

Original Article

miR-145 targets the SOX11 3'UTR to suppress endometrial cancer growth

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Abstract: To explore the functions of SOX (Sex determining Region Y-related HMG-box) family genes in endometrial cancer (EC) and determine the influence of miR-145/SOX11 on EC cell functions. The relationship between miR-145 and SOX11 was confirmed using TargetScan, miRNA databases and dual-luciferase reporter gene assays. The expression of SOX11 mRNA in tissue specimens was examined using RT-qPCR, while SOX11 protein expression in tissues and cell lines were detected through immunohistochemistry (IHC) and western blotting. After transfection using Lipofectamine 2000, the proliferation, migration, invasion and apoptosis of ECC-1 and HEC-1-A cells were assessed through colony formation, transwell and flow cytometry assays. The correlation of SOX11 expression with the prognosis outcomes of patients was analyzed using Kaplan-Meier analysis and the log-rank test. SOX11 showed high expression in EC, which is negatively correlated with a poor prognostic outcome of EC patients. The expression of miR-145 was lower in EC tissues than in adjacent tissues. MiR-145 significantly reduced the expression of SOX11. In ECC-1 cells, miR-145 suppressed the propagation, migration, and invasion of cells and promoted cell apoptosis. MiR-145 also inhibited the proliferation, migration, and invasion of HEC-1-A cells and facilitated cell apoptosis by inhibiting SOX11. MiR-145 targeted site 3 (3615) of the SOX11 3'UTR to affect the expression of SOX11. MiR-145 and its target gene SOX11 could serve as diagnostic markers for EC. MiR-145 targets the SOX11 3'UTR to inhibit its expression and suppress the propagation and metastasis of EC cells.

Keywords: SOX11, miR-145, endometrial cancer

Introduction

Endometrial cancer (EC) is the leading cause of one of the most common gynecological malignancies, with more than 280,000 cases diagnosed each year worldwide. The incidence of EC has increased by 21% since 2008, and the prevalence of this disease has reached more than 2.7% among women [1]. Reflecting the rapid growth of invasive cells, EC rapidly develops [2]. According to previous studies, radical surgery might be the only effective cure for EC in the precursor stage of lesions, suggesting the significance of early diagnosis for EC [3]. The determination of a potential mechanism that regulates the initiation and progression of EC is therefore valuable in early diagnosis.

The SOX (SRY-related HMG-box) gene family, containing nearly 20 SOX genes, is a conserved

group of transcription factors [4]. Most of these factors have critical functions in the determination of cell fate and cell differentiation [5]. SOX11 is an intronless gene encoding a transcription factor of the SOX family [6] which plays a role in tumorigenesis [7]. The aberrant regulation of SOX11 has been observed in diverse tumor cell lines. Studies have demonstrated that abnormal SOX11 expression is correlated with diverse cancers, such as EC, malignant glioma, medulloblastoma, and ovarian cancer [8]. Additionally, SOX11 is upregulated in EC cells, which is also a prognostic marker for recurrence-free survival of patients with EC [9]. However, the current understanding of the mechanism between SOX11 and EC is far from adequate.

MicroRNAs (miRNAs) are small non-coding RNAs involved in the regulation of post-transcriptional gene expression.

Table 1. Primers used for RT-qPCR

Primers	Sequence
SOX11 (F)	5'-GGTGGATAAGGATTGGATTTCG-3'
SOX11 (R)	5'-GCTCCGGCGTGCACTAGT-3'
GAPDH (F)	5'-AGTAGAGGCAGGGATGATG-3'
GAPDH (R)	5'-TGGTATCGTGAAGGACTC-3'
hsa-miR-145 (F)	5'-GTCCAGTTTTCCAGGAATCCCT-3'
hsa-miR-145 (R)	5'-GCTGTCAACATACGCTACGTAACG-3'
U6 (F)	5'-GGTCGGGCAGGAAAGAGGGC-3'
U6 (R)	5'-GCTAATCTTCTCTGTATCGTTCC-3'

F: forward primer; R: reverse primer.

