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Silence of FOXD2-AS1 inhibited the proliferation and invasion of esophagus cells by regulating miR-145-5p/CDK6 axis

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Summary. This study aimed to investigate the function of long non-coding RNA FOXD2 adjacent opposite strand RNA 1 (lncRNA FOXD2-AS1) during the progression of esophagus cancer (EC) and explore its underlying molecular mechanisms. The level of FOXD2-AS1 in EC tissues and paracancerous tissues was detected by using RT-qPCR; ROC curve was used to evaluate the diagnostic value of FOXD2-AS1 for EC. In addition, CCK8 assay and immunofluorescence staining assay were used to detect the proliferation of Eca-109 and TE-1 cells. To investigate the function of FOXD2-AS1 on cell apoptosis and cell cycle, flow cytometry was performed. To detect the invasion ability of EC cells, transwell invasion assay was performed. Starbase3.0 and Targetscan were used to predict the target genes of FOXD2-AS1 and miR-145-5p, and protein expressions were detected with western blot. We found FOXD2-AS1 was significantly upregulated in EC tissues compared with adjacent normal tissues, which was positively correlated with clinicopathological parameters of patients with EC. Downregulation of FOXD2-AS1 inhibited the proliferation and invasion by inducing apoptosis of EC cells. Moreover, FOXD2-AS1 may regulate the expression of CDK6 by targeting miR-145-3p. Meanwhile, silencing of FOXD2-AS1 caused G1 phase arrest of EC cells by reducing the expression of CDK6. In conclusion, silening FOXD2-AS1 significantly inhibited the proliferation and invasion of EC cells by regulating the miR-145-5p/CDK6 axis. Therefore, FOXD2-AS1 might be used as diagnostic biomarker and therapeutic target for EC.

Key words: Esophagus cancer, Long non-coding RNA FOXD2-AS1, miR-145-5p, CDK6, Proliferation, Cell cycle

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Introduction

Esophagus cancer (EC) is the sixth leading cause of cancer related death around the world with high morbidity rate, and the number of deaths has increased by 500% during the last 30 years (Hagen et al., 2012; Parry et al., 2017). Although great advances have made on the technologies of chemotherapy, radiotherapy and surgery, the 5 year survival rate of patients with EC remains low (Yu-Suo et al., 2015). Thus, it is urgent to investigate the molecular mechanisms of EC with the purpose of diagnosing and treating EC.

Long-noncoding RNAs (LncRNAs) are a class of RNAs with a length of more than 200 nucleotides. Emerging evidence has demonstrated that lncRNAs played essential roles in the onset and progression of a variety of cancers by regulating expression of genes (Wang and Chang, 2011; Hu et al., 2018). What's more, it has been demonstrated that lncRNAs could be used as molecular targets for diagnosis, therapy and prognosis in a number of cancers (Silva et al., 2015; Chandra Gupta

and Nandan Tripathi, 2017). For example, focally amplified lncRNA on chromosome1 (FALEC) was upregulated in the blood plasma of patients with cervical cancer, and overexpression of FALEC enhanced the proliferation and invasion of HeLa cells. In addition, FALEC could be used as a biomarker for diagnosis and prognosis of cervical cancer (Li et al., 2020). LncRNA HOXA-AS2, an oncogene, was up-regulated in non-small cell lung cancer; overexpression of HOXA-AS2 may promote the malignant progression of non-small cell lung cancer (Cui et al., 2019). A recent study proved that FOXD2-AS1 could serve as a predicted marker for survival rate of patients with EC (Bao et al., 2018). However, the mechanism by which FOXD2-AS1 regulates the progression of EC remains unclear.

MicroRNAs (miRNAs) are a class of non-coding RNAs shorter than 25 nucleotides, and previous studies proved that miRNAs played critical roles in the pathogenesis and development of numerous kinds of cancers (Bertoli et al., 2015; Lan et al., 2015; Lin and Gregory, 2015). For example, miR-592 was significantly upregulated in tumor tissues compared with the adjacent normal tissues in patients with thyroid cancer; forced expression of miR-592 may promote the proliferation, migration and invasion of thyroid cancer cells (Luo et al., 2019). Emerging evidence indicated that lncRNAs may regulate the progression of cancers via sponging miRNAs. For instance, Long noncoding RNA nuclear paraspeckle assembly transcript 1 (NEAT1) regulated the progression of esophageal squamous cell carcinoma (ESCC) by sponging miR-129 (Huo et al., 2017). In addition, miR-145-5p was reported as a tumor suppressor in various types of cancers, including ovarian cancer (Hang et al., 2019), non-small lung cancer (Lu et al., 2018), prostate cancer (Ozen et al., 2015), and endometrial cancer (Wu et al., 2020). However, the function of miR-145-5p during the progression of esophageal cancer remains unclear. In present study, we found miR-145-5p may be regulated by LncFOXD2-AS1 and played essential role in the progression of EC.

Material and methods

Cell culture

Eca-109 and TE-1 EC cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured with Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (DMEM, Gibco, CA, USA) at 37°C with 5% CO_2.

The prediction of target genes

Starbase 3.0 (http://starbase.sysu.edu.cn/index.php) was adopted to predict the target miRNAs of FOXD2-AS1. Targetscan Human (http://www.targetscan.org/vert_72/) was used to predict the target genes of miRNA.

Clinical specimens

EC tissues and the para-carcinoma tissues were obtained from patients with EC who received treatments in our hospital. All of the experiments were approved by the ethics committee of Yancheng Third People's Hospital, and informed consent was signed patients or a legal guardian. None of the patients received preoperative interventional therapy or systemic chemotherapy before surgery.

CCK8 assay

The viability of EC cells was examined by using Cell Counting Kit-8 (CCK8, APExBIO, Houston, TX, USA). Cells were seeded in 96-well plates at a density of 5×10^3 cells/well and cultured in 100 µl cell culture medium. After 48 h of incubation, 10 µl CCK8 was added into each well for 2 h. Finally, the absorbance at 450 nm was assessed by using a plate reader (model 680; Bio-Rad, Hertfordshire, UK).

Wound healing assay

To detect the migration ability of cells, Eca-109 cells (2×10^5 cells per well) were seeded into 12-well culture plate overnight at 37°C. At 80% confluence, a wound area was made with a 20 µL pipette tip in the cell monolayer. The width of the wound area was photographed at 0 h and 24 h using the microscope.

