MiR-181c affects estrogen-dependent endometrial carcinoma cell growth by targeting PTEN

Lili Zhuang¹ *, Hongmei Qu² *, Jianxiang Cong¹, Huangguan Dai¹ and Xiaoyan Liu¹

¹ Department of Center for Reproductive Medicine, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, China
² Department of Obstetrics, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, China

Abstract. MicroRNAs (miRNAs), which is a type of non-coding and single-stranded small molecule RNA, bind either completely or incompletely to 3'-UTR of the target gene mRNA to inhibit mRNA translation or degradation. In our study, we aimed to explore the roles and mechanisms of miR-181c in the apoptosis of RL95-2 human endometrial carcinoma cells. Cell activity and apoptosis were detected by cell counting Kit-8 (CCK-8) assay and flow cytometry (FCM), respectively. Related mRNAs and proteins expression was determined by quantitative real-time reverse transcription PCR (qRT-PCR) and western blot assays, respectively. The binding capacity of PTEN-3'-UTR and miR-181c was assessed by luciferase reporter assay. The obtained results suggested that E2 evidently increased the cell activity of RL95-2 cells. In addition, miR-181c inhibitor suppressed the cell viability and enhanced the apoptosis capacity of E2-induced RL95-2 cells and distinctly reduced the miR-181c expression. We also found that miR-181c could bind to PTEN-3'-UTR and miR-181c inhibitor up-regulated the expression level of PTEN in E2-induced RL95-2 cells. Besides, overexpression of PTEN markedly promoted the apoptosis of E2-induced RL95-2 cells through regulating the Bax and Bcl-2 expression, and modulated the expression of AKT pathway, p53 and Cyclin D. In conclusion, our findings revealed that miR-181c affected the estrogen-dependent endometrial carcinoma cell growth by targeting PTEN. The potential effects of miR-181c on the apoptosis of E2-induced RL95-2 cells suggest that miR-181c could be an effective target for endometrial carcinoma therapies.

Key words: miR-181c, Estradiol, Endometrial carcinoma, PTEN, RL95-2

ENDOMETRIAL CARCINOMA, which is an epithelial malignancy that originates in the glands of endometrium, accounts for 20% to 30% of the female genital tract malignancies [1]. The cause of endometrial carcinoma is not yet clear, and researchers believe that changes in life and eating habits, obesity, infertility, hormone replacement therapy and diabetes are all factors contributing to endometrial carcinoma [2]. Surgical treatment is currently the main method for treating endometrial carcinoma [3]. Postoperatively, appropriate adjuvant therapy is determined according to the patient’s pathological results and clinical stages. Long-term effects of therapies using high estrogens without progesterone antagonism, which also involves long-term estrogen replacement therapy, polycystic ovary syndrome and functional ovarian tumors that secrete estrogen, may increase the risk of developing endometrial carcinoma [4]. However, to the best of our knowledge, the exact mechanism of estrogen-induced endometrial carcinoma is not yet clear.

Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is the earliest discovered tumor suppressor gene with phosphatase activity that can maintain homeostasis and normal substance metabolism [5]. A recent study has suggested that PTEN deficiency or low expression is closely related to tumor progression and poor prognosis [6]. PTEN was cloned by the three research groups at the same time in 1979, which has a high frequency of deletions and mutations in multiple advanced and metastatic tumors including bladder cancer [7], non-small cell lung cancer [8] and breast cancer [9]. In addition, some studies also suggested that PTEN was closely associated to endometrial carcinoma, for example, Sakurada reported that introduction of wild-type PTEN by application of adenovirus-mediated gene transfer technique could induce cell apoptosis [10]. Nevertheless, the possible mechanisms of PTEN in the growth of endometrial carcinoma were little studied.
MicroRNAs (miRNAs), which was a type of non-coding and single-stranded small molecule RNA, bind either completely or incompletely to the 3′-UTR of the target gene mRNA to inhibit mRNA translation or degradation [11]. According to statistics, the translation of more than 60% of protein-coding genes that affects cell proliferation, differentiation, apoptosis and development was regulated by miRNAs [12, 13]. miRNAs are abnormally expressed in many diseases, including cancers [14]. Moreover, miRNAs also can be used as oncogenes or tumor suppressor genes and it participated in the development of tumors [15]. Several studies reported that miR-181c played important roles in regulating different tumor progression [16, 17]. Gao reported that miR-181c-5p helped treat pancreatic cancer cell chemoresistance via inactivating the Hippo signaling [18]. Zhao suggested that upregulation of miR-181c could inhibit chemoresistance via targeting ST8SIA4 in chronic myelocytic leukemia [19]. As for Endometrial carcinoma, Devor suggested that Dysregulation of miR-181c expression affected recurrence of endometrial endometrioid adenocarcinoma by regulating Notch2 expression [20]. Xiong also reported that they were the first to discover dysregulation of hsa-miR-181c-3p in Endometrioid endometrial carcinoma by analyzing integrated microRNA and mRNA transcriptome sequencing [21]. miRNAs have been increasingly found to modulate PTEN, for example, research showed that miR-214 could be fully complementarily paired with PTEN 3′-UTR to suppress the translation of PTEN, therefore activating the AKT pathway [22]. Thus, the exploration of miRNAs in tumor progression and therapy still attracted much research attention. Moreover, the regulating effects of miR-181c/PTEN on endometrial carcinoma remain undefined.

In the present investigation, we will explore the effects and molecular mechanisms of miR-181c and PTEN on apoptosis of estradiol (E2)‐induced endometrial carcinoma cells RL95-2. The study provided theoretical and experimental evidence for miRNA regulation in endometrial carcinoma therapy and finds new therapeutic target to endometrial carcinoma.

**Materials and Methods**

**Cell culture and reagents**

The human endometrial carcinoma cell line (RL95-2) was offered by Cell Bank of Chinese Academy of Sciences. In our study, RL95-2 cells were incubated in the Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Carlsbad, CA, USA; 12100046) supplemented with 10% fetal bovine serum (FBS, Gibco; 10099-141) in a 5% CO₂ atmosphere at 37°C. E2 was purchased from Hangzhou Hairui Chemical Co., Ltd. (purity of E2 ≥ 98%, Hangzhou, Zhejiang, China; HR131794). miR-181c inhibitor NC and miR-181c inhibitor (Cat. no. 4464084) were obtained from Invitrogen (Thermo Fisher Scientific, Shanghai, China). The PTEN mRNA was cloned into the pcDNA3.1(+) empty vector (Invitrogen, Shanghai, China) as the overexpression PTEN plasmids.

**Grouping**

For the first section of our experiments, 6 treatment groups were prepared as follows: none treatment group (Control), RL95-2 cells transfected with inhibitor, empty vector group (Inhibitor NC), RL95-2 cells treated with miR-181c inhibitor group (Inhibitor), RL95-2 cells treated with E2 group (E2), RL95-2 cells treated with E2, and then transfected with inhibitor empty vector group (E2 + Inhibitor NC) and RL95-2 cells treated with E2, and then treated with miR-181c inhibitor group (E2 + Inhibitor). For the second section of our experiments, 6 treatment groups were established as follows: none treatment group (Control), RL95-2 cells transfected with empty vector group (NC), RL95-2 cells transfected with overexpression PTEN plasmids group (PTEN), RL95-2 cells treated with E2 group (E2), RL95-2 cells treated with E2 and then transfected with empty vector group (E2 + NC) as well as RL95-2 cells treated with E2 and then transfected with overexpression PTEN plasmids group (E2 + PTEN).

