFAP1-AS1) is considered an oncogenic lncRNA, little is known about the role of AFAP1-AS1 in cervical cancer. In the present study, we found that AFAP1-AS1 was elevated and hypomethylated in cervical cancer and was associated with a poor prognosis of patients with cervical cancer by analyzing the Cancer RNA-Seq Nexus (CRN), MethCH and UCSC XENA databases. Subsequently, we knocked AFAP1-AS1 expression down using siRNAs in cervical cancer cells. Wound healing experiments and matrigel invasion experiments revealed that the downregulation of AFAP1-AS1 suppressed the migration and invasion of cervical cancer cells. Furthermore, western blot analysis demonstrated that the antitumor effects induced by the silencing of AFAP1-AS1 were mainly mediated through the regulation of the Rho/Rac signaling pathway and epithelial-mesenchymal transition (EMT)-related genes. Taken together, the findings of the present study indicate that the expression level of AFAP1-AS1 may be involved in the development of cervical cancer. Thus, AFAP1-AS1 may be a novel prognostic biomarker and a potential therapeutic target for patients with cervical cancer.

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Abbreviations: AFAP1-AS1, AFAP1 antisense RNA1; ANOVA, analysis of variance; ATCC, American Type Culture Collection; CRN, Cancer RNA-Seq Nexus; EMT, epithelial-mesenchymal transition; GEO, Gene Expression Omnibus; lncRNAs, long non-coding RNAs; OS, overall survival; PI, propidium iodide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SE, standard error; siRNAs, short interfering RNAs; SRA, Sequence Read Archive; TCGA, The Cancer Genome Atlas

Key words: long non-coding RNAs, AFAP1 antisense RNA1, cervical cancer, prognosis, migration and invasion

Introduction

Cervical cancer, one of the most common malignant tumors affecting the female population, brings a serious problem to affect public women's health (1). The morbidity and mortality associated with cervical cancer has significantly decreased over the past three decades. However, cervical cancer remains the third most commonly diagnosed type of cancer among women worldwide and some patients with this disease have a poor prognosis (2-4). Therefore, novel and effective therapeutic strategies for cervical cancer are urgently required. Accumulating evidence has indicated that the development of cervical cancer is a multi-step process, including the abnormal expression of oncogenes and tumor suppressor genes (5-7). Despite advances in cervical cancer, the precise molecular mechanisms of carcinogenesis and progression underlying cervical cancer are only partly understood (5). Therefore, the identification of the specific molecular biomarkers and

detailed underlying mechanisms, which may contribute to the development of novel diagnostic and treatment strategies for patients with cervical cancer is critical.

Long non-coding RNAs (lncRNAs) are a distinct subclass of RNA transcripts that more than 200 nucleotides in length (8). An increasing number of individual lncRNAs have been determined to exert a multitude of effects within cells, such as modulating gene expression at the epigenetic, transcriptional, post-transcriptional and translational levels under both physiological and pathological conditions (9-13). The aberrant expression of lncRNAs has been linked to tumor initiation and progression (14-18).

The findings from our previous studies (19-24), as well as other studies (25-27) have reported that lncRNA AFAP1-AS1 is upregulated in several tumors and functions as an oncogene. However, the expression level, epigenetic state and function of AFAP1-AS1 in cervical cancer remain unclear. Thus, in the present study, we firstly analyzed the related databases and found that AFAP1-AS1 expression was increased and that it was hypomethylated in cervical cancer. Furthermore, to evaluate the potential biological functions of AFAP1-AS1, we performed loss-of-function experiments and functional analyses. The findings of the present study may provide novel insight concerning the specific role of AFAP1-AS1 in the development of cervical cancer.

Materials and methods

Gene expression data and DNA methylation. Cancer RNA-Seq Nexus (CRN) is a database of phenotype-specific transcriptome profiling in cancer cells. Using this database, we systematically obtained RNA-seq datasets concerning cervical cancer tissues from The Cancer Genome Atlas (TCGA), Sequence Read Archive (SRA) and NCBI Gene Expression Omnibus (GEO) (28). The expression data of AFAP1-AS1 in cervical cancer was downloaded from this database. The DNA methylation data of cervical cancer was downloaded from the MethHC database (http://methhc.mbc.nctu.edu.tw/php/index.php), data which came from TCGA (29). The data used to draw the survival curve were all from the UCSC XENA database (http://xena.ucsc.edu/).