Evaluation of cell invasion ability

To detect the invasion ability of cells, Eca-109 cells were collected and suspended in DMEM without FBS at a density of 5×10^4 cells/ml. Then, 200 µl cell suspension was added into the upper chambers of transwell which were coated with Matrigel. After that, the bottom chamber was filled with DMEM with 20% FBS. Forty eight hours later, the invasive cells were fixed with 4% paraformaldehyde (PFA, Sigma, NY, USA) and stained with crystal violet.

Cell apoptosis assay

Annexin V-FITC/PI Apoptosis Detection Kit (YEASEN, Shanghai, China) was used to examine the cell apoptosis. Cells were collected and washed with 1×PBS 3 times, and suspended in 400 µl binding buffer. Then, cell suspension were incubated with 10 µl Annexin V-FITC and 10 µl PI at 37°C for 30 min. Finally, cell apoptosis was detected with flow cytometry.

Real-time fluorescence quantitative RT-qPCR

Total RNAs were extracted by Trizol reagent (Thermo Fisher scientific, Waltham, CA, USA) and the concentration was evaluated using Nano drop 2000. 2 µl RNA was used for reverse transcription (RT reagent Kit,

Vazyme, Nanjing, China) and the real-time quantification polymerase chain reaction was implemented by using SYBR Green (Vazyme, Nanjing, China). The primers were used as follows: FOXD2-AS1-forward: 5'- TATGTGGTAGGGGACTCGCT -3' FOXD2-AS1-reverse: CCCTCTTCCCACGAACA ACA-3' GAPDH-forward: 5'- CCACCCCCAATGTCT CTGTT-3', GAPDH-reverse: 5'- ATGGATGAACGG CAATCCCC-3'.

Western blot

RIPA buffer (Abcam, Cambridge, MA, USA) with protease inhibitor cocktail Sigma Aldrich (St. Louis, MO, USA) was adopted to extract proteins; the concentration of protein was assessed with BCA Kit (Thermo Fisher Scientific, NY, USA). 30 µl of each sample was separated by SDS-page electrophoresis. Primary antibodies were used as follows: anti-CDK6 (Abcam, ab124821), anti-cyclinD1 (Abcam, ab16663), anti-Bcl-2 (Abcam, ab185002), anti-Bax (Abcam, ab182733), anti-cleaved caspase 3 (Abcam, ab13847), anti-p-p65 (Abcam, ab86299) and anti-MMP2 (Abcam, ab97779).

Luciferase assay

Mimic-NC, mimic-miR-145-5p, psi FOXD2-AS1 WT, psi FOXD2-AS1 MUT and Mimic-NC, mimic-miR-145-5p, psi CDK6 WT, psi CDK6 MUT were co-transfected into Eca-109 cells. Forty eight hours later, the activities of luciferase were evaluated by Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Data statistics

All of the Results were analyzed by using the GraphPad Prism Software (San Diego, CA, USA). Statistical comparisons were subjected to one way ANOVA followed by Tukey's test. Each experiment was repeated more than three times. P<0.05 was considered as statistically significant.

Results

The diagnostic value of FOXD2-AS1 in EC

To investigate the function of FOXD2-AS1 during the progression of EC, we first detected the expression of FOXD2-AS1 in tumor tissues and the adjacent normal tissues of patients with EC. As revealed in Fig. 1A, the level of FOXD2-AS1 in cancer tissues was significantly

Table 1. FOXD2-AS1 expression and clinical parameters of patients with esophagus cancer.

Parameters	Number	FOXD2-AS	p value
Age			0.939
≤50	17	1.762±0.832	
>50	13	1.741±0.591	
Tumor volume			
≤5 cm	19	1.846±0.742	0.369
>5 cm	11	1.594±0.702	
Lymph node metastasis			0.080
N0	18	1.564±0.553	
N1-N3	12	2.037±0.877	
Distant metastasis			0.019*
M0	22	1.569±0.425	
M1	8	2.259±1.114	
TNM stage			0.001**
I-II	21	1.449±0.348	
III-IV	9	2.464±0.891	

*P<0.05, **P<0.01; student's t test.

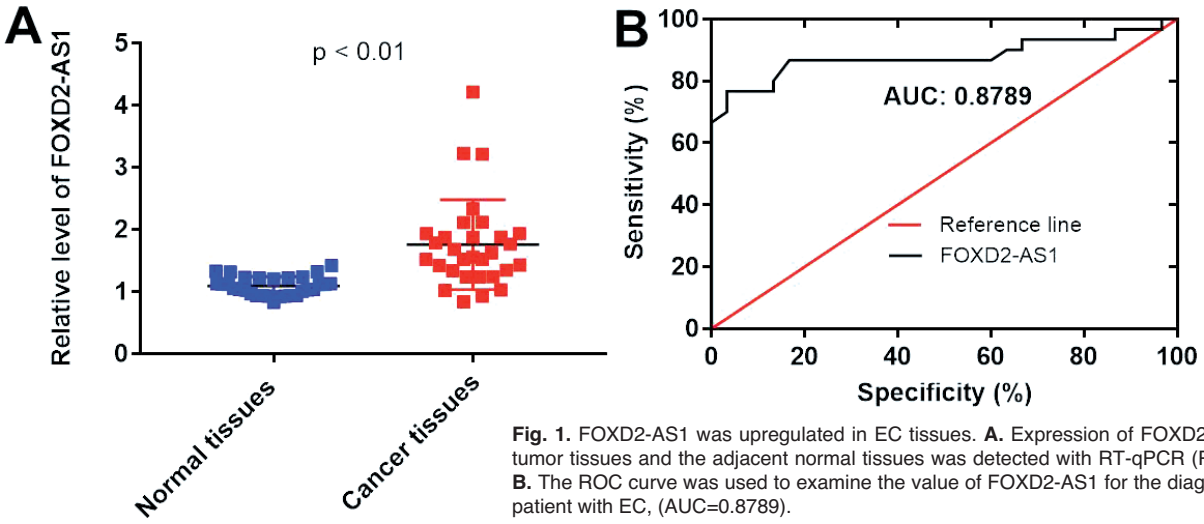

Fig. 1. FOXD2-AS1 was upregulated in EC tissues. **A.** Expression of FOXD2-AS1 in tumor tissues and the adjacent normal tissues was detected with RT-qPCR (P<0.01). **B.** The ROC curve was used to examine the value of FOXD2-AS1 for the diagnosis of patient with EC, (AUC=0.8789).

upregulated compared with that in adjacent normal tissues. In addition, FOXD2-AS1 expression was positively correlated with clinicopathological parameters including distant metastasis and TNM stage (Table 1). Meanwhile, receiver operation characteristic (ROC) curve was used to examine the value of FOXD2-AS1 in the diagnosis of EC (Fig. 1B). These data indicated that the level of FOXD2-AS1 in EC was upregulated, which was associated with a poor prognosis of EC.