**Cell viability analysis**

The cell viability of cultured RL95-2 cells was detected by cell counting Kit-8 (CCK-8; Beyotime, Shanghai, China; C0037). About 5 × 10^3 cells/mL cultured RL95-2 cells in the logarithmic phase were sowed into the 96-well plates (Beyotime, Shanghai, China; FPT011). Afterwards, the cells were maintained in a 5% CO₂ atmosphere at 37°C for 12 h and then respectively treated with different concentrations of E2 (100 μL of 10^{-10}, 10^{-9} and 10^{-8} mol/L E2) or with the treatment groups as described above. The control group was added with 100 μL complete medium as a comparison. The cells were then incubated for 24 h. 10 μL of CCK reagent was supplemented into the wells of 96-well plates. After that, the cells were incubated for 3 h. A microplate reader (BIORAD, CA, USA) was used to record the absorbance at 450 nm.

**Luciferase reporter assay**

Cultured RL95-2 cells were trypsinized by 0.25% Trypsin (Beyotime, Shanghai, China; C0205). After centrifugation, the cells were re-suspended in the DMEM supplemented with 10% FBS. Next, the cultured RL95-2 cells were sowed into the 48-well plates at a final density
of $4 \times 10^4$ cells/well. After 24 hours of incubation, the corresponding psiCHECK-2 vector (Invitrogen, Shanghai, China) of miR-181c, reporter vector of PTEN 3′-UTRs and pRL-TK were co-transfected into the cultured RL95-2 cells. After 48 hours of transfection, the luciferase activity was detected by the Dual-Luciferase Reporter Assay System based on the specification (Promega). The determination of luciferase activity was carried out using a multi-functional microplate reader (BIO-RAD, CA, USA).

**Apoptosis analysis**

The apoptosis ability of RL95-2 cells was assessed by the flow cytometry (FCM). Cultured RL95-2 cells were trypsinized by 0.25% Trypsin (Beyotime, Shanghai, China; C0205) after being washed by PBS. The supernatant was discarded and the incubation buffer was used to suspend the RL95-2 cells for evaluation at a final density of $1 \times 10^6$ cells/mL. Annexin V-FITC and propidium iodide (PI) (Beyotime, Shanghai, China; C1063, ST512) were then maintained with RL95-2 cells for 15 min in the dark at room temperature. FACSCalibur (BD Biosciences, San Diego, CA, USA) was performed to assess the cell apoptosis.

**Western blot analysis**

Cultured RL95-2 cells were seeded into 6-well plate to reach a final cell concentration at $1 \times 10^5$ cells/mL, and then the cells were incubated at constant temperature for 24 h. The medium was discarded, and the cells were washed three times by PBS. 1 mL PBS was added to the cells. After that, the cells were transferred to a 1.5 mL enzyme-free EP tube. After being centrifuged for 5 min at 4°C, the supernatant was discarded. 1 μL of the lysate and PMSF mixture (previously configured in the ratio of PMSF: lysate = 1:100) were added to the EP tube. After being blown, the EP tube was placed on ice and shaken every 5 min for 6 times. Next, the concentration of protein was detected. Having centrifuged for 20 min at 4°C, 1 × Buffer solution was added to the EP tube, which was stored in a freezer at –80°C. Protein concentration was measured using Bradford Protein Assay kit (Bio-Rad Laboratories, Inc.). The proteins lysates were then segregated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Beyotime, Shanghai, China; FFP28). The blotting was performed on specific antibodies (anti-PTEN, Dilution, 1:10,000; Abcam, ab32199; rabbit anti-human; anti-Bax, Dilution, 1:500; Abcam, ab53154, rabbit anti-human; anti-Bcl-2, Dilution, 1:1,000; Abcam, ab32124, rabbit anti-human; anti-p-AKT, Dilution, 1:500; Abcam, ab8933, rabbit anti-human; anti-AKT, Dilution, 1:500; Abcam, ab8805, rabbit anti-human; anti-p53, Dilution, 1:1,000, Abcam, ab32049, rabbit anti-human; anti-Cyclin D, Dilution, 1:10,000, Abcam, ab134175, rabbit anti-human; anti-β-actin, Dilution, 1:2,500, Abcam, ab8227; rabbit anti-human). Horseradish peroxidase-conjugated secondary antibodies (Dilution, 1:5,000; Abcam, ab205718, goat anti-rabbit) were supplemented and incubated at room temperature for 1 h. Enhanced chemiluminescent reagents (Millipore, Billerica, MA, USA) using an ECL system (Amersham Pharmacia, Piscataway, NJ, USA) were performed on the evaluation of results.