Cell lines and siRNAs. The HeLa cell line (ATCC no. CCL-2) is the most widely usedcervical cancer cell line. Due to its strong ability to proliferate, invade and migrate, we selected it for further research. The HeLa cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc., Waltham, MA, USA), penicillin and streptomycin (both from Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in a humidified incubator with 5% CO_2 at 37˚C. For gene knockdown, the cells were seeded and cultured overnight. Subsequently, 20 nM AFAP1-AS1 siRNA or scramble control (NC) siRNA (Guangzhou RiboBio Co., Ltd., Guangzhou, China) were transfected into the cervical cancer cells using Lipofectamine 3000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The two siRNAs used in this manuscript had no off-target effect and exhibited the optimal knockdown efficiency in previous studies by us, as well as other groups (19,30). The cycling conditions of PCR were as follows: First, 10 min at 95˚C. Denaturation at 95˚C for 15 sec, annealing at 60˚C for 30 sec, and elongation at 70˚C for 10 sec. After 40 cycles, the PCR tubes were incubated 5 min at 70˚C. The sequences were as follows: AFAP1-AS1 siRNA1, 5'-GGG CTTCAATTTACAAGCATT-3' and AFAP1-AS1 siRNA2, 5'-CCTATCTGGTCAACACGTATT-3'. The abovementioned nucleotide sequences were synthesized by Guangzhou RiboBio Co., Ltd.

Scratch test. The cervical cancer cells were seeded and cultured in 6-well culture plates following transfection for 24 h. When the cells grew to 90% confluence, a 10 μl tip was used to create a scratch. Images were captured (magnification, x20) at different time points (0, 24 and 48 h) using a microscope (The Cell Culture Laboratory Solution CKX53; Olympus, Tokyo, Japan). The ocular ruler was performed to measure the gap width in each group at the identified time point. All the wounds in the experimental group had the same width at the 0 h (31-33).

Transwell invasion assay. The invasive capacity was detected by Transwell invasion assays (34-36). The HeLa cells ($1x10^5$) were transfected with AFAP1-AS1 siRNA or NC siRNA and were plated on the top chamber of a 24-well Transwell insert (8-μm pore size; BD Biosciences, San Jose, CA, USA). Matrigel (20 μl) (2 mg/ml; BD Biosciences) was placed on the top well. The bottom well contained 800 μl completed RPMI-1640 medium (containing with 15% FBS). Following 48 h of incubation at 37˚C, the cells on the upper surface were removed using a cotton swab, while the cells that had invaded through the matrigel were fixed in 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet (Merck KGaA, Darmstadt, Germany) for 10 min at room temperature. Invasive tumor cells were observed and calculated under a light microscope (The Cell Culture Laboratory Solution CKX53; Olympus) and 5 randomly low power (x20) fields were selected for each test.

MTT cell viability assay. MTT assay was used to assess cell viability (37-40). The transfected cells were seeded into 96-well plates at a density of $5x10^3$ cells/well. Subsequently, 100 μl complete RPMI-1640 medium were added per well and cultured for 0, 1, 2, 3, 4 and 5 days. MTT reagent (20 μl of 1 mg/ml; Sigma-Aldrich; Merck KGaA) was then added to each well followed by incubation for 4 h. The absorbance was detected at 450 nm using a spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA).

RNA isolation and RT-qPCR. The HeLa cells were harvested after being transfected with the siRNAs for 36 h and total RNA was extracted using TRIzol reagent (Invitrogen/Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The RNA was reversed into cDNA using the 5X All-In-One RT Master Mix Reagent kit (Abm Canada Inc., Milton, ON, Canada) according to the kit protocol. cDNA was subjected to RT-qPCR using the SYBR Premix Ex Taq II kit (Takara Bio, Inc., Shiga, Japan). Reactions were carried out on the CFX96 Real-Time PCR Detection System (185-5196; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Fold changes of lncRNA were calculated using the $2^{-\Delta\Delta Cq}$ method, with β-actin

as an internal control (41). The sequences of the primers were as follows: AFAP1-AS1 forward, 5'-AATGGTGGTAGGAGG GAGGA-3' and reverse, 5'-CACACAGGGGAATGAAGA GG-3'; β-actin forward, 5'-TCACCAACTGGGACGACA TG-3' and reverse, 5'-GTCACCGGAGTCCATCACGAT-3'.

Cell cycle detection. Cell cycle distribution was detected by flow cytometry. When the cells were transfected with the siRNAs for 48 h, the cells were collected and fixed in 75% ethanol at -20˚C overnight. The fixed cells were then washed thrice with phosphate-buffered saline (PBS) before being incubated at room temperature with RNase A for 20 min. These cells were stained using a propidium iodide (PI) staining kit (BD Biosciences) and incubated in the dark for 30 min at 4˚C. A Beckman flow cytometer (Beckman Coulter, Inc.) was used for cell cycle analysis. Three independent experiments were conducted.

