European Review for Medical and Pharmacological Sciences 2019; 23: 3261-3269

Long non-coding RNA cancer susceptibility candidate 2 inhibits the cell proliferation, invasion and angiogenesis of cervical cancer through the MAPK pathway

X.-W. WANG, W. ZHANG

Department of Gynecology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, China

Abstract. – OBJECTIVE: Increasing evidence has indicated that long non-coding RNA cancer susceptibility candidate 2 (CASC2) is aberrantly expressed and acts as a key regulator in various types of cancer. However, few reports have mentioned the precise function of CASC2 in cervical cancer.

PATIENTS AND METHODS: In the present study, CASC2 levels in cervical cancer tissues and cell lines were measured by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The proliferation, migration, and invasion of cervical cancer cells were detected by cell counting kit-8 (CCK-8) and transwell assays. Angiogenesis was evaluated by tube formation assays of human umbilical vein endothelial cells (HUVECs). The protein levels of the components of the mitogen-activated protein kinase (MAPK) pathway, including the c-Jun N-terminal kinase (JNK) and the extracellular-signal-regulated kinase-1 (ERK1), in cell lines of cervical cancer were detected by Western blot.

RESULTS: CASC2 levels in human cervical cancer tissues and cell lines were significantly downregulated compared to para-cancerous tissues and the immortalized human keratinocyte line HaCaT. Compared to scramble group, CASC2 overexpression significantly inhibited the proliferation, migration, invasion, and angiogenesis of cervical cancer cells. However, the inhibition of CASC2 had the opposite effect. Moreover, the expression of p-JNK and p-ERK1 levels in the MAPK pathway was significantly decreased in pcDNA3.1-CASC2 group.

CONCLUSIONS: Overexpression of CASC2 may inhibit the development of cervical cancer through the inactivation of the MAPK pathway and may be a potential therapeutic target for the treatment of patients with cervical cancer.

Key Words:
Long non-coding RNA, CASC2, Cervical cancer, MAPK pathway.

Introduction

Cervical cancer is the third most common cancer and the fourth leading cause of cancer death among women worldwide[1]. In recent years, with the spread of cervical cancer screening and cervical cancer vaccines, the incidence and mortality of cervical cancer have greatly decreased in many countries. Despite these changes, cervical cancer remains a major public health problem, especially in developing countries[2]. The migration and invasion of cancer cells are closely related to the mortality of cervical cancer patients. Thus, it is particularly urgent to predict the specific tumour markers of the biological behaviour of cervical cancer.

Long non-coding RNAs are non-coding RNAs longer than 200 nucleotides with minimal protein coding ability[3]. In recent years, lncRNA has been reported to be involved in the development of cancer. LncRNA SPRY4-IT1 can promote the proliferation of human breast cancer cells by upregulating the ZNF703 expression[4]. LncRNA-ATB is involved in the progression and prognosis of colorectal cancer[5]. LINC00472 inhibits proliferation and promotes apoptosis of lung adenocarcinoma cells, which might provide a novel insight into a potential therapeutic approach for lung adenocarcinoma[6]. The lncRNA cancer susceptibility candidate 2 (CASC2) is located on chromosome 10q26 and was originally identified as a downregulated gene in endometrial cancer and a tumour suppressor gene with three different splice variant transcripts: CASC2a, CASC2b, and CASC2c[7]. Reports show that exogenously expressed CASC2a in undifferentiated endometrial cancer cells significantly inhibits tumour growth[8]. LncRNA CASC2 significantly inhibits human glioma malignancies, which may be a novel therapeutic target for the treatment of glioma[9]. Moreover, long

Corresponding Author: Wei Zhang, MD; e-mail: zw6676@163.com

noncoding RNA CASC2 inhibits metastasis and epithelial cells to the mesenchymal transition of lung adenocarcinoma[10]. However, the exact mechanism of lncRNA CASC2 in cervical cancer remains obscure. Therefore, we explored the effect of lncRNA CASC2 on the development of cervical cancer and its mechanism, providing new ideas for the diagnosis and treatment of cervical cancer.

Patients and Methods

Reagents and Instruments

The cervical cancer cell lines HeLa and SiHa, immortalized human keratinocyte line HaCaT and HUVECs were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), pcDNA3.1 vector, and Lipofectamine 3000 were purchased from Invitrogen (Carlsbad, CA, USA). The Midiprep kit was purchased from Qiagen (Hilden, Germany), and the cell counting kit-8 (CCK-8) was purchased from Beyotime Biotechnology (Shanghai, China). Anti-p-ERK1, p-JNK, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and the enhanced chemiluminescence (ECL) reagent kit was purchased from Millipore (Billerica, MA, USA). The PCR kit was purchased from Toyobo (Kita-ku, Osaka, Japan). The Western blot instrument was purchased from Bio-Rad (Hercules, CA, USA), and the PCR instrument was purchased from Roche (Basel, Switzerland).

Cervical Samples

The fresh cervical cancer and adjacent tissues (3 cm beyond cancer) were obtained from 50 patients with cervical cancer who underwent radical hysterectomy between 2015 and 2017 in the Department of Gynaecology. A diagnosis of cervical cancer was confirmed independently by 3 experienced pathologists. All tissue samples were washed with sterile phosphate-buffered saline (PBS) and stored at −80°C for use. No radiation therapy or chemotherapy was given before the surgery. This study was approved by the Ethics Committee of our hospital, and informed consent was obtained from each patient participating in the investigation.