Downregulation of FOXD2 inhibited the proliferation of EC

In order to examine the effect of FOXD2-AS1 on the proliferation of EC cells, we next downregulated FOXD2-AS1 using siRNAs (Fig. 2A,B). Next, CCK8 assay was conducted to determine the proliferative ability of Eca-109 cells (Fig. 2C) and TE-1 cells (Fig. 2D). The results indicated that downregulation of FOXD2-AS1 significantly inhibited the proliferation of Eca-109 and TE-1 cells. In addition, the quantification of Ki67 positive cells revealed that downregulation of FOXD2-AS1 significantly decreased the number of Ki67 positive cells (Fig. 2E,F). All these data suggested downregulation of FOXD2 inhibited the proliferation of EC.

Downregulation of FOXD2-AS1 induced cell apoptosis and inhibited the migration and invasion of Eca-109 cells

To determine the effect of FOXD2-AS1 on Eca-109 cell apoptosis, flow cytometry was performed. The results showed that inhibition of FOXD2-AS1 notably induced the apoptosis of Eca-109 cells (Fig. 3A,B). In addition, downregulation of FOXD2-AS1 markedly decreased the expression of Bcl-2, but increased the levels of Bax and cleaved caspase 3 in Eca-109 cells (Fig. 3C-F). Furthermore, wound healing assay and transwell invasion assay were implemented to test the effect of FOXD2-AS1 on the migration and invasion of Eca-109 cells. The results indicated that downregulation of FOXD2-AS1 prominently attenuated the migration and invasion of Eca-109 cells (Fig. 4A-D).

FOXD2-AS1 regulated the proliferation, apoptosis migration and invasion of Eca-109 cells by regulating miR-145-5P/CDK6 axis

To explore the molecular mechanisms by which FOXD2-AS1 regulated the proliferation, apoptosis, migration and invasion of Eca-109 cells, Starbase3.0 was used to predict the target miRNAs of FOXD2-AS1. The data indicated that miR-145-5p was the binding target of FOXD2-AS1 (Fig. 5A,B). Meanwhile, Targetscan was used to predict the target genes of miR-145-5p, which suggested CDK6 was the binding target of miR-145-5p (Fig. 5A,C). Next, the luciferase assay was implemented to determine the interplay between miR-145-5p, FOXD2-AS1 and CDK6. The results of luciferase assay proved that FOXD2-AS1 directly interacted with miR-145-5p, and miR-145-5p directly interacted with CDK6 (Fig. 5D,E). In addition, the level of miR-145-5p in cancer tissues was significantly downregulated compared with that in adjacent normal tissues, which was negatively correlated with the expression of FOXD2-AS1 (Fig. 5F,G). Our findings

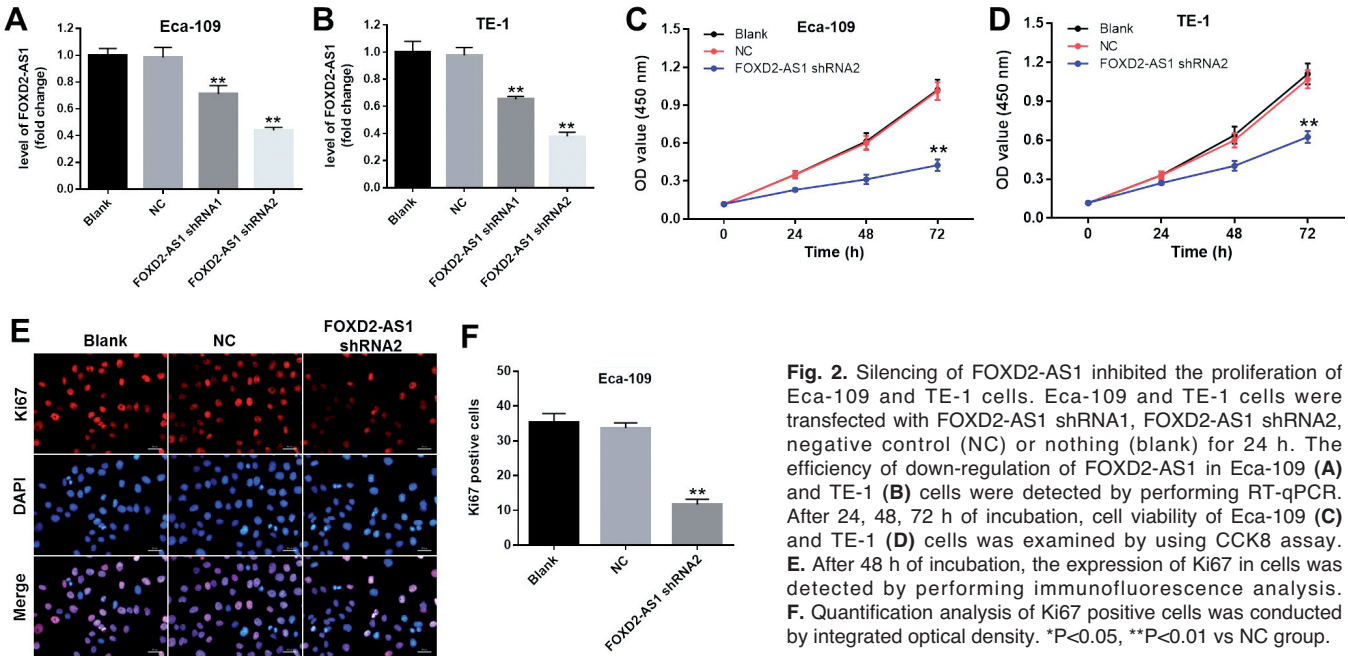

Fig. 2. Silencing of FOXD2-AS1 inhibited the proliferation of Eca-109 and TE-1 cells. Eca-109 and TE-1 cells were transfected with FOXD2-AS1 shRNA1, FOXD2-AS1 shRNA2, negative control (NC) or nothing (blank) for 24 h. The efficiency of down-regulation of FOXD2-AS1 in Eca-109 **(A)** and TE-1 **(B)** cells were detected by performing RT-qPCR. After 24, 48, 72 h of incubation, cell viability of Eca-109 **(C)** and TE-1 **(D)** cells was examined by using CCK8 assay. **E.** After 48 h of incubation, the expression of Ki67 in cells was detected by performing immunofluorescence analysis. **F.** Quantification analysis of Ki67 positive cells was conducted by integrated optical density. *P<0.05, **P<0.01 vs NC group.

suggested that FOXD2-AS1 may regulate the expression of CDK6 via sponging with miR-145-5p.