Western blot analysis. Total protein in the HeLa cells was extracted using RIPA extraction reagent (Beyotime Institute of Biotechnology, Shanghai, China) supplemented with a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Protein from the HeLa cells was quantified by the BCA method. To separate the protein, 30-50 μg protein per lane was loaded and electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the protein bands were transferred onto PVDF membranes. The membranes were blocked with 5% skim milk for 1 h at room temperature and then incubated with primary anti-Rho GDP-dissociation inhibitor 1 (RHOGDI, 1:1,000; cat. no. 14282-1-AP), anti-Rac Family Small GTPase 2 (RAC2, 1:1,000; cat. no. 10735-1-AP), anti-RAB1B (1:800; cat. no. 17824-1-AP), anti-RAB11A (1:800; cat. no. 20229-1-AP), anti-profilin 1 (PFN1, 1:1,000; cat. no. 11680-1-AP) and anti-LIM and SH3 protein 1 (LASP1, 1:1,000; cat. no. 10515-1-AP) antibodies (ProteinTech, Inc., Rosemont, IL, USA) or E-cadherin (1:1,000; cat. no. 3195), Zonula occludens-1 (ZO-1, 1:1,000; cat. no. 8193), Vimentin (1:1,000; cat. no. 5741), β-catenin antibodies (1:1,000; cat. no. 8480) (Cell Signaling Technology, Inc., Danvers, MA, USA) overnight at 4˚C. The following day, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG-HRP; cat. no. SC-2005; and goat anti-rabbit IgG-HRP, cat. no. SC-2005; 1:2,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 30 min at 37˚C. Immunoreactive bands were visualized using an ECL detection reagent (Amersham; GE Healthcare Life Sciences, Chalfont, UK). β-actin (1:1,000; cat. no. 4970; Cell Signaling Technology, Inc.) expression was used as a housekeeping gene control.

Statistical analysis. All the experimental data are represented as the means ± standard error (SE) and processed using SPSS statistical software, version 19.0 (SPSS, Inc., Chicago, IL, USA). Overall survival (OS) was analyzed using the Kaplan-Meier method and the results of the analysis were considered significant if log-rank test yielded a value of $P<0.05$. Significance between 2 groups was evaluated by a Student's t-test. Pearson's correlation analysis was used to assess the correlation between AFAP1-AS1 expression and its methylation. For the comparison of multiple groups, analysis of variance (ANOVA) with Dunnett's post hoc t-test was performed. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Upregulated AFAP1-AS1 expression is associated with a poor prognosis of patients with cervical cancer. Firstly, the expression level of AFAP1-AS1 in cervical cancer samples was analyzed using TCGA data from the CRN database. The data suggested that AFAP1-AS1 was more abundant in the cervical cancer tissues compared to the normal tissues ($P<0.001$; Fig. 1A). Moreover, a high AFAP1-AS1 expression was positively associated with the TNM stage ($P<0.05$; Fig. 1B). To validate whether the expression levels of AFAP1-AS1 were associated with the OS of patients with cervical cancer, we analyzed the patient outcome data from the UCSC XENA database. This analysis revealed that compared to patients with a low expression of AFAP1-AS1, patients with a high AFAP1-AS1 expression had a shorter survival time ($P<0.05$; Fig. 1C).

Hypomethylation of AFAP1-AS1 is associated with a poor prognosis of patients with cervical cancer. DNA methylation is an important part of epigenetic inheritance and is involved in the transcriptional expression of lncRNAs (42,43). In the present study, to investigate whether the expression of AFAP1-AS1 is influenced by DNA methylation, we downloaded the methylation data of AFAP1-AS1 in cervical cancer from the human pan-cancer method database, MethCH. We found that the average methylation level of the AFAP1-AS1 promoter region was lower in the cervical cancer samples when compared with the non-tumor cervical cancer samples ($P<0.01$; Fig. 2A). In addition, the results of Pearson's correlation analysis revealed that the expression level of AFAP1-AS1 negatively correlated with its methylation level ($r=-0.33$, $P<0.001$; Fig. 2B). The survival analysis was constructed using the UCSC XENA database, and we identified that the hypomethylation of AFAP1-AS1 was associated with a poor overall survival of patients with cervical cancer ($P=0.05$; Fig. 2C). These data suggested that the high expression of AFAP1-AS1 may be related to the hypomethylation of its promoter region. In addition, the hypomethylation of the AFAP1-AS1 promoter region and the high expression levels of AFAP1-AS1 may act as independent prognostic indicators of patients with cervical cancer.