Cell Culture

The cervical cancer cell lines HeLa and SiHa and immortalized human keratinocyte line HaCaT were cultured in DMEM (Gibco; Thermo Fisher Scientific, Shanghai, China) containing 10% FBS (HyClone; GE Healthcare, Logan, UT, USA), 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37°C in a 5% of CO_2 incubator.

Plasmid Construction and Cell Transfection

CASC2 siRNA (si-CASC2) and negative control siRNA (si-NC) were purchased from GenePharma (Shanghai, China). CASC2 overexpression vector (pcDNA-CASC2) and the control Vector (pcDNA-N1) were purchased from Generay Biotechnology (Shanghai, China). Both the siRNA and plasmid were effectively transfected into cells as described previously[11]. Briefly, after 24 h culture in DMEM, cells (10,000 cells/well) were transfected with either siRNA or CASC2 overexpression vector using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. At 48 h post-transfection, the cells were harvested for proliferation assays, transwell assays or Western blot.

Cell Proliferation Assay

Cell Counting Kit-8 (CCK-8) assays were used to evaluate cell proliferation. Transfected HeLa or SiHa cells were seeded onto 96-well plates (5×10^3 cells/well) and incubated in DMEM containing 10% FBS at 37°C. The optical density was measured at 450 nm using a microtiter plate reader every other day (0, 24, 48, and 72 h). Results were taken as the average of three replicates in the same conditions.

Transwell Assays

For transwell assays, cervical cancer cells (5×10^4 cells/well) were cultured in a serum-free medium in the upper chamber with or without Matrigel-coated wells, and then 10% of serum was added to the lower chamber. Cells were incubated at 37°C in 5% of CO_2. After 48 h, all the cells in the upper chamber were removed using a cotton swab. Then, the migrated and invaded cells on the surface of the inferior chamber were fixed by methanol and stained with 0.1% of crystal violet (Sigma-Aldrich, St. Louis, MO) and counted using a cell counter.

Tube Formation Assays

A HUVEC capillary-like tube formation assay was performed to explore the effect of CASC2 on angiogenesis *in vitro,* as previously described[12]. First, 2×10^4 HUVECs/well treated with the condi-

tioned medium derived from the cervical cancer cells transfected with si-CASC2, pcDNA-CASC2 or negative control were seeded into the Matrigel-coated 96-well plates and incubated in DMEM containing 10% of FBS. After incubating for 36 h at 37°C in a 5% CO_2 atmosphere, the network of tubular structures was imaged under a microscope (Olympus, Melville, NY, USA) and the total tube length was calculated using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). Each experiment was replicated at least 3 times.

Quantitative Real Time-Polymerase Chain Reaction

As previously described, Total RNA was extracted from cervical cancer tissues or cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA)[13]. The PCR primers were designed as follows using Premier 6.0: CASC2, forward: 5'-GCACATTGGACGGTGTTTCC-3' and reverse: 5'-CCCAGTCCTTCACAGGTCAC-3'; GAPDH, forward: 5'-AGAAGGCTGGGGCTCATTTG-3'; and reverse: 5'-AGGGGCCATCCACAGTCTTC-3'. The cycling conditions for the amplification were designed as follows: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, annealing at 60°C for 10 s, and then, elongation at 72°C for 30 s for a total of 40 cycles. Relative gene expression was analysed using the $2^{-\Delta\Delta Ct}$ method and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was normalized as an internal control. Each experiment was replicated at least 3 times.

Western Blot Assays

Total proteins were extracted from cervical cancer cells using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) with a protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Equal amounts of protein were separated by 10% of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to the polyvinylidene difluoride (PVDF) membrane. Then, 5% of fat-free milk was blocked for 1 h. PVDF membrane and primary antibody p-JNK (1:500), p-ERK1 (1:500), and β-actin (1:1000) were incubated overnight at 4°C, and then incubated with HRP-conjugated secondary antibody (1:5000) for 2 h at room temperature. The proteins were visualized using Enhanced Chemiluminescence Luminol reagent (PerkinElmer Inc., Boston, MA, USA), and band intensities were quantified using the ImageJ software. β-actin was used as an internal control.

Statistical Analysis

Statistical analysis was performed using SPSS version 19.0 (Chicago, IL, USA). Data were expressed as the mean ± SD. Differences between two groups were analysed using the Student's *t*-tests, and multi-group comparisons of the means were carried out by the one-way analysis of variance (ANOVA) test with post hoc Student-Newman-Keuls tests. A p-value of <0.05 was considered statistically significant.

Results

CASC2 Level was Downregulated in Cervical Cancer Tissues and Cell Lines

The expression of CASC2 in the tissues of cervical cancer patients and the cervical cancer cell lines were detected by qRT-PCR. Compared to the para-carcinoma tissue, CASC2 expression was significantly downregulated in cervical cancer tissues ($p<0.01$, Figure 1A). In addition, the CASC2 levels in HeLa or SiHa cells were significantly reduced compared to the immortalized human keratinocyte line HaCaT ($p<0.01$, Figure 1B).

CASC2 Inhibits the Proliferation of HeLa or SiHa Cells in vitro

To investigate the role of CASC2 in the development of cervical cancer, we examined the effect of CASC2 on the proliferation of HeLa or SiHa cells. First, the overexpression or inhibition of CASC2 after transfection with pcDNA-CASC2 or si-CASC2 cervical cancer cells was measured using qRT-PCR (Figure 2A and 2C). The CCK8 assay showed that CASC2 overexpression significantly inhibited the proliferation of HeLa or SiHa cells compared to scramble group, while the inhibition of CASC2 resulted in a significant increase (Figure 2B and 2D). Therefore, our results indicate that CASC2 inhibits the proliferation of cervical cancer cells *in vitro*.