Downregulation of FOXD2-AS1 enhanced the expression level of CDK6

Finally, western blot analysis was further conducted to detect the effect of FOXD2-AS1 on protein expressions. The results showed that down-regulation of FOXD2-AS1 prominently reduced the expressions of CDK6, Cyclin D1, p-p65 and MMP2 (Fig. 6A-E). In addition, cell cycle distribution analysis revealed that inhibition of FOXD2-AS1 caused G1 arrest of Eca-109 cells (Fig. 6F,G). These data suggest that inhibition of FOXD2-AS1 caused G1 arrest of Eca-109 cells by increasing the expression of CDK6.

Discussion

In this study, we proved that FOXD2-AS1 was significantly upregulated in tumor tissues of EC. Downregulation of FOXD2-AS1 significantly inhibited the proliferation, migration and invasion of EC cells via increasing cell apoptosis. In addition, FOXD2-AS1 modulated the expression level of CDK6 by directly interacting with miR-145-3p. Thus, our findings suggest that FOXD2-AS1 played critical roles in the proliferation, migration, invasion and apoptosis of EC cells by regulating the cell cycle.

It is well known that long non-coding RNAs play essential roles in the development of cancers through regulating synthesis of protein and maintenance of cellular homeostasis (Guttman et al., 2009). FOXD2-AS1 has been reported to be aberrantly expressed in many types of cancers, including hepatocellular carcinoma (Liu et al., 2019), bladder cancer (An et al., 2018), melanoma (Ren et al., 2019), nasopharyngeal carcinoma (Chen et al., 2018), gastric cancer (Yang et al., 2019) and EC (Hu et al., 2019). For example, aberrant expression of FOXD2-AS1 was found in hepatocellular carcinoma (HCC) tissues and high expression of FOXD2-AS1 was negatively associated with the survival rate of patients with HCC. In addition, restoration of FOXD2-AS1 could significantly enhance the proliferation and migration of HCC cells by regulating the miR-206/ANXA2 axis (Chang et al., 2018). In nasopharyngeal carcinoma (NPC), upregulation of FOXD2-AS1 indicated unfavorable prognosis of patients. In contrast, deficiency of FOXD2-AS1 may be able to inhibit the proliferation of NPC cells by modulating the miR-363-5p/S100A1 axis (Chen et al., 2018). The correlation between FOXD2-AS1 and EC

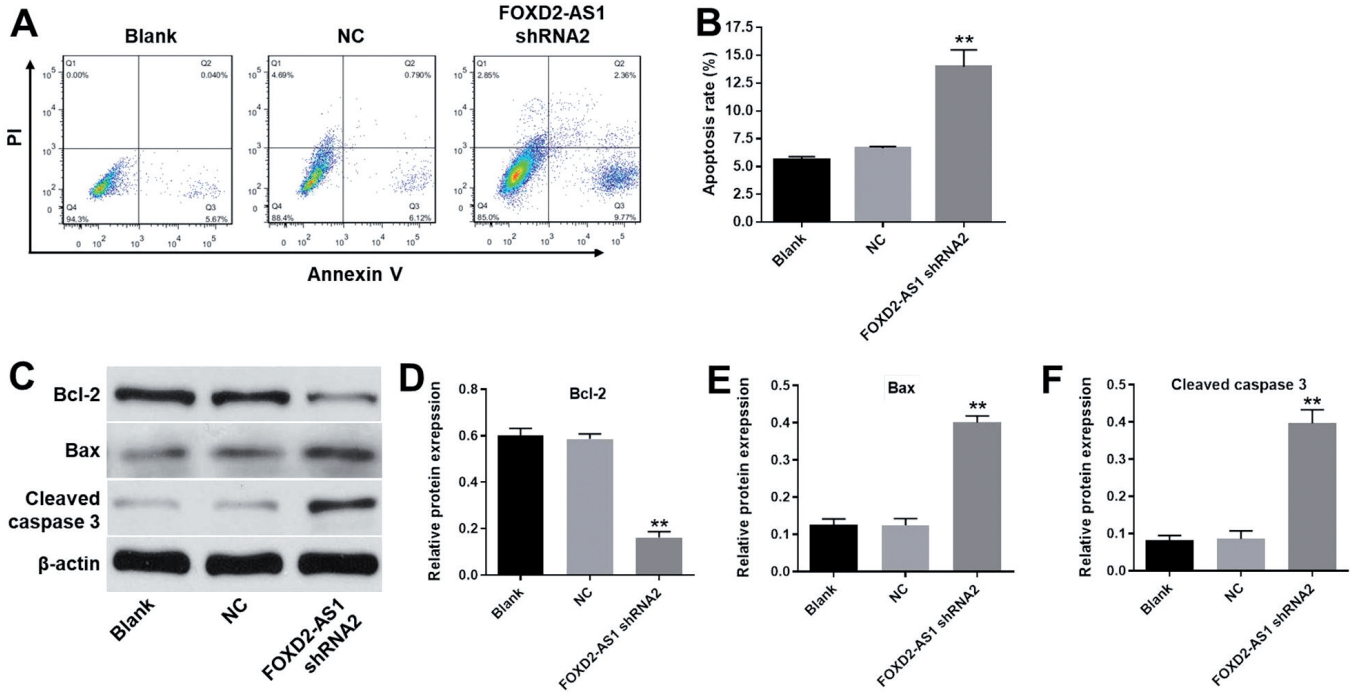

Fig. 3. Downregulation of FOXD2-AS1 induced apoptosis of Eca-109 cells. Eca-109 cells were transfected with FOXD2-AS1 shRNA2, NC or blank for 72 h. **A.** The flow cytometry analysis of the status of EC cells which were double stained with Annexin V and PI. X axis: the level of Annexin-V FITC fluorescence; Y axis: the PI fluorescence. **B.** The quantification of apoptosis cells. **C.** The expressions of Bcl-2, Bax and cleaved caspase 3 in Eca-109 cells were detected by Western blot analysis. β-actin was used as an internal control. **D, E, F.** The relative expressions of Bcl-2, Bax and cleaved caspase 3 in cells were quantified. **P<0.01 vs NC group.