Tumor cell migration and invasion are suppressed following the knockdown of AFAP1-AS1. To elucidate the role of AFAP1-AS1 in cervical cancer, the AFAP1-AS1 expression was downregulated by transfection of the cervical cancer cell line, HeLa, with two validated siRNAs targeting AFAP1-AS1 (siRNA1 and siRNA2). We examined the knockdown efficiency of these two siRNAs by RT-qPCR which yielded satisfactory results. AFAP1-AS1 expression was knocked down by at least 65% in the HeLa cells (both siRNAs, $P<0.01$; Fig. 3A). The functional experiments were performed to examine the phenotypic alterations induced by the silencing of AFAP1-AS1 in the cervical cancer cells. The

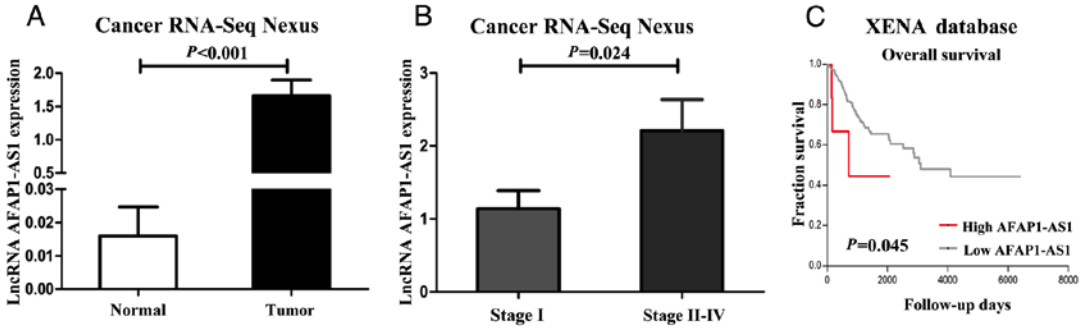

Figure 1. A high AFAP1-AS1 expression is associated with a poor prognosis of patients with cervical cancer. (A) AFAP1-AS1 expression as analyzed by TCGA cervical cancer cohort data from the Cancer RNA-Seq Nexus database, was upregulated in cervical cancer tissues (Tumor) compared to non-tumor tissues (Normal) (P<0.001). (B) AFAP1-AS1 expression was higher in stage II-IV lesions than in stage I lesions (P=0.024). (C) The cervical cancer patients with a higher AFAP1-AS1 expression have a shorter survival time. The overall survival data were collected from the TCGA datasets and analyzed using a Kaplan-Meier curve (P=0.045). AFAP1-AS1, AFAP1 antisense RNA1.

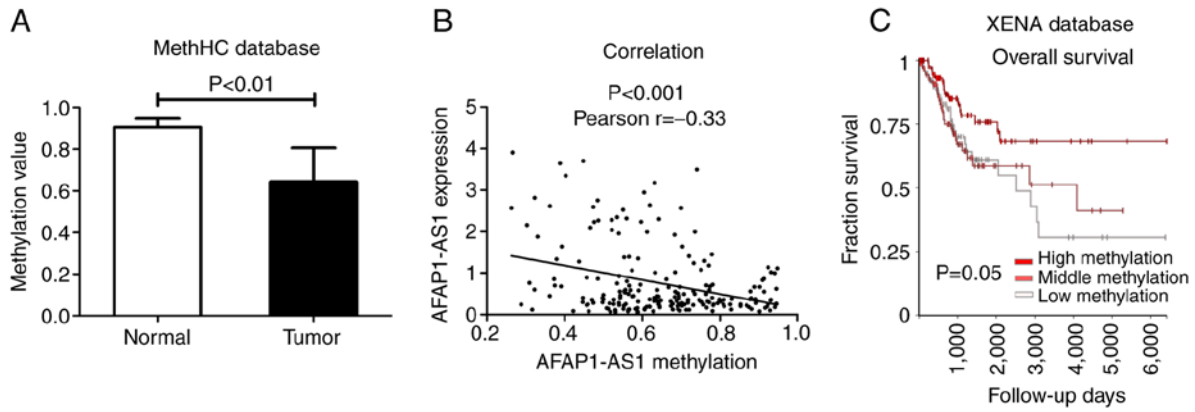

Figure 2. Hypomethylated AFAP1-AS1 is associated with a poor prognosis of patients with cervical cancer. (A) Comparisons of AFAP1-AS1 methylation level between cancer tissues (Tumor) and non-cancerous tissues (Normal) of cervical cancer involved in the TCGA based on the MethHC database (P<0.01). (B) A negative correlation between AFAP1-AS1 methylation level and AFAP1-AS1 expression in cervical cancer (r=-0.33, P<0.001). (C) Kaplan-Meier curve between groups with AFAP1-AS1 high, middle and low methylation (P=0.05). This graph was conducted by XENA. AFAP1-AS1, AFAP1 antisense RNA1.