scriptional genes [10]. Previous studies verified that after binding to the 3'-untranslated regions (3'UTRs) of mRNAs, some target genes can be regulated by miRNAs [11]. MiRNAs play an important role in tumor angiogenesis, cell activities and other biological processes [12]. Moreover, studies have shown that miRNAs serve as oncogenes or tumor suppressors in different tumor environments [13]. This information emphasizes the critical effects of miRNAs in tumor progression and provides a new insight into the molecular mechanisms underlying carcinogenesis. MiR-145, transcribed from the miRNA cluster at chromosome 5q32, has been demonstrated as a tumor suppressor of many human cancers, such as EC, prostate cancer, bladder cancer, colon cancer, and ovarian cancer [14]. Studies have reported that this molecule inhibits the expression of *OCT4*, thereby suppressing the growth of EC cells [15]. Although evidence has highlighted the importance of miR-145 as a tumor suppressor in EC, the precise molecular mechanisms remain largely unknown.

In the present study, we hypothesized that there was a correlation between SOX family genes and the miRNA of EC cells. The results demonstrated that *SOX11* is a target gene of miR-145. EC progression could be inhibited by promoting miR-145 or downregulating the expression of *SOX11*. This discovery will improve the development of treatment for EC patients.

Materials and methods

Clinical samples and cell culture

A total of 23 pairs of EC and adjacent tissue specimens were collected from patients undergoing surgery at the First Affiliated Hospital of

Zhengzhou University. All tissue samples were frozen in a guanidinium thiocyanate solution and stored at -80°C. The research was ratified through the First Affiliated Hospital of Zhengzhou University, and written informed consent was obtained from all participants prior to the study. Human EC cell lines (ECC-1, HEC-1-A and RL95-2) and the human embryonic kidney cell line HEK293 were obtained from BeNa Culture Collection (BNCC; China). The cells were cultured in RPMI-1640 medium (Gibco, NY, USA) supplemented with 10% FBS (Gibco, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Gibco).

RT-qPCR

Total RNA was isolated from EC cells using a total RNA purification kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity, quality and concentration of RNA were assessed using a NanoDrop 2000 (ThermoFisher Scientific, Wilmington, DE, USA). The TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and the TaqMan high-capacity cDNA Kit (Applied Biosystems) were applied to reverse transcribe miRNA and mRNA, respectively. *GAPDH* was used as a reference gene for *SOX11*, and U6 was used as an internal control for miR-145. The RT-qPCR was performed using the StepOnePlus real-time PCR system (Life Technologies). The PCR thermal cycling conditions were 94°C for 3 min, followed by 45 cycles at 94°C for 5 s and 60°C for 30 s. The primers used in RT-qPCR were designed and generated by Sangon Biotech (Shanghai, China). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are indicated in **Table 1**.

Immunohistochemistry (IHC) assay

The sections were deparaffinized in xylene and washed in phosphate-buffered saline (PBS) for 5 min, followed by incubation in 10% bovine serum albumin (BSA) at 37°C for 20 min. Afterwards, the protein block solution was discarded and the tissue specimens were incubated with the anti-*SOX11* antibody (ab154138, 1:500, Abcam, Cambridge, MA, USA) and anti-*GAPDH* antibody (ab9484, 5 µg/ml, Abcam) overnight at 4°C. After washing twice with PBS for 10 min, the tissues were incubated with an HRP-conjugated goat anti-mouse IgG H&L anti-

Table 2. SOX11 mutant 3'UTR construction primer sequences

Mutation site	Sequences
Site1 (F)	5'-GATGGAAGGTTTGTCTCGATTTTAG-3'
Site1 (R)	5'-CTAAAAATCGAGAACAAACCTTCCATC-3'
Site2 (F)	5'-CGCATCGCCAATAGTCGGTCTGTGTCGAG-3'
Site2 (R)	5'-CTGCGACAACAGGACCGACTATTGGCGATGCG-3'
Site3 (F)	5'-CAGGTTCTCAATATTTCTGGAGTTG-3'
Site3 (R)	5'-CAACTCCAGAAAATATTGAGAACCTG-3'
Site4 (F)	5'-GTATAATACACATGTAGTCTTCAATAAAAG-3'
Site4 (R)	5'-CTTTTATTGAAGACTACATGTGATTATAC-3'
Site5 (F)	5'-CGTTACATTTCCCTTCCAGTAATTATC-3'
Site5 (R)	5'-GATAAATTACTGGAAGGGGAAATGTAACG-3'

F: forward primer; R: reverse primer.

body (1:2000) for 30 min. All sections were counterstained with hematoxylin (Richard Allan Scientific, Kalamazoo, MI, USA). The color reaction was performed using the peroxidase substrate diaminobenzidine (Vector Laboratories, Burlingame, CA, USA) and observed under a microscope (Bio-Rad, Hercules, CA, USA).