was reported by a previous study (Bao et al., 2018). It was demonstrated that FOXD2-AS1 might serve as a biomarker for prediction of the prognosis of patients with ESCC due to its overexpression in the tumor tissues of ESCC (Bao et al., 2018). However, the molecular mechanisms remain unclear. In the present study, we found FOXD2-AS1 may be able to regulate the expression of CDK6 by sponging miR-145-5p.

Accumulating studies indicate that miR-145-5p may act as a tumor suppressor to regulate the proliferation, migration, invasion and apoptosis of cancer cells (Chang et al., 2017; Wang et al., 2018). The expression of miR-145-5p was significantly downregulated in hepatocellular carcinoma cell lines compared with normal cell lines. Restoration of miR-145-5p inhibited proliferation and metastasis, but induced the apoptosis of hepatocellular cancer cells by directly targeting RAB18 (Wang et al., 2019). In addition, expression of miR-145-5p was associated with the Epithelial-mesenchymal transition (EMT) of gastric cancer ,and overexpression of miR-145-5p inhibited the migration and invasion of gastric cancer cells by targeting zinc-finger E-box binding homeobox2 (ZEB2) (Jiang et al., 2016). Moreover, a previous study demonstrated that the expression of miR-145-5p was down-regulated in the tumor tissues of ESCC; force expression of miR-145-5p is able to significantly attenuate the proliferation, migration and invasion of ESCC cells by targeting the Sp1/NF-kB signaling pathway (Mei et al., 2017). Our data indicated that downregulation of FOXD2-AS1 significantly reduced the expression of p-p65 in Eca-109 cells. These data indicated that downregulation of FOXD2-AS1 inhibited the growth of Eca-109 cells via inactivation of p65. What's more, miR-145-5p also repressed the expression of cell cycle related genes, such as cyclin D1, cyclin A2 and cyclin E1 (Mei et al., 2017). In our work, we found miR-145-5p functioned as a target of FOXD2-AS1, and regulated the expressions of CDK6 and cyclin D1.

CDK6, a member of Cyclin-dependent kinases (CDKs), which are a family of serine /threonine protein kinases, is associated with many cellular processes

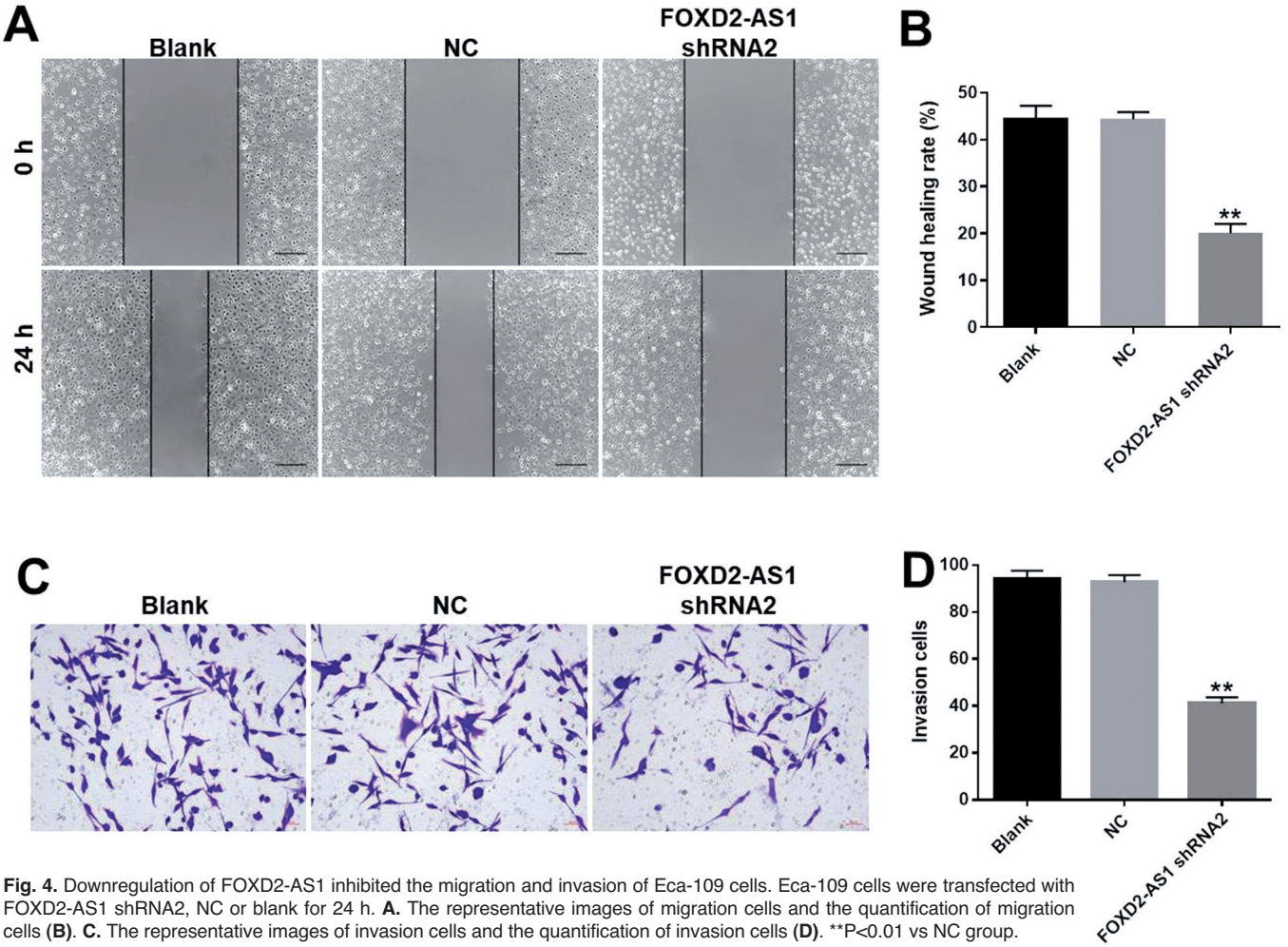

Fig. 4. Downregulation of FOXD2-AS1 inhibited the migration and invasion of Eca-109 cells. Eca-109 cells were transfected with FOXD2-AS1 shRNA2, NC or blank for 24 h. **A.** The representative images of migration cells and the quantification of migration cells **(B)**. **C.** The representative images of invasion cells and the quantification of invasion cells **(D)**. **P<0.01 vs NC group.