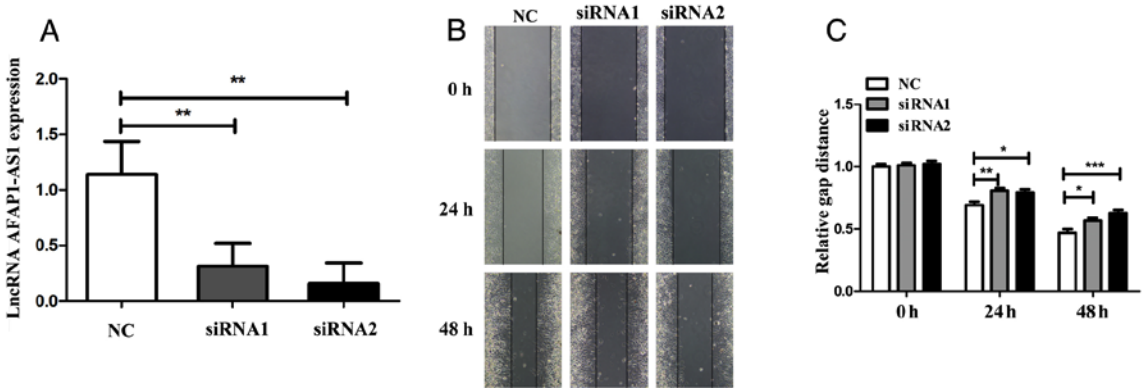

Figure 3. Cell migration is suppressed after AFAP1-AS1 knockdown. (A) Both siRNAs were effective in knocking down AFAP1-AS1 expression. siRNA1 and siRNA2 markedly suppressed AFAP1-AS1 expression compared with the scrambled control siRNA (NC) in the HeLa cells. (B) The migratory capacity of HeLa cells was suppressed after AFAP1-AS1 knockdown. The migration distance of the tumor cells were measured at 0, 24 and 48 h. (C) The migration rate was calculated at identified time points. The AFAP1-AS1 siRNA treated cells displayed wider gap. *P<0.05, **P<0.01 and ***P<0.001 vs. NC. AFAP1-AS1, AFAP1 antisense RNA1.

scratch test results revealed that the migratory capacity of the AFAP1-AS1-silenced cells was suppressed at 24 and 48 h following transfection with siRNA compared to the si-control group (24 h, siRNA1 vs. control, P<0.01; siRNA2 vs. control, P<0.05; 48 h, siRNA1 vs. control, P<0.05; siRNA2 vs. control, P<0.001; Fig. 3B and C). Moreover, as shown in Fig. 4, the number of invaded cells was also decreased in the AFAP1-AS1 siRNA-transfected cancer cells compared with

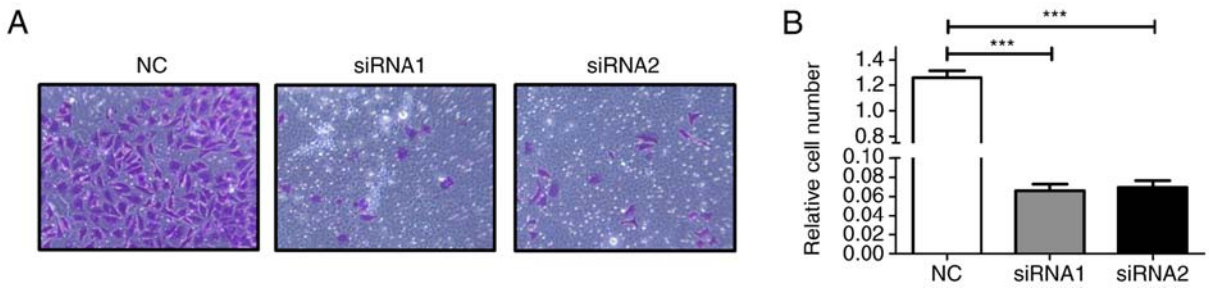

Figure 4. AFAP1-AS1 knockdown inhibits cell invasion. (A) HeLa cells were treated with AFAP1-AS1 siRNA or control siRNA, respectively. The cells were then added to the top of the matrigel invasion chambers. The results revealed that the number of invasion tumor cells was reduced after AFAP1-AS1 knockdown. (B) The representative graph of each group was presented. ***P<0.001. AFAP1-AS1, AFAP1 antisense RNA1.