Western blot analysis

Total protein was extracted from the cells using ice-cold RIPA lysis buffer (Solarbio Inc., China), and the protein concentration was quantified using a bicinchoninic acid (BCA) assay kit (Solarbio). The proteins were separated using 10% SDS-PAGE (Bio-Rad) and transferred onto a polyvinylidene difluoride (PVDF) membrane, which was then blocked for 2 h in 5% nonfat milk in PBS-Tween-20 (PBST). The membranes were incubated with primary antibodies against SOX11 (ab154138, 1:1000, Abcam) and GAPDH (ab9484, 1 µg/ml, Abcam) at 4°C overnight and then washed three times with Tris-buffered saline-Tween 20 (TBST). Next, the membranes were incubated in HRP-conjugated goat anti-mouse IgG secondary antibody (1:2000) for 1 h, followed by washing twice with TBST. An enhanced chemiluminescence (ECL) western blot detection system (Millipore, Bedford, MA, USA) was applied for signal detection. The optical density (OD) of the protein bands was analyzed using ImageJ software.

Cell transfection and grouping

ECC-1 and HEC-1-A cells were seeded onto 6-well plates (1×10^5 cells/well) and cultured in RPMI-1640 medium (10% FBS) until 70% con-

fluence. MiR-145 mimics, SOX11 siRNA and SOX11 cDNA were synthesized by GenePharma Inc. (Shanghai, China). ECC-1 cells were transfected with SOX11 siRNA and miR-145 mimics, while HEC-1-A cells were transfected with SOX11 cDNA, SOX11 siRNA and miR-145 mimics using Lipofectamine 2000 (Invitrogen). The ECC-1 cells were assigned to three groups: a control group (transfected with blank plasmids), an si-SOX11 group (transfected with SOX11 siRNA) and a miR-145 group (transfected with miR-145 mimics). The experiment for HEC-1-A cells was performed using the following four groups: a control group (non-transfection), a cDNA-SOX11 group (transfected with SOX11 cDNA), an si-SOX11 group (transfected with SOX11 siRNA) and a cDNA-SOX11+miR-145 group (co-transfected with SOX11 cDNA and miR-145 mimics). The transfection efficiency of each group in two types of EC cells was detected at 24 h after transfection.

Cell proliferation assay

For cell proliferation analysis, a colony formation assay was conducted to detect EC cell proliferation. Cells at the logarithmic growth phase were digested with 0.25% trypsin, dissociated and suspended in RPMI-1640 supplemented with 10% FBS. The cells in each group were inoculated onto dishes at 50, 100 and 200 cells/dish containing 10 ml of prewarmed (37°C) culture medium. The cells were maintained in an incubator (5% CO₂) for 24 h after transfection. The cell culture was not terminated until colonies were visible. After discarding the supernatant, the cells were washed twice in PBS and fixed with 4% paraformaldehyde solution for 20 min and stained using 0.1% crystal violet for 15 min. The colonies were detected using an inverted microscope ($\times 200$).

Transwell assay

To examine cell migration, the cells were plated onto a 24-well transwell chamber (Corning, NY, USA). Matrigel (Collaborative Research, Bedford, Massachusetts, USA) was first prepared by dilution with serum-free medium (100 µL) and then added to the upper chamber. After trypsinization, the cells were diluted in serum-free medium at 24 h post transfection and sub-