**Quantitative real-time reverse transcription PCR (qRT-PCR) analysis**

The cultured RL95-2 cells were fully lysed with 1 mL of Trizol (Beyotime, Shanghai, China; R0016) and allowed to maintain at room temperature for 5 min. 200 μL chloroform (Aladdin, Shanghai, China; C112210) was added to each lysate. After being vigorously shaken, the lysates were placed at room temperature for 3 min. The supernatant was aspirated to another RNase-free centrifuge tube after the centrifugation for 15 min at 4°C. 500 μL isopropanol (Aladdin; I112015) was added to the supernatant obtained from each lysate. The liquid was mixed gently by inverting the tube and placed for 10 min at room temperature. After being centrifuged for 10 min at 4°C, the supernatant was discarded. 1 mL of 75% ethanol (Aladdin; A171299) was added, and then the supernatant was discarded by being centrifuged for 10 min at 4°C. After all supernatant has been aspirated, the RNA sediment was dried in a clean bench for 5 min. 20 μL DEPC water was added to the sediment, and then the RNA was suspended in DEPC water and incubated at 50–60°C for 10 min. After the RNA sediment has been completely dissolved, the RNA was quantified. RNA was reverse transcribed to cDNA by Reverse Transcription Kit (Beyotime; D7168L) according to the direction. qRT-PCR analysis was carried out on ABI 7500 Thermocycler (Applied Biosystems, Foster City, CA, USA). PCR cycles were as following: 2 min pretreatment at 94°C, 94°C for 30 s, 63°C for 30 s, 72°C for 1 min (35 cycles), a final extension at 72°C for 7 min and held at 4°C. The primers were designed by Invitrogen (Shanghai, China) as following: miR-181c, forward: 5′-CCGAAATTTTGGCAGAGCTTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); PTEN, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-CGCCCTCTGACTGGGAATAGT-3′ (product: 238 bp); Bax, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); PTEN, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-CGCCCTCTGACTGGGAATAGT-3′ (product: 238 bp); Bax, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); PTEN, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); Bax, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); PTEN, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp); Bax, forward: 5′-ACCCACCACAGCTAGAACTT-3′ and reverse: 5′-GGGAAACCATCGACCCTTGTA-3′ (product: 162 bp).
CACTTGACCATCT-3' and reverse: 5'-TAACATCGC CGCAGACAAAC-3' (product: 205 bp). β-actin was used as the control of the input RNA level.

**Statistical analysis**

The results in our research were shown as mean ± SE of at least three independent experiments. The experimental data from CCK-8, qRT-PCR and western blot assays were analyzed by Kruskal-Wallis and Tukey’s test using IBM SPSS statistical software (version 19). The statistical significance was defined as \( p < 0.05 \).

**Results**

**E2 evidently accelerated the cell viability of RL95-2 cells, and enhanced the expression levels of miR-181c**

In the current investigation, we measured the cell viability of RL95-2 cells treated with different concentrations of E2 for the purpose of testing the effect of E2. The results revealed that the cell viability of RL95-2 cells treated with 10\(^{-8}\) and 10\(^{-6}\) mol/L E2 were distinctly higher than that in control group (Fig. 1A; \( p < 0.05 \)). Thus, we confirmed that E2 had the function of leading to a distinct high cell viability of RL95-2 cells. Furthermore, we also determined in the following investigation that the most effective concentration of E2 to RL95-2 cells was 10\(^{-8}\) mol/L. The expression levels of miR-181c in RL95-2 cells treated with E2 were also detected. qRT-PCR data indicated that the expression levels of miR-181c in RL95-2 cells treated with 10\(^{-8}\) and 10\(^{-6}\) mol/L E2 were obviously higher than control (Fig. 1B; \( p < 0.01 \)). Hence, we affirmed that E2 could up-regulate the miR-181c expression in RL95-2 cells. As Fig. 1 shown, 10\(^{-8}\) mol/L concentration of E2 already significantly induced cell viability and remarkably increased miR181c expression, and this concentration had successfully established an E2-induction endometrial carcinoma proliferation model. Such a concentration has been used in a previous study, in which it could induce endometrial cancer cell proliferation [23]. Therefore, 10\(^{-8}\) mol/L of E2 was selected to be used in the following experiments.