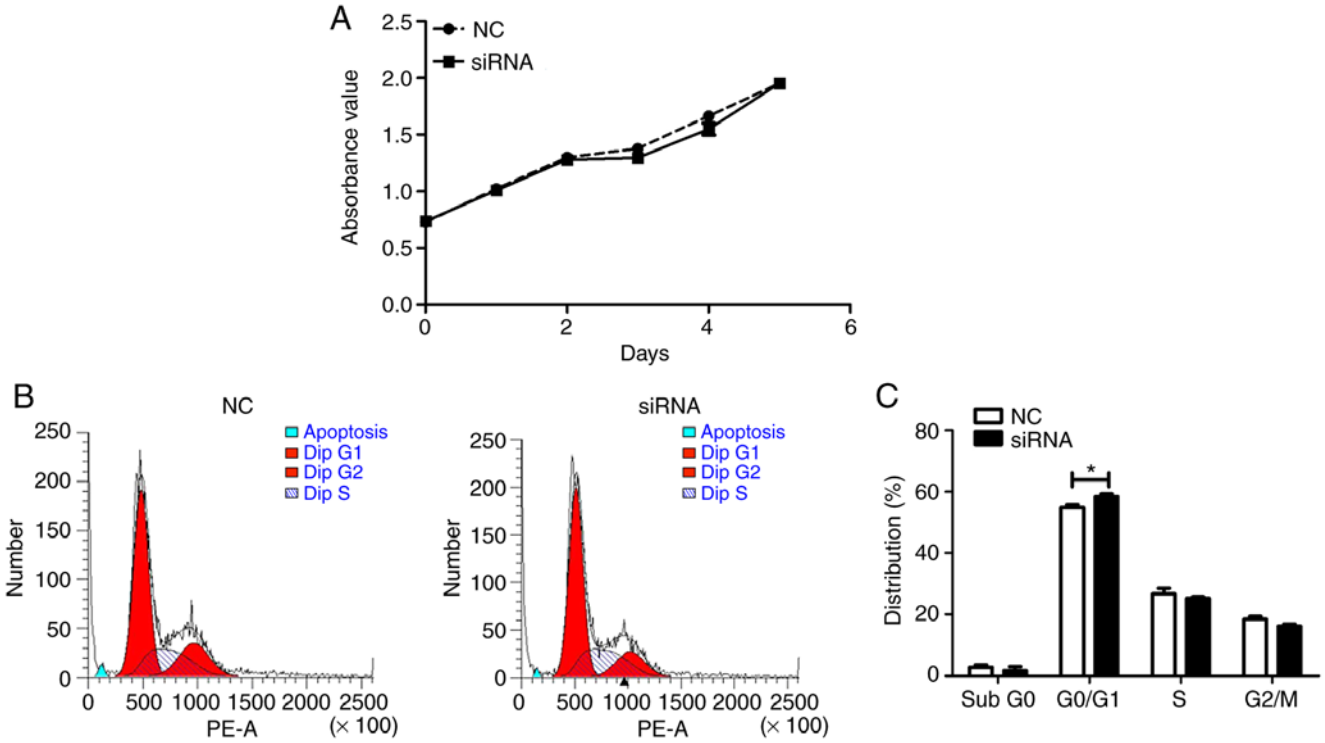

Figure 5. AFAP1-AS1 knockdown does affect cell viability and apoptosis, but slightly affects cell cycle distribution (A). The cell viability of each group at different time points was detected by MTT assay. Flow cytometric analysis was performed to (B) the analyze cell cycle and (C) apoptosis. However, no obvious difference was observed in cell apoptosis between AFAP1-AS1 knockdown group and NC group. In contrast, AFAP1-AS1 knockdown slightly, yet significantly influenced the cell cycle. *P<0.05. AFAP1-AS1, AFAP1 antisense RNA1.

the si-control group (both siRNAs, P<0.001; Fig. 4). However, we noted that there was no significant association between the expression of AFAP1-AS1 and cell viability and cell apoptosis. By contrast, AFAP1-AS1 knockdown slightly, yet significantly increased the number of cells in the G0/G1 phase in the present study (P<0.05; Fig. 5).

AFAP1-AS1 is involved in the regulation of the Rho/Rac signaling pathways. To investigate the potential mechanisms of AFAP1-AS1 regarding its promoting effect on the migration and invasion of cervical cancer cells, we examined the expression levels of several key molecules of the Rho/Rac signaling pathways by western blot analysis. The results suggested that the knockdown of AFAP1-AS1 increased the expression of RHOGDI, PFN1, RAB11A and RAC2, while it decreased the expression of RAB1B and LASP1 (Fig. 6).

Silencing of AFAP1-AS1 affects the expression of EMT-related genes. In order to further investigate the mechanisms underlying the role of AFAP1-AS1 in cervical cancer development, we also detected changes in the protein levels of some EMT-related genes. The results revealed that following the knockdown of AFAP1-AS1, the expression of ZO-1 increased, while the expression of Vimentin and β-catenin decreased. However, E-cadherin expression was not markedly altered (Fig. 7).

Discussion

In recent years, an increasing number of studies have reported that non-coding RNAs, including lncRNAs, miRNAs and circular RNAs (circRNAs) act as tumor suppressors or promoters in tumorigenesis and pathogenesis (44-46).