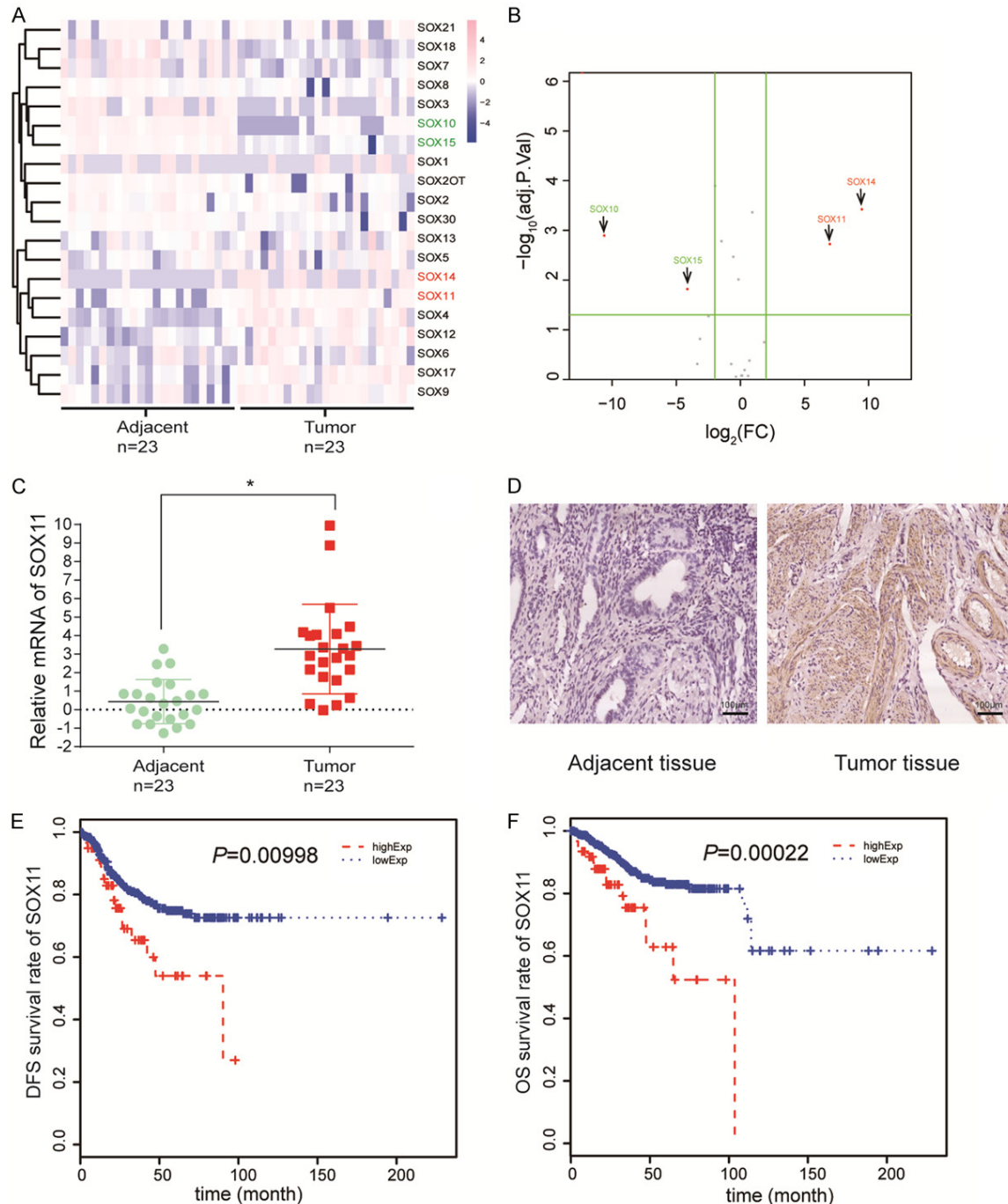


Figure 1. SOX11 was highly expressed in EC tissues. A, B. The heat map shows the significantly differentially expressed genes in 23 pairs of EC and adjacent tissues. SOX11 and SOX14 were the most significantly overexpressed genes, whereas SOX10 AND SOX15 were the most significantly underexpressed genes in the 23 pairs of EC samples analyzed by heat map and volcano plot. C, D. The expression of SOX11 in tumor tissues was higher than that in adjacent tissues analyzed by RT-qPCR and immunohistochemistry (IHC). * $P < 0.05$, compared with adjacent tissues. E, F. EC patients with high SOX11 expression had lower disease-free survival (DFS) and overall survival (OS) rates.