CASC2 Inhibits the Migration, Invasion and Tube Formation of Cervical Cancer Cells in vitro

To investigate the effect of CASC2 on the migration and invasion of cervical cancer cells, HeLa or SiHa cells transfected with pcDNA-CASC2 or si-CASC2 were used for transwell assays. As shown in Figure 3, the density of HeLa or SiHa

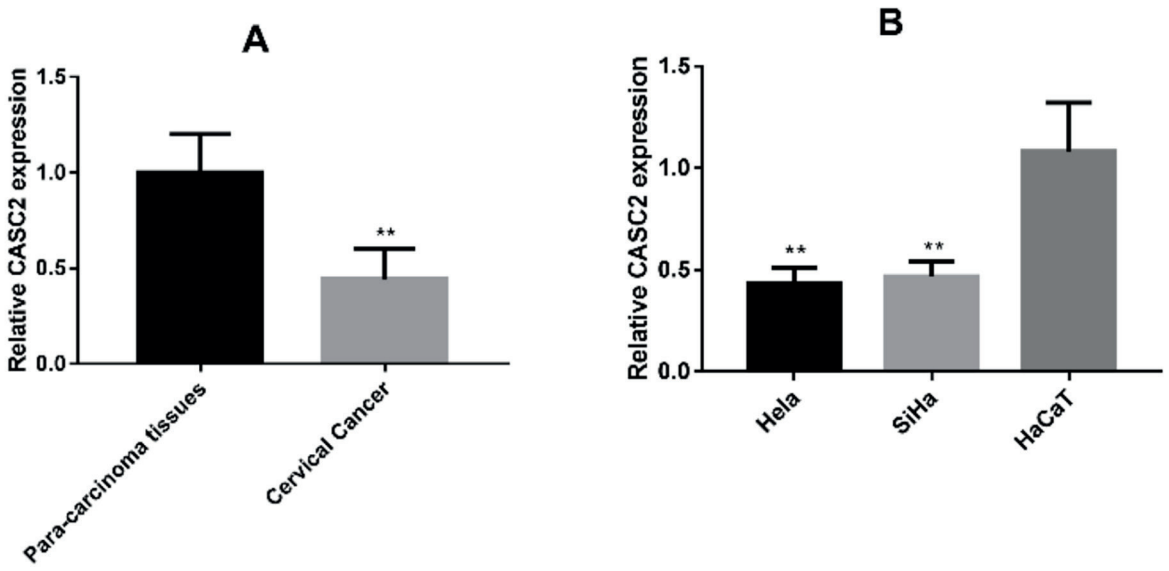

Figure 1. LncRNA CASC2 level is decreased in cervical cancer tissues and cell lines. Relative CASC2 level in cervical cancer tissues and para-carcinoma tissues were detected by qRT-PCR (**A**). Relative CASC2 levels in HeLa, SiHa and immortalized human keratinocyte HaCaT cells were detected by qRT-PCR (**B**). Data are expressed as the mean ± SD. GAPDH is used as an internal control. * p <0.05, ** p <0.01.

cells in the lower chamber was significantly lower than that of scramble group, suggesting that CASC2 overexpression significantly inhibited the migration and invasion of cervical cancer cells, while in si-CASC2 group, the migration and invasion abilities of HeLa or SiHa cells were significantly enhanced. Furthermore, the formation of capillary-like structures in HUVECs treated with conditioned medium derived from cervical cancer cells transfected with pcDNA-CASC2 was significantly decreased. However, cervical cancer cells transfected with si-CASC2 formed better organized capillary-like structures in HUVECs (Figure 4), suggesting that CASC2 acted as a tumour suppressor inhibiting cell migration, invasion, and angiogenesis *in vitro*.

CASC2 Mediates the Development of Cervical Cancer Through the MAPK Pathway

To investigate the regulatory mechanisms of CASC2 in cervical cancer, we examined whether CASC2 affects the MAPK signaling pathway, particularly the ERK and JNK proteins, which are normally activated to enhance cell proliferation and metastasis in human tumours. As shown in Figure 5, Western blot analysis showed that in HeLa cells transfected with pcDNA-CASC2, p-JNK, and p-ERK1 protein levels were significantly decreased compared to scramble group (p <0.05). In contrast, there was a significant increase in p-JNK and p-ERK1 expression in si-CASC2 group (p <0.05). Therefore, CASC2 mediates the development of cervical cancer through the MAPK pathway.

Discussion

A large number of studies show that lncRNA plays an important role in the development of human cervical cancer. Long non-coding RNA BLACAT1 promotes cell proliferation, migration, and invasion in cervical cancer through the activation of the Wnt/β-catenin signaling pathway[14]. LncRNA CCAT-1 promotes the proliferation of cervical cancer cells through the Wnt signaling pathway, indicating that lncRNA plays a key role in the proliferation of cervical cancer cells[15]. Reducing the level of lncRNA MALAT1 in Caski cells inhibited cell migration and reduced tumour growth in cervical cancer[16]. Furthermore, Kim et al[17] reported that lncRNA HOTAIR may represent a novel biomarker for predicting cervical cancer recurrence and prognosis and is expected to be a new target for cervical cancer treatment. Nevertheless, the role of lncRNA CASC2 in cervical cancer remains unclear.