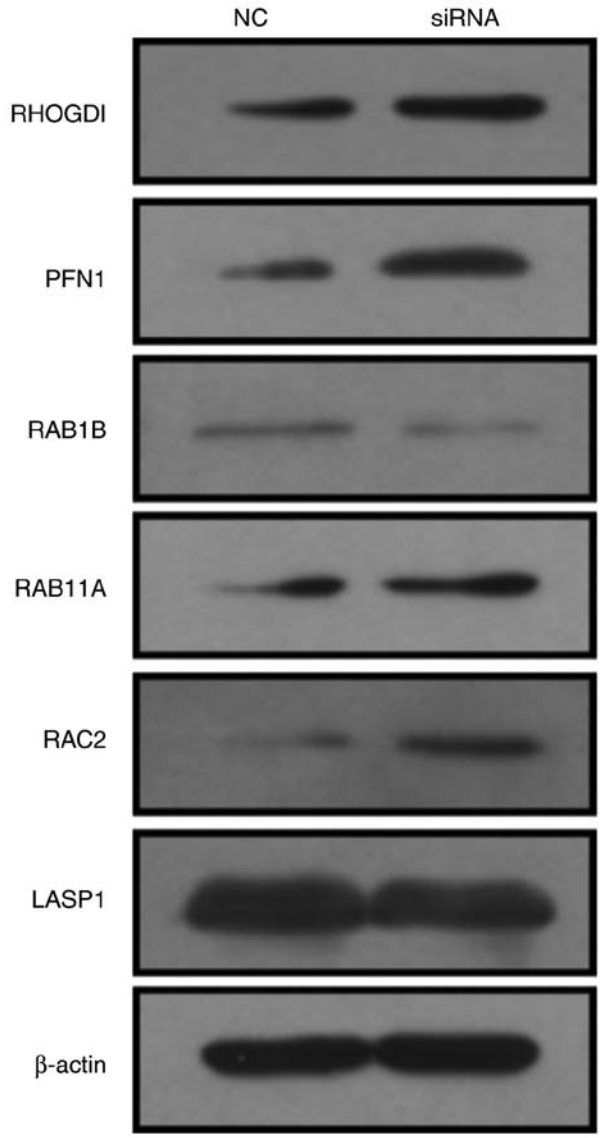

Figure 6. The expression levels of Rho/Rac GTPase family members were altered after AFAP1-AS1 knockdown. Cells were transfected with AFAP1-AS1 siRNA or control siRNA, respectively. The protein levels of Rho/Rac GTPase family members were detected by western blot analysis. The protein levels of RHOGDI, PFN1, RAB11A and RAC2 were elevated, while RAB1B and LASP1 protein levels were decreased. AFAP1-AS1, AFAP1 antisense RNA1.

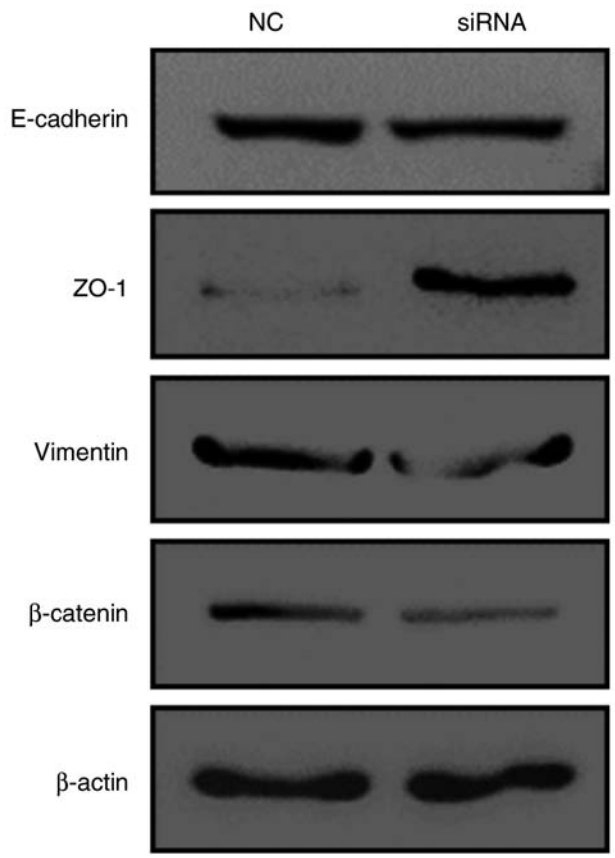

Figure 7. The expression of EMT-related genes were decreased after AFAP1-AS1 knockdown. The expression levels of vimentin and β-catenin were downregulated, while those of ZO-1 were upregulated in si-AFAP1-AS1-transfected HeLa cells compared to the si-control group. E-cadherin expression was not markedly altered. EMT, epithelial-mesenchymal transition; AFAP1-AS1, AFAP1 antisense RNA1; ZO-1, Zonula occludens-1.