sequently inoculated onto the upper chamber. A total of 600 μ l of DMEM (10% FBS) was added to the lower chamber. Non-migratory cells were removed after incubation overnight using a cot-

ton swab, while the migratory cells in the lower chamber were fixed and stained with methyl alcohol and 0.1% crystal violet. The method for the cell invasion assay was similar to that used

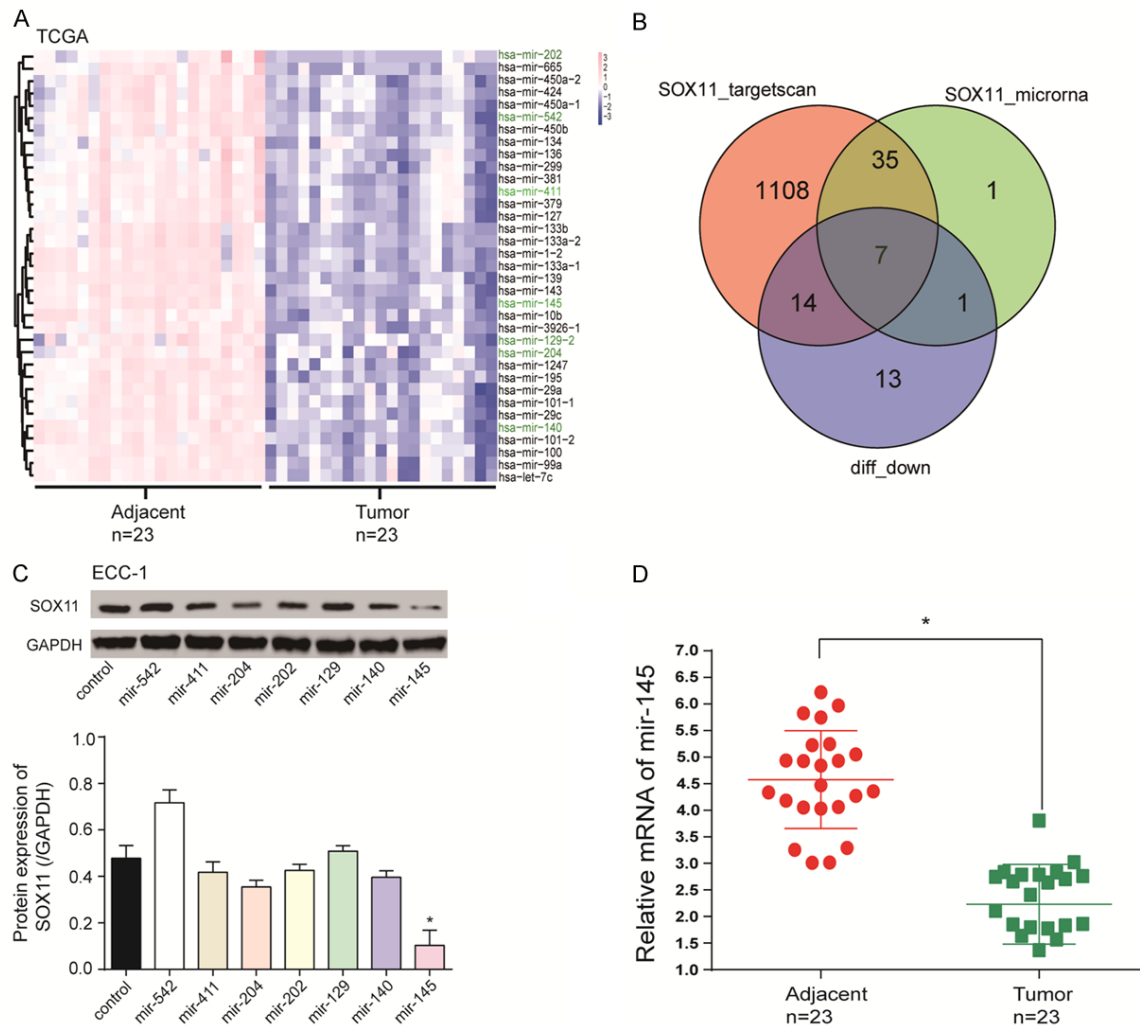