CASC2 is a recently discovered lncRNA that has been shown to modulate the proliferation of

non-small cell lung cancer cells and is associated with prognosis in patients with non-small cell lung cancer. In the present study, lncRNA CASC2 is significantly downregulated in cervical cancer tissues and cell lines. In addition, a CCK8 assay showed that CASC2 overexpression significantly inhibited the proliferation of cervical cancer cells. Transwell assays revealed that CASC2 overexpression markedly inhibited the migration and invasion of cervical cancer cells. The tube formation assay showed that CASC2 overexpression of the cervical cancer cells significantly decreased the capillary-like structure of HUVECs. These data suggest that CASC2 may be an inhibitor in the development of cervical cancer.

The mitogen-activated protein kinase (MAPK) signaling pathway mediates various biological events in cells such as gene expression, cell proliferation, differentiation, apoptosis, migration, and invasion[18]. Recent studies have shown that lncRNA can regulate tumour progression through the MAPK pathway. LncRNA DBH-AS1 promotes

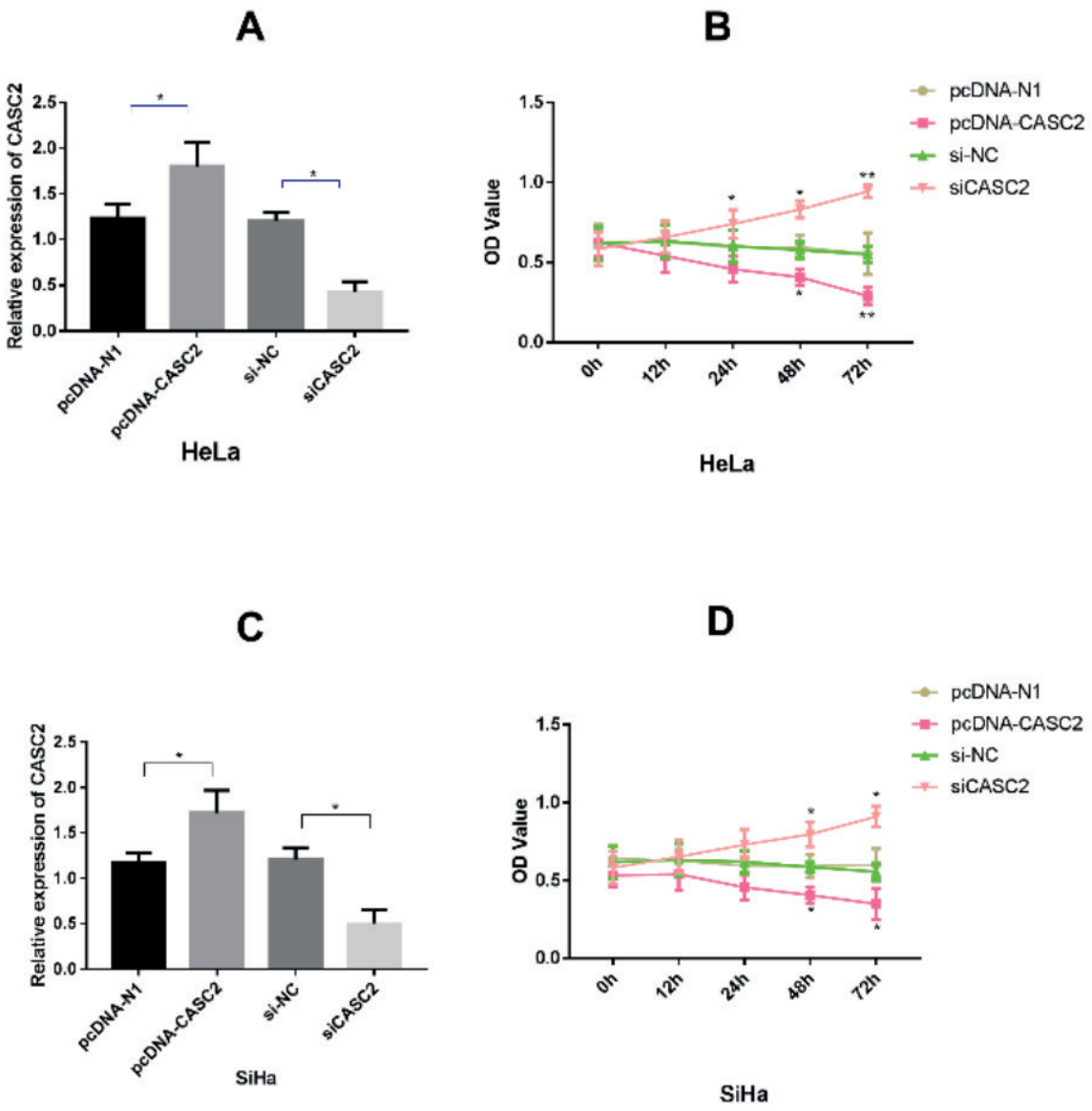

Figure 2. Overexpressed CASC2 inhibits the proliferation of cervical cancer cells *in vitro*. The overexpression or inhibition of CASC2 after transfection into cervical cancer cells was confirmed using qRT-PCR (**A, C**). Effect of pcDNA-CASC2 or si-CASC2 on the proliferation of SiHa and HeLa cells (**B, D**). Data are expressed as the mean ± SD. GAPDH is used as an internal control. * p <0.05, ** p <0.01.