including cell cycle, genetic transcription, translation, cell apoptosis, and neurogenesis (Malumbres, 2014). As a subunit of CDK6/cyclin D complex, CDK6 played critical roles in the progression of G1 to S cell cycle (Malumbres and Barbacid, 2005). Aberrant expression of CDK6 was found in several types of cancers (Solomon et al., 2015). For instance, upregulation of CDK6 in ovarian cancer induced the resistance to platinum-based therapies, while silence of CDK6 enhanced the sensitivity of ovarian cancer cells to

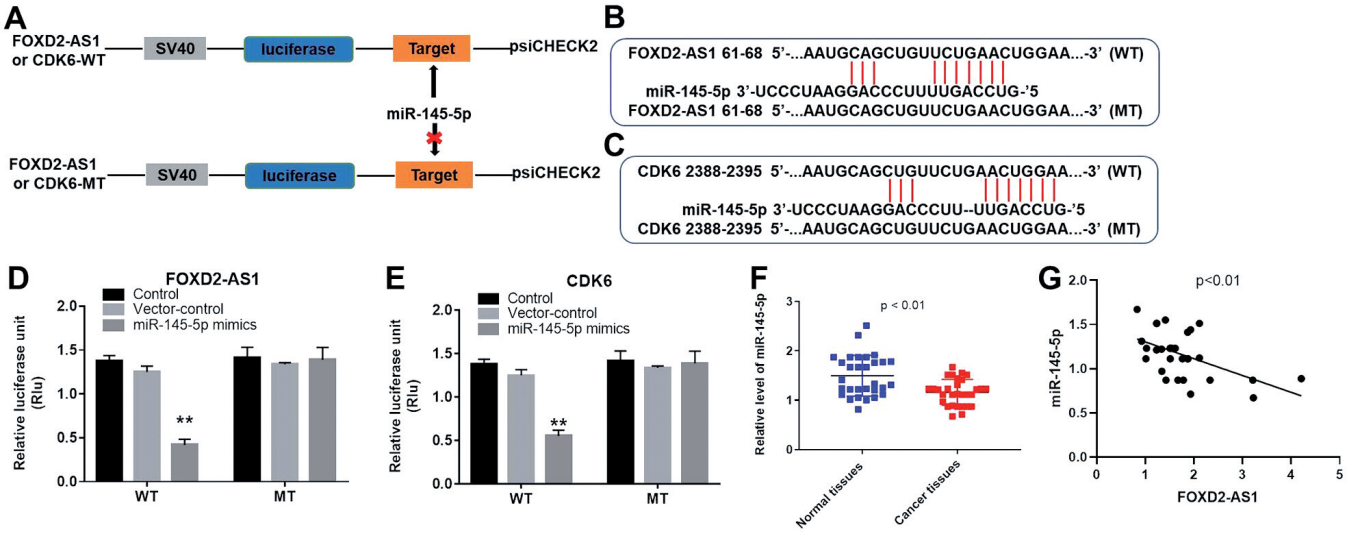

Fig. 5. FOXD2-AS1 regulated the progression of EC by modulating miR-145-5p/CDK6 axis. **A.** Diagrammatic drawing of molecular mechanism by which FOXD2-AS1 regulated the progression of EC. **B.** The binding sites between FOXD2-AS1 and miR-145-5p. **C.** The biding sites between miR-145-5p and CDK6. **D.** Relative luciferase activity of reporter plasmids carrying WT or MT FOXD2-AS1 3'-UTR in Eca-109 cells following co-transfection with miR-145-5p mimics were measured using dual luciferase reporter assay. **E.** Relative luciferase activity of reporter plasmids carrying WT or MT CDK6 3'-UTR in Eca-109 cells following co-transfection with miR-145-5p mimics were measured using dual luciferase reporter assay. **F.** Expression of miR-145-5p in tumor tissues and the adjacent normal tissues was detected with RT-qPCR. **G.** The correlation between FOXD2-AS1 and miR-145-5p was analyzed with linear regression. **P<0.01 vs NC group.