**miR-181c inhibitor suppressed the cell activity of E2-induced RL95-2 cells, and distinctly reduced the miR-181c expression**

Due to the regulating ability of E2 on miR-181c expression in RL95-2 cells, we further speculated whether miR-181c and its inhibitor also could affect the cell activity of RL95-2 cells induced by E2. Thus, the cell viabilities of RL95-2 cells treated with inhibitor NC, miR-181c inhibitor, E2, E2 + inhibitor NC and E2 + miR-181c inhibitor were measured. On the basis of the CCK-8 data, we noted that the cell viability of RL95-2 cells treated with E2 was evidently higher than control. However, the cell viability of RL95-2 cells in E2 + miR-181c inhibitor group was distinctly reduced (Fig. 2A; \( p < 0.05 \)). Therefore, it was proved that miR-181c inhibitor could suppress the cell activity of E2-induced RL95-2 cells. Moreover, the expression levels of miR-181c in RL95-2 cells from each treatment group were also evaluated. qRT-PCR results showed that the expression level of miR-181c in E2-induced RL95-2 cells could be markedly reduced by miR-181c inhibitor (Fig. 2B; \( p < 0.01 \)). Hence, we could draw a conclusion that miR-181c inhibitor suppressed the cell viability of E2-induced RL95-2 cells and distinctly reduced the miR-181c expression.

**miR-181c inhibitor significantly enhanced the apoptosis capacity of E2-induced RL95-2 cells**

As miR-181c inhibitor could obviously inhibit the cell viability...
viability of E2-induced RL95-2 cells, we therefore studied whether miR-181c inhibitor also affected the cell apoptosis capacity of E2-induced RL95-2 cells. Hence, the cell apoptosis capacities of RL95-2 cells treated with all of the treatment groups were assessed by FCM. As FCM data shown, the proportion of apoptosis cell number of RL95-2 cells treated with miR-181c inhibitor was obviously higher than that in control group. In addition, it was also showed that miR-181c inhibitor markedly promoted the apoptosis of RL95-2 cells induced by E2 (Fig. 2C–D; \( p < 0.05 \)). These outcomes indicated that miR-181c inhibitor could enhance the apoptosis ability of E2-induced RL95-2 cells, to some extent.

**miR-181c specifically bound to PTEN-3’-UTR and miR-181c inhibitor up-regulated the expression level of PTEN in E2-induced RL95-2 cells**

Targetscan revealed that the binding site for miR-181c in the 3’-UTR of PTEN is UUGAAUGU (Fig. 3A). Luciferase reporter assay was carried out in our investigation in order to confirm the binding of miR-181c in the PTEN-3’-UTR. Based on the data, we found that miR-181c markedly inhibited the expression of the Renilla luciferase reporter, which carries the wild-type putative target sites of PTEN, compared with control group (Fig. 3B; \( p < 0.01 \)). The obtained results suggested that PTEN contained an effective miR-181c binding site in its 3’-UTRs. Hence, western blot and qRT-PCR assays
were performed in the current study to confirm whether the treatment of miR-181c inhibitor into RL95-2 cells affected the expression level of PTEN. The results showed that PTEN expression was significantly increased by miR-181c inhibitor in E2-induced RL95-2 cells (Fig. 4A–B; \( p < 0.01 \)). Similarly, the qRT-PCR data also indicated that the PTEN expression in E2-induced RL95-2 cells was significantly enhanced under the treatment with miR-181c inhibitor (Fig. 4C; \( p < 0.01 \)). Therefore, these results suggested that PTEN may be a functional target of miR-181c in RL95-2 cells.