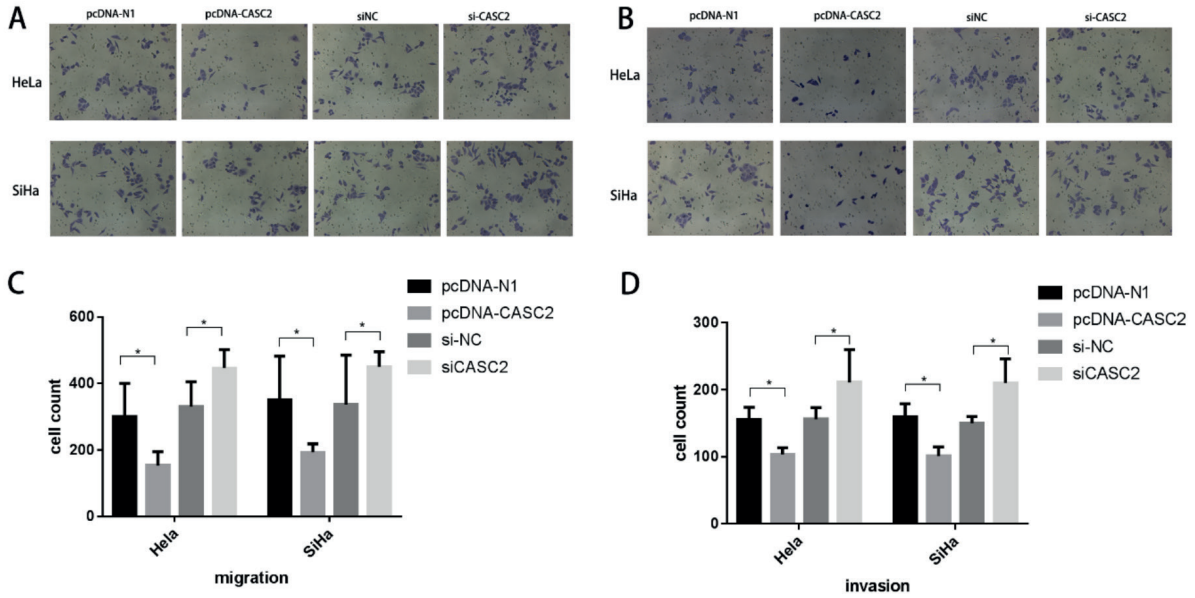

Figure 3. CASC2 inhibits the migration and invasion of cervical cancer cells. Migration and invasion capacity were measured by transwell assays after were transfection with si-CASC2, si-NC, pcDNA-CASC2 or pcDNA-N1 (***A, B***). Histograms depict the migrated or invaded cells in the lower chambers (***C, D***) (magnification × 200). Data are expressed as the mean ± SD. * p <0.05, ** p <0.01.