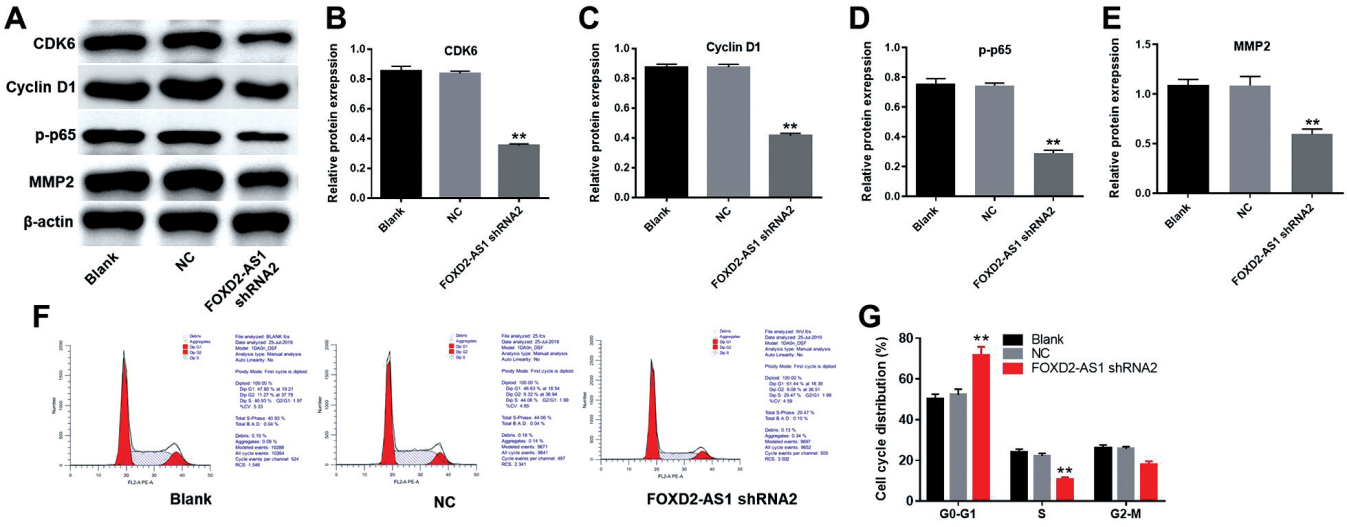

Fig. 6. Silencing of FOXD2-AS1 inhibited the expression of CDK6. Eca-109 cells were transfected with FOXD2-AS1 shRNA2 or NC. **A.** The expressions of CDK6, cyclin D1, p-p65 and MMP2 in Eca-109 cells were detected by Western blot analysis. β-actin was used as an internal control. **B-E.** The relative expressions of CDK6, cyclin D1, p-p65 and MMP2 were quantified. **F.** Flow cytometry analysis of cell cycle status of Eca-109 cells which were stained by propidium iodide (PI). **G.** The quantification of cells in each cell phase. **P<0.01 vs NC group.

platinum (Dall'Acqua et al., 2017). In addition, CDK6 was reported to be required for the proliferation of esophagus adenocarcinoma cells (EACs), and inhibiting its activity suppressed the proliferation of EACs (Ismail et al., 2011). In agreement with previous studies, we found downregulation of FOXD2-AS1 induced EC cells G1 phase arrest by inhibiting the expressions of CDK6 and cyclin D1.

To sum up, our findings proved that FOXD2-AS1 functioned as an oncogene in EC by regulating miR-145-5p/CDK6 axis. FOXD2-AS1 might be used as diagnostic biomarker and therapeutic target for EC.

Author's contribution. Study design, literature research, experimental study was performed by Woda Shi, Zhengya Gao and Wencai Wang; data acquisition, data analysis and statistical analysis was performed by Jianxiang Song. All the authors were responsible as guarantors of the integrity of the entire study, manuscript preparation and manuscript editing.

Competing interests. None.

References

An Q., Zhou L. and Xu N. (2018). Long noncoding RNA FOXD2-AS1 accelerates the gemcitabine-resistance of bladder cancer by sponging miR-143. Biomed. Pharmacother. 103, 415-420.

Bao J., Zhou C., Zhang J., Mo J., Ye Q., He J. and Diao J. (2018). Upregulation of the long noncoding RNA FOXD2-AS1 predicts poor prognosis in esophageal squamous cell carcinoma. Cancer Biomark. 21, 527-533.

Bertoli G., Cava C. and Castiglioni I. (2015). MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. Theranostics 5, 1122-1143.

Chandra Gupta S. and Nandan Tripathi Y. (2017). Potential of long non-coding RNAs in cancer patients: From biomarkers to therapeutic targets. Int. J. Cancer 140, 1955-1967.

Chang Y., Yan W., Sun C., Liu Q., Wang J. and Wang M. (2017). miR-145-5p inhibits epithelial-mesenchymal transition via the JNK signaling pathway by targeting MAP3K1 in non-small cell lung cancer cells. Oncol. Lett. 14, 6923-6928.

Chang Y., Zhang J., Zhou C., Qiu G., Wang G., Wang S., Chang X., Li X. and Fan L. (2018). Long non-coding RNA FOXD2-AS1 plays an oncogenic role in hepatocellular carcinoma by targeting miR206. Oncol. Rep. 40, 3625-3634.

Chen G., Sun W., Hua X., Zeng W. and Yang L. (2018). Long non-coding RNA FOXD2-AS1 aggravates nasopharyngeal carcinoma carcinogenesis by modulating miR-363-5p/S100A1 pathway. Gene 645, 76-84.

Cui T.J., Lin G.S., Dai Y. M., Zheng J.P., Chen Z., Chen Q., Zheng Y. and Lin X. (2019). LncRNA HOXA-AS2 regulates microRNA-216a-5p to promote malignant progression of non-small cell lung cancer. Eur. Rev. Med. Pharmacol. Sci. 23, 264-273.

Dall'Acqua A., Sonego M., Pellizzari I., Pellarin I., Canzonieri V., D'Andrea S., Benevol S., Sorio R., Giorda G., Califano D., Bagnoli M., Militello L., Mezzanzanica D., Chiappetta G., Armenia J., Belletti B., Schiappacassi M. and Baldassarre G. (2017). CDK6 protects epithelial ovarian cancer from platinum-induced death via FOXO3 regulation. EMBO Mol. Med. 9, 1415-1433.

Guttman M., Amit I., Garber M., French C., Lin M.F., Feldser D., Huarte M., Zuk O., Carey B.W., Cassady J.P., Cabili M.N., Jaenisch R., Mikkelsen T.S., Jacks T., Hacohen N., Bernstein B.E., Kellis M., Regev A., Rinn J.L., and Lander E.S. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458, 223-227.

Van Hagen P., Hulshof M.C.C.M., Van Lanschot J.J.B., Steyerberg E.W., Van Berge Henegouwen M.I., Wijnhoven B.P.L., Richel D.J., Nieuwenhuijzen G.A.P., Hospers G.A.P., Bonenkamp J.J., Cuesta M.A., Blaisse R.J., Busch O.R., ten Kate F.J., Creemers G.J., Punt C.J., Plukker J.T., Verheul H.M., Spillenaar Bilgen E.J., van Dekken H., van der Sangen M.J., Rozema T., Biermann K., Beukema J.C., Piet A.H., van Rij C.M., Reinders J.G., Tilanus H.W., van der Gaast A. and CROSS Group. (2012). Preoperative chemoradiotherapy for esophageal or junctional cancer. N. Engl. J. Med. 366, 2074-2084.

Hang W., Feng Y., Sang Z., Yang Y., Zhu Y., Huang Q. and Xi X. (2019). Downregulation of miR-145-5p in cancer cells and their derived exosomes may contribute to the development of ovarian cancer by targeting CT. Int. J. Mol. Med. 43, 256-266.