Overexpression of PTEN markedly promoted the apoptosis of E2-induced RL95-2 cells through regulating the Bax and Bcl-2 expression

We proved in our above investigation that PTEN might be a functional target of miR-181c in RL95-2 cells, and therefore tested the apoptosis ability of E2-induced RL95-2 cells treated with empty vector, overexpression PTEN plasmids, E2, E2 + empty vector and E2 + overexpression PTEN plasmids. FCM data showed that overexpression of PTEN could markedly enhance the apoptosis of RL95-2 cells (Fig. 5A–B; \( p < 0.01 \)). The
result was similar to a study, in which introduction of wild-type PTEN by applying adenovirus-mediated gene transfer technique could induce cell apoptosis [10]. Moreover, overexpression of PTEN also could increase cell apoptosis in E2-induced RL95-2 cells, to some extent (Fig. 5A–B; p < 0.01). Hence, the expression levels of apoptosis-associated proteins, which involve Bax and Bcl-2 in RL95-2 cells from each group, were also determined by western blot and qRT-PCR assays. Western blot data showed that the protein expression level of Bax was significantly enhanced, while Bcl-2 was reduced in E2-induced RL95-2 cells by overexpression of PTEN (Fig. 5C–D; p < 0.01). It was also revealed that overexpression of PTEN markedly up-regulated the Bax expression, however, it down-regulated the Bcl-2 expression in E2-induced RL95-2 cells (Fig. 5E; p < 0.01).

**Overexpression of PTEN modulating the expression of AKT pathway, p53 and Cyclin D in E2-induced RL95-2 cells**

To further investigate the associated mechanisms through which PTEN affected the apoptosis capacity of E2-induced RL95-2 cells, we therefore determined the expression levels of phosphorylated AKT, AKT, p53 and
Cyclin D in RL95-2 cells from each group. According to the western blot data, we found that the expression levels of p-AKT ($p < 0.05$) and Cyclin D ($p < 0.01$) in E2-induced RL95-2 cells were distinctly reduced under transfection with overexpression PTEN plasmids (Fig. 6A–B). However, compared with other groups, a sharp increase in p53 expression in E2-induced RL95-2 cells transfected with overexpression PTEN plasmids was observed in our research ($p < 0.05$). These findings suggested that overexpression of PTEN might affect the apoptosis of E2-induced RL95-2 cells via modulating the expression levels of AKT pathway, p53 and Cyclin D.

**Discussion**

Estrogens are the most important steroid hormone produced by the ovaries and placenta, and the adrenal cortex also produces a small amount of estrogens. Estrogens regulate many functions, for example, female reproductive system, cardiovascular system, nervous system, bone excretory system and immune response, in human organism [24]. Estrogens play a major role in physiological changes or pathological changes, including endometrial carcinoma, breast cancer, ovarian cancer and other tumors, in women [9, 25, 26]. Estradiol (E2) is the main component of estrogen. Yaquchi suggested that E2 could increase the growth of endometrial carcinoma Ishikawa cells [27]. In the current study, we assessed the cell viability of endometrial carcinoma cell RL95-2 treated with different concentrations of E2, and the results revealed that E2 obviously enhanced the cell viability of RL95-2 cells in a dose-dependent manner. Moreover, we thereby determined in the following investigation that the most effective concentration of E2 to RL95-2 cells was $10^{-8}$ mol/L.