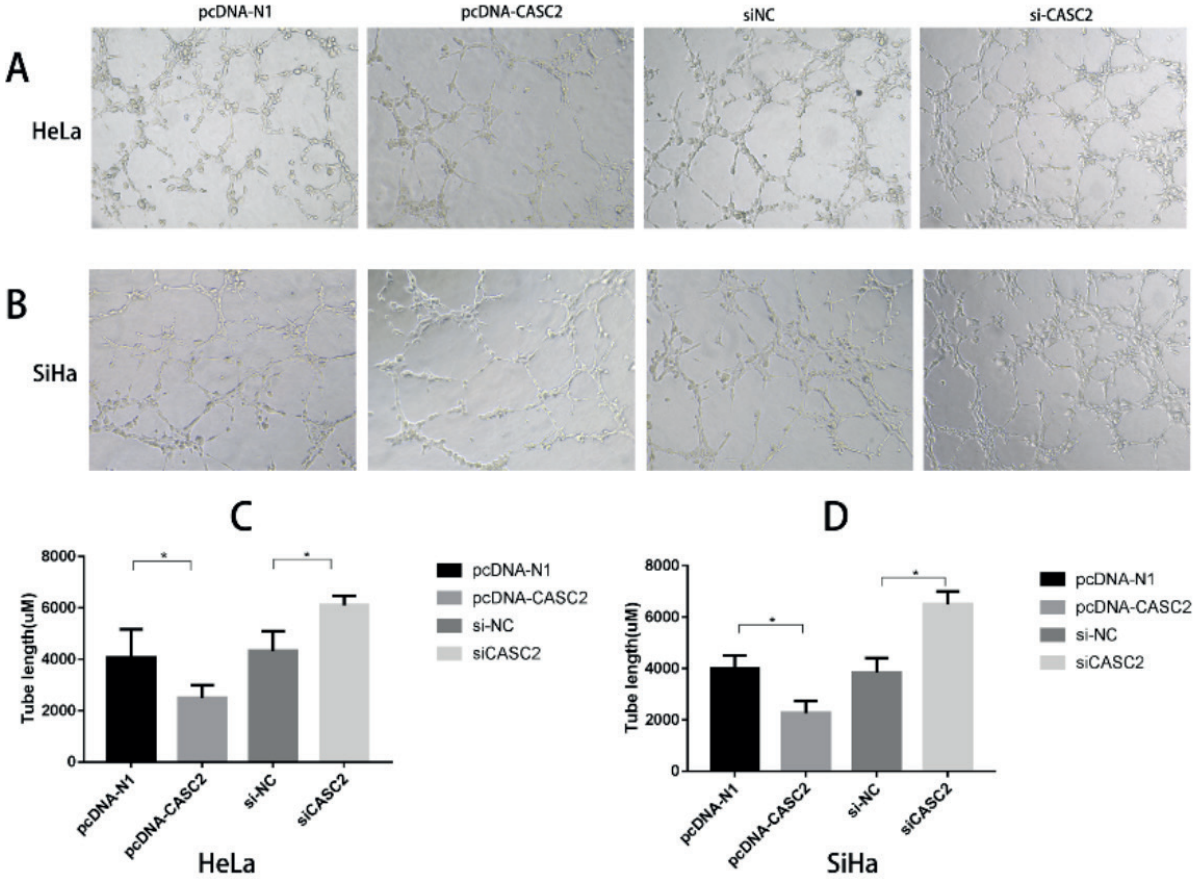

Figure 4. CASC2 inhibited tube formation in HUVECs. Micrographs depict the tubular structures shaped by HUVECs cultured with conditioned medium derived from HeLa and SiHa cells transfected with si-CASC2, si-NC, pcDNA-CASC2 or pcDNA-N1 (***A, B***) (magnification × 150). Histograms represent the total tube length measured in each well (***C, D***). Data are expressed as the mean ± SD. * p <0.05, ** p <0.01.

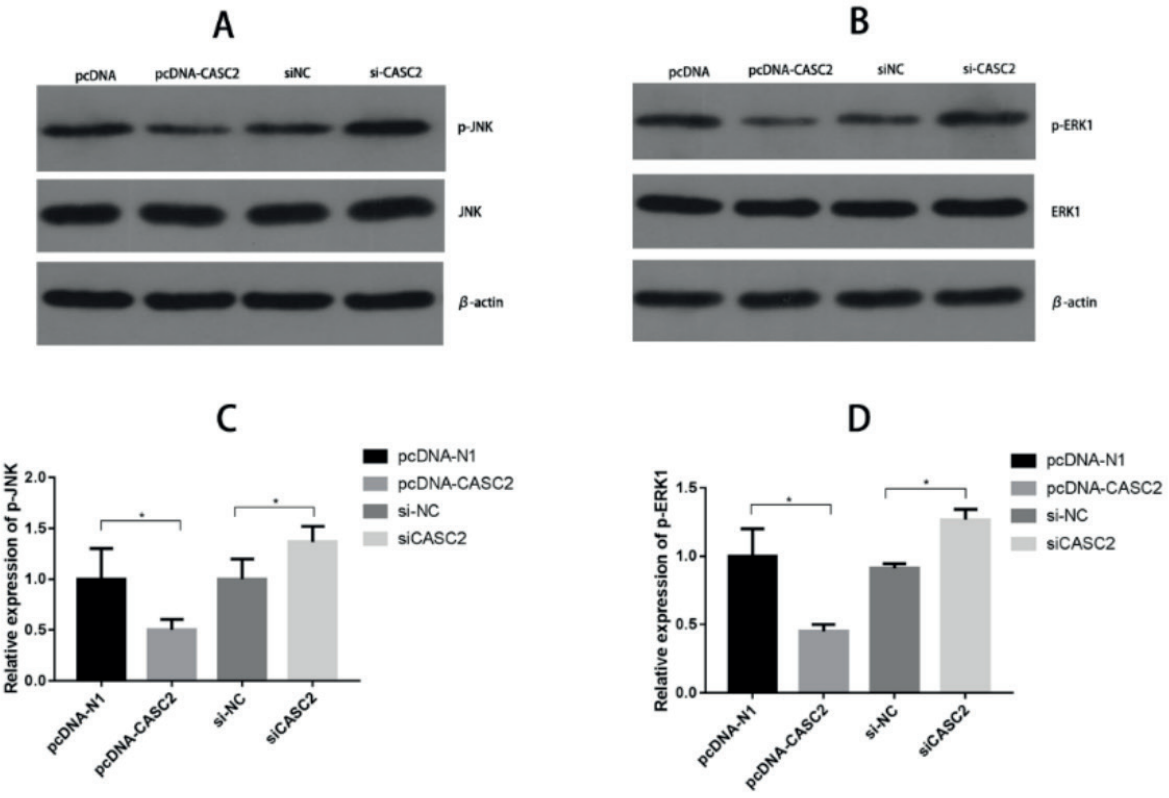

Figure 5. CASC2 significantly inhibits the expression of ERK1 and JNK proteins. After transfection of HeLa cells with si-CASC2, si-NC, pcDNA-CASC2 or pcDNA-N1, the expression of ERK1 and JNK proteins in HeLa cells was analysed by Western blot (**A, B**). Histograms depict the expression of ERK1 and JNK proteins in HeLa cells (**C, D**). β-actin is used as an internal control. Data are expressed as the mean ± SD. * p <0.05, ** p <0.01.

cell proliferation and survival of hepatocellular carcinoma by activating the MAPK pathway[19]. CARLo-5 serve as a pro-oncogenic lncRNA that promotes the proliferation of gastric cancer and activates the ERK/MAPK pathway[20]. LncRNA MALAT1 can suppress glioma cell growth by inactivating ERK/MAPK signaling[21]. LncRNA BANCR promotes the proliferation and migration of lung cancer through the MAPK pathway[22]. To further study the molecular mechanism by which CASC2 inhibits the development of cervical cancer, we examined the effect of CASC2 on ERK and JNK protein levels in cervical cancer cells, which are in the MAPK signaling pathway. It was found that CASC2 overexpression significantly inhibited the level of p-JNK and p-ERK1, suggesting that lncRNA CASC2 inhibits cervical cancer progression via regulating the inactivation of the MAPK pathway. However, other components of the MAPK signaling pathway, such as p38, ERK2, and ERK5, are yet to be further tested. In addition, we will further improve animal experiments and demonstrate detailed molecular mechanisms in combination with *in vivo* experiments.