Hu G., Niu F., Humburg B.A., Liao K., Bendi V.S., Callen S., Fox H.S. and Buch S.J.O. (2018). Molecular mechanisms of long noncoding RNAs and their role in disease pathogenesis. Oncotarget 9, 18648-18663.

Hu Q., Tai S. and Wang J. (2019). Oncogenicity of lncRNA FOXD2-AS1 and its molecular mechanisms in human cancers. Pathol. Res. Pract. 215, 843-848.

Huo Q., Ge C., Tian H., Sun J., Cui M., Li H., Zhao F., Chen T., Xie H., Cui Y., Yao M. and Li J. (2017). Dysfunction of IKZF1/MYC/MDIG axis contributes to liver cancer progression through regulating H3K9me3/p21 activity. Cell Death Dis. 8, e2766.

Ismail A., Bandla S., Reveiller M., Toia L., Zhou Z., Gooding W.E., Kalatskaya I., Stein L., D´Souza M. and Godfrey T.E. (2011). Early G1 cyclin-dependent kinases as prognostic markers and potential therapeutic targets in esophageal adenocarcinoma. Clin. Cancer Res. 17, 4513-4522.

Jiang S.B., He X.J., Xia Y.J., Hu W.J., Luo J.G., Zhang J. and Tao H. Q. (2016). MicroRNA-145-5p inhibits gastric cancer invasiveness through targeting N-cadherin and ZEB2 to suppress epithelial–mesenchymal transition. Oncotargets Ther. 9, 2305-2315.

Lan H., Lu H., Wang X. and Jin H. (2015). MicroRNAs as potential biomarkers in cancer: opportunities and challenges. Biomed Res. Int. 2015, 125094.

Li X., Luo Y., Liu L., Cui S., Chen W., Zeng A., Shi Y. and Luo L. (2020). The long noncoding RNA ZFAS1 promotes the progression of glioma by regulating the miR-150-5p/PLP2 axis. J. Cell Physiol. 235, 2937-2946.

Lin S. and Gregory R.I. (2015). MicroRNA biogenesis pathways in cancer. Nat. Rev. Cancer. 15, 321-333.

Liu X., Fu Q., Li S., Liang N., Li F., Li C., Sui C., Dionigi G. and Sun H. (2019). LncRNA FOXD2-AS1 functions as a competing endogenous RNA to regulate TERT expression by sponging miR-7-5p in thyroid cancer. Front Endocrinol. (Lausanne). 10, 207.

Lu Q., Shan S., Li Y., Zhu D., Jin W. and Ren T. (2018). Long noncoding RNA SNHG1 promotes non-small cell lung cancer progression by up-regulating MTDH via sponging miR-145-5p. FASEB J. 32, 3957-3967.

Luo Y., Hao T., Zhang J., Zhang M., Sun P. and Wu L. (2019). MicroRNA-592 suppresses the malignant phenotypes of thyroid cancer by regulating lncRNA NEAT1 and downregulating NOVA1.

Int. J. Mol. Med. 44, 1172-1182.
Malumbres M. (2014). Cyclin-dependent kinases. Genome Biol. 15, 122.
Malumbres M. and Barbacid M. (2005). Mammalian cyclin-dependent kinases. Trends Biochem. Sci. 30, 630-641.
Mei L.L., Wang W.J., Qiu Y.T., Xie X.F., Bai J. and Shi Z.Z. (2017). miR-145-5p suppresses tumor cell migration, invasion and epithelial to mesenchymal transition by regulating the Sp1/NF-kappaB signaling pathway in esophageal squamous cell carcinoma. Int. J. Mol. Sci. 18.
Ozen M., Karatas O.F., Gulluoglu S., Bayrak O.F., Sevli S., Guzel E., Ekici I.D., Caskurlu T., Solak M., Creighton C.J. and Ittmann M. (2015). Overexpression of miR-145-5p inhibits proliferation of prostate cancer cells and reduces SOX2 expression. Cancer Invest. 33, 251-258.
Parry K., Ruurda J.P., van der Sluis P.C. and van Hillegersberg R. (2017). Current status of laparoscopic transhiatal esophagectomy for esophageal cancer patients: a systematic review of the literature. Dis. Esophagus. 30, 1-7.
Ren W., Zhu Z. and Wu L. (2019). FOXD2-AS1 correlates with the malignant status and regulates cell proliferation, migration, and invasion in cutaneous melanoma. J. Cell Biochem. 120, 5417-5423.
Silva A., Bullock M. and Calin G. (2015). The clinical relevance of long non-coding RNAs in cancer. Cancers (Basel). 7, 2169-2182.
Solomon T., Mingfeng Y., Malika K., Bich Thuy L. and Shudong W. (2015). Targeting CDK6 in cancer: State of the art and new insights. Cell Cycle. 14, 3220-3230.
Wang B., Dong W. and Li X. (2019). miR-145-5p acts as a novel tumor suppressor in hepatocellular carcinoma through targeting RAB18. Technol. Cancer Res. Treat. 18, 1533033819850189.
Wang K.C. and Chang H.Y. (2011). Molecular mechanisms of long noncoding RNAs. Mol. Cell. 43, 904-914.
Wang S., Xue X., Wang R., Li X., Li Q., Wang Y., Xie P., Kang Y., Meng R. and Feng X. (2018). CircZNF609 promotes breast cancer cell growth, migration, and invasion by elevating p70S6K1 via sponging miR-145-5p. Cancer Manag. Res. 10, 3881-3890.
Wu X., Han Y., Liu F. and Ruan L. (2020). Downregulations of miR-449a and miR-145-5p act as prognostic biomarkers for endometrial cancer. J. Comput. Biol. 27, 834-844.
Yang Z., Sun Y., Liu R., Shi Y. and Ding S. (2019). Plasma long noncoding RNAs PANDAR, FOXD2-AS1, and SMARCC2 as potential novel diagnostic biomarkers for gastric cancer. Cancer Manag. Res. 11, 6175-6184.
Yu-Suo T., Xiao-Wei W., Xi-Lei Z., Zi-Hao L., Tong-Xin Y., Wei-Hong S., Hai-Wei X., Jin L., Qing-Quan W. and Xiu-Feng C.J.M.C. (2015). Identification of the long non-coding RNA POU3F3 in plasma as a novel biomarker for diagnosis of esophageal squamous cell carcinoma. Mol. Cancer 14, 1-13.

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