Accumulating evidence indicates that the abnormal expression of lncRNAs is associated with tumorigenesis and progression (43,47-52). The TCGA database includes genome, transcriptome, epigenetic, proteomic and clinical phenotypic data of 34 tumors, which is one of the most authoritative cancer databases worldwide (53-55). Based on the TCGA database, researchers developed the MethCH, XENA and CRN databases. These provide numerous transcriptome, epigenetic and clinical phenotypic data for data mining about human malignant tumors. The mining of these data is a frequently used and convenient method for the exploration of gene or lncRNA expression and function (43).

In the present study, we combined the MethCH, XENA and CRN databases to investigate whether the transcription level and methylation level of lncRNA AFAP1-AS1 differ in cervical cancer. We found that AFAP1-AS1 was upregulated and hypomethylated in cervical cancer tissues compared to normal tissues, which is consistent with the findings of a previous study showing that the increased expression of AFAP1-AS1 was due to its promoter demethylation in esophageal carcinoma (30). Moreover, AFAP1-AS1 upregulation and hypomethylation were associated with a shorter survival time of patients with cervical cancer. In addition, some studies have revealed that lncRNA AFAP1-AS1 is enriched in several tumors and functions as an oncogene (19,20,30,56,57). Thus, we hypothesized that AFAP1-AS1 may also function as an oncogene in cervical cancer. Subsequent functional experiments confirmed that cell migration and invasion were significantly suppressed following the knockdown of AFAP1-AS1 in cervical cancer cells, while no effect on cell proliferation or cell apoptosis *in vitro* were observed. However, AFAP1-AS1 knockdown slightly affected cell cycle distribution.

The Rho/Rac GTPase family is an important member of the Ras superfamily. It regulates cytoskeletal remodeling by affecting the polymerization and stability of microtubules and microfilaments. The Rho/Rac GTPase family has been reported to regulate cell adhesion and motility (58-60). Several studies have found that Rho/Rac GTPase family members are involved in the migration and invasion of various tumors, including colorectal cancer and ovarian cancer (61,62). Moreover, two recent studies indicated that lncRNA AFAP1-AS1 was involved

in the regulation of Rho/Rac GTPase family members (19,56). In the present study, we determined that the RHOGDI, PFN1, RAB11A and RAC2 protein levels were elevated, while the RAB1B and LASP1 protein levels were suppressed after the silencing of AFAP1-AS1 in HeLa cells. These results were consistent with those of previous studies on lung cancer and nasopharyngeal carcinoma (19,20). However, the change in RAC2 protein levels was contrary to the findings of a previous study on liver cancer (56). This suggests that AFAP1-AS1 may modulate the Rho/Rac pathway through different mechanisms in different types of tumors.

EMT is a vital malignant phenotype in tumor cell migration and invasion which promotes tumor development (48,63). A number of studies have indicated that lncRNAs play important roles in the EMT process in tumors (64-69). In the present study, to identify whether AFAP1-AS1 regulates the EMT process in cervical cancer as well, the protein levels of some EMT-related genes were measured by western blot analysis. The data suggested that AFAP1-AS1 knockdown downregulated the EMT-related genes vimentin and β-catenin, while it upregulated ZO-1 in cervical cancer cells. Our data indicated that AFAP1-AS1 may function as a tumor promoter by affecting the EMT process in HeLa cells. β-catenin is an important member of the Wnt/β-catenin signaling pathway. We demonstrated that AFAP1-AS1 knockdown decreased the protein levels of β-catenin. Nevertheless, it is not clear whether the Wnt/β-catenin signaling pathway suggests a downstream of AFAP1-AS1 in cervical cancer. This question warrants further investigation. In addition, only one cell line was used in the present study to explore the function and mechanism of AFAP1-AS1 in cervical cancer. The lack of multiple cell lines in the present study was a limitation, and that the results should be confirmed in further experimental models.

Taken together, the findings of the present study revealed that AFAP1-AS1 was significantly hypomethylated and upregulated in cervical cancer. And hypomethylation and upregulation of AFAP1-AS1 are both associated with a poor outcome of patients with cervical cancer. We further confirmed that AFAP1-AS1 knockdown suppressed the migration and invasion of HeLa cells. The inhibition of AFAP1-AS1 negatively regulated the Rho/Rac pathway and EMT-related gene protein products. The identified lncRNA AFAP1-AS1 may be a novel target for cervical cancer therapy.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article or are available from the corresponding author on reasonable request.

Authors' contributions

HB and ZG mainly performed the experiments, analyzed the data and wrote the paper. ZL, LS, CG, XL, QL, WZ, KC and MZ helped with the experiments and analyzed the data. BX, XL, QL and LF helped with the data acquisition. BX, XL, WX, LF, ZZ, SZ and FX helped with the paper writing. GL, WX, ZZ, SZ and FX carried out the experiment design and manuscript drafting. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All patient data analyzed in the present study were downloaded from online databases. Thus, no ethics approval was required.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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