Previous investigations have suggested that miR-181c attenuated the proliferation, invasion or self-renewal abilities in breast cancer, colorectal cancer and glioblastoma [28-30]. However, little is known about the roles and mechanisms of miR-181c in the growth of endometrial carcinoma cells. Our results showed that E2 distinctly up-regulated the miR-181c expression in RL95-2 cells. In addition, we also found that miR-181c inhibitor reduced the miR-181c expression, suppressed the cell viability and enhanced the apoptosis ability in E2-induced RL95-2 cells. These results indicated that miR-181c might play an important role in the E2-dependent growth of RL95-2 cells. The results were similar to other miRNAs in regulating the progression of endometrial cancer. Wang reported that miR-15a-5p could suppress endometrial cancer cell growth by inhibiting WNT3A via Wnt/β-catenin signaling [31]. By conducting *in vitro* and *in vivo* experiments, Su reported that miR-142 also could suppress endometrial cancer proliferation by targeting Cyclin D1 [32].

PTEN is often considered as a tumor suppressor in various cancers, including breast cancer, prostate cancer and gastric cancer [33, 34]. Studies reported that PTEN was closely related to the progression of endometrial cancer, for example, Bian suggested that PTEN deficiency could sensitize endometrioid endometrial cancer [35]. Zhu revealed the importance of the miR-494-3p/PTEN/Pi3K/AKT axis in the progression of endometrial cancer [36]. In our study, we found that overexpression of PTEN could induce cell apoptosis in RL95-2 cells and E2-induced RL95-2 cells. In addition, the 3’-UTR of PTEN also has been demonstrated to bind to several miRNAs, such as miR-21, miR-216 and miR-217 [37-39]. To the best of our knowledge, there are various
targets of miR-181c in modulating biological functions of different tumors or diseases, for instance, ATXN3 in Lymphocytes from SCA3 patients, Notch2 in Pediatric High-Grade Gliomas and Bcl211 in Parkinson [40–42]. Our results confirmed that miR-181c inhibitor significantly up-regulated the expression level of PTEN in E2-induced RL95-2 cells, indicating that miR-181c could target PTEN in E2-induced RL95-2 cells. Apart from PTEN, Devor also found that increased NOTCH2 via loss of miR-181c is a significant component contributing to endometrioid adenocarcinoma recurrence [20].

To further explore the exact function of PTEN targeted by miR-181c in E2-dependent growth of RL95-2 cells, we determined the apoptosis capacity of E2-induced RL95-2 cells transfected with overexpression PTEN plasmids. The FCM data proved that overexpression of PTEN markedly promoted the apoptosis of E2-induced RL95-2 cells. Furthermore, the related apoptosis mechanisms were also investigated. The expression levels of Bax and Bcl-2 in E2-induced RL95-2 cells were measured, and the results indicated that overexpression of PTEN significantly enhanced the Bax expression, while reduced the Bcl-2 expression in E2-induced RL95-2 cells. These outcomes proved that overexpression of PTEN accelerated the apoptosis of E2-induced RL95-2 cells by modulating the expression levels of Bax and Bcl-2. Moreover, studies have suggested previously that AKT pathway played a pivotal role in the apoptosis mechanisms of tumor cells [43–45]. Hence, we also determined the expression levels of phosphorylated AKT, AKT, p53 and Cyclin D in RL95-2 cells. The western blot data showed that overexpression of PTEN could markedly reduce the phosphorylation of AKT in E2-induced RL95-2 cells. Besides, we also proved that overexpression of PTEN significantly up-regulated the p53 expression, while down-regulated the expression level of Cyclin D in E2-induced RL95-2 cells. These results suggested that overexpression of PTEN affected the AKT pathway and the expression levels of p53 and Cyclin D in E2-induced RL95-2 cells.

Taken together, miR-181c affected the growth of E2-induced RL95-2 cells by targeting PTEN and regulating the expression levels of AKT, p53 and Cyclin D.

Conclusion

In conclusion, our findings revealed that miR-181c affected the growth of E2-induced RL95-2 cells by targeting PTEN. The potential effects of miR-181c and PTEN on estrogen-dependent endometrial carcinoma cell growth suggested that miR-181c might be an effective target for endometrial carcinoma therapies.

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Disclosure of Conflict-of-Interest

The authors declare no conflicts of interest.

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