CASC2 also exerts its oncosuppressor activities with the modulation of the mTOR pathway and the regulation of autophagy[23,24]. Moreover, mTOR pathway upregulation often results in a synergistic action with heightened MAPK activity, either due to mutation or endogenous activators such as pro-inflammatory and pro-oncogenic cytokines, as macrophage migration inhibitory factor, has been implicated in the pathogenesis of cancer and infectious diseases and autoimmune diseases[25-28]. Furthermore, CASC2 may inhibit cancer development inactivating the TGF-ß signaling pathway, which is also involved in fibrosis. Thus, long non-coding RNA CASC2 on TGF-ß may act as a powerful oncogenic cytokine and be beneficial in the prevention and treatment of fibrosis[29-31]. Other evidence has shown that CASC2 regulates carcinoma cell oncogenesis through ceRNA mechanisms[32,33]. Thus, we propose that long non-coding RNA CASC2 may represent a

pleiotropic pharmacological target for several pathologies in addition to cancer such as autoimmune diseases, infectious diseases, and fibrosis.

Conclusions

We found that CASC2 is significantly down-regulated in cervical cancer tissues and cell lines. Overexpression of CACS2 can inhibit the proliferation, invasion, and angiogenesis of cervical cancer cells *in vitro*. Furthermore, CACS2 leads to inactivation of the MAPK signaling pathway. Therefore, CACS2 may be a tumour suppressor that inhibits the development of cervical cancer through the MAPK pathway.

Conflict of Interests

The Authors declare that they have no conflict of interests

Authors' contributions

Xiwen Wang performed the experiments and wrote the paper, Wei Zhang designed the study and reviewed the manuscript. All authors discussed the results and approved the final manuscript.

References

1) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
2) Kodama J, Seki N, Masahiro S, Kusumoto T, Nakamura K, Hongo A, Hiramatsu Y. Prognostic factors in stage IB-IIB cervical adenocarcinoma patients treated with radical hysterectomy and pelvic lymphadenectomy. J Surg Oncol 2010; 101: 413-417.
3) Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.
4) Shi Y, Li J, Liu Y, Ding J, Fan Y, Tian Y, Wang L, Lian Y, Wang K, Shu Y. The long noncoding RNA SPRY4-IT1 increases the proliferation of human breast cancer cells by upregulating ZNF703 expression. Mol Cancer 2015; 14: 51.
5) Iguchi T, Uchi R, Nambara S, Saito T, Komatsu H, Hirata H, Ueda M, Sakimura S, Takano Y, Kurashige J, Shinden Y, Eguchi H, Sugimachi K, Maehara Y, Mimori K. A long noncoding RNA, lncRNA-ATB, is involved in the progression and prognosis of colorectal cancer. Anticancer Res 2015; 35: 1385-1388.
6) Su C, Shi K, Cheng X, Han Y, Li Y, Yu D, Liu Z. Long noncoding RNA LINC00472 inhibits proliferation and promotes apoptosis of lung adenocarcinoma cells via regulating miR-24-3p/DEDD. Technol Cancer Res Treat 2018; 17: 1533033818790490.
7) Baldinu P, Cossu A, Manca A, Satta MP, Sini MC, Rozzo C, Dessole S, Cherchi P, Gianfrancesco F, Pintus A, Carboni A, Deiana A, Tanda F, Palmieri G. Identification of a novel candidate gene, CASC2, in a region of common allelic loss at chromosome 10q26 in human endometrial cancer. Hum Mutat 2004; 23: 318-326.
8) Baldinu P, Cossu A, Manca A, Satta MP, Sini MC, Palomba G, Dessole S, Cherchi P, Mara L, Tanda F, Palmieri G. CASC2a gene is down-regulated in endometrial cancer. Anticancer Res 2007; 27: 235-243.
9) Wang P, Liu YH, Yao YL, Li Z, Li ZQ, Ma J, Xue YX. Long non-coding RNA CASC2 suppresses malignancy in human gliomas by miR-2. Cell Signal 2015; 27: 275-282.
10) Wang D, Gao ZM, Han LG, Xu F, Liu K, Shen Y. Long noncoding RNA CASC2 inhibits metastasis and epithelial to mesenchymal transition of lung adenocarcinoma via suppressing SOX4. Eur Rev Med Pharmacol Sci 2017; 21: 4584-4590.
11) Li X, Song Y, Liu F, Liu D, Miao H, Ren J, Xu J, Ding L, Hu Y, Wang Z, Hou Y, Zhao G. Long non-coding RNA MALAT1 promotes proliferation, angiogenesis and immunosuppressive properties of mesenchymal stem cells by inducing VEGF and IDO. J Cell Biochem 2017; 118: 2780-2791.
12) Lee J, Song J, Kwon ES, Jo S, Kang MK, Kim YJ, Hwang Y, Bae H, Kang TH, Chang S, Cho HJ, Kim SC, Kim S, Koh SS. CTHRC1 promotes angiogenesis by recruiting Tie2expressing monocytes to pancreatic tumors. Exp Mol Med 2016; 48: e261.
13) Zhou J, Huang H, Tong S, Huo R. Overexpression of long non-coding RNA cancer susceptibility 2 inhibits cell invasion and angiogenesis in gastric cancer. Mol Med Rep 2017; 16: 5235-5240.
14) Wang CH, Li YH, Tian HL, Bao XX, Wang ZM. Long non-coding RNA BLACAT1 promotes cell proliferation, migration and invasion in cervical cancer through activation of Wnt/β-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 3002-3009.
15) Zhang J, Gao Y. CCAT-1 promotes proliferation and inhibits apoptosis of cervical cancer cells via the Wnt signaling pathway. Oncotarget 2017; 8: 68059-68070.
16) Guo F, Li Y, Liu Y, Wang J, Li Y, Li G. Inhibition of metastasis-associated lung adenocarcinoma transcript 1 in caski human cervical cancer cells suppresses cell proliferation and invasion. Acta Biochim Biophys Sin (Shanghai) 2010; 42: 224-229.
17) Kim HJ, Lee DW, Yim GW, Nam EJ, Kim S, Kim SW, Kim YT. Long non-coding RNA HOTAIR is associated with human cervical cancer progression. Int J Oncol 2015; 46: 521-530.
18) Meng X, Zhang S. MAPK cascades in plant disease resistance signaling. Annu Rev Phytopathol 2013; 5: 245-266.

19) Huang JL, Ren TY, Cao SW, Zheng SH, Hu XM, Hu YW, Lin L, Chen J, Zheng L, Wang Q. HBx-related long non-coding RNA DBH-AS1 promotes cell proliferation and survival by activating MAPK signaling in hepatocellular carcinoma. Oncotarget 2015; 6: 33791-33804.

20) Zhang Y, Ma M, Liu W, Ding W, Yu H. Enhanced expression of long noncoding RNA CARLo-5 is associated with the development of gastric cancer. Int J Clin Exp Pathol 2014; 7: 8471-8479.

21) Han Y, Wu Z, Wu T, Huang Y, Cheng Z, Li X, Sun T, Xie X, Zhou Y, Du Z. Tumor-suppressive function of long noncoding RNA MALAT1 in glioma cells by downregulation of MMP2 and inactivation of ERK/MAPK signaling. Cell Death Dis 2016; 7: e2123.

22) Jiang W, Zhang D, Xu B, Wu Z, Liu S, Zhang L, Tian Y, Han X, Tian D. Long non-coding RNA BANCR promotes proliferation and migration of lung carcinoma via MAPK pathways. Biomed Pharmacother 2015; 69: 90-95.

23) Jiang C, Shen F, Du J, Fang X, Li X, Su J, Wang X, Huang X, Liu Z. Upregulation of CASC2 sensitized glioma to temozolomide cytotoxicity through autophagy inhibition by sponging miR-193a-5p and regulating mTOR expression. Biomed Pharmacother 2018; 97: 844-850.

24) Zhang S, Leng T, Zhang Q, Zhao Q, Nie X, Yang L. Sanguinarine inhibits epithelial ovarian cancer development via regulating long non-coding RNA CASC2-EIF4A3 axis and/or inhibiting NF-κB signaling or PI3K/AKT/mTOR pathway. Biomed Pharmacother 2018; 102: 302-308.

25) Presti M, Mazzon E, Basile MS, Petralia MC, Bramanti A, Colletti G, Bramanti P, Nicoletti F, Fagone P. Overexpression of macrophage migration inhibitory factor and functionally-related genes, D-DT, CD74, CD44, CXCR2 and CXCR4, in glioblastoma. Oncol Lett 2018; 16: 2881-2886

26) Caporali S, Alvino E, Lacal PM, Levati L, Giurato G, Memoli D, Caprini E, Antonini Cappellini GC, D'Atri S. Targeting the PI3K/AKT/mTOR pathway overcomes the stimulating effect of dabrafenib on the invasive behavior of melanoma cells with acquired resistance to the BRAF inhibitor. Int J Oncol 2016; 49: 1164-1174.

27) Chen D, Mao C, Zhou Y, Su Y, Liu S, Qi WQ. PF-04691502, a dual PI3K/mTOR inhibitor has potent pre-clinical activity by inducing apoptosis and G1 cell cycle arrest in aggressive B-cell non-Hodgkin lymphomas. Int J Oncol 2016; 48: 253-260.

28) Fagone P, Mazzon E, Cavalli E, Bramanti A, Petralia MC, Mangano K, Al-Abed Y, Bramati P, Nicoletti F. Contribution of the macrophage migration inhibitory factor superfamily of cytokines in the pathogenesis of preclinical and human multiple sclerosis: in silico and in vivo evidences. J Neuroimmunol 2018; 322: 46-56.

29) Zhang Y, Zhu M, Sun Y, Li W, Wang Y, Yu W. Up-regulation of lncRNA CASC2 suppresses cell proliferation and metastasis of breast cancer via inactivating of the TGF-ß signaling pathway. Oncol Res 2019; 27: 379-387.

30) Papageorgis P, Stylianopoulos T. Role of TGFβ in regulation of the tumor microenvironment and drug delivery (review). Int J Oncol 2015; 46: 933-943.

31) Fagone P, Mangano K, Mammana S, Pesce A, Pesce A, Caltabiano R, Giorlandino A, Portale TR, Cavalli E, Lombardo GA, Coco M, Puleo S, Nicoletti F. Identification of novel targets for the diagnosis and treatment of liver fibrosis. Int J Mol Med 2015; 36: 747-752.

32) Gao Z, Wang H, Li H, Li M, Wang J, Zhang W, Liang X, Su D, Tang J. Long non-coding RNA CASC2 inhibits breast cancer cell growth and metastasis through the regulation of the miR-96-5p/SYVN1 pathway. Int J Oncol 2018; 53: 2081-2090.

33) Zhao L, Zhang Y, Zhang Y. Long non-coding RNA CASC2 regulates hepatocellular carcinoma cell oncogenesis through miR-362-5p/Nf-κB axis. J Cell Physiol 2018; 233: 6661-6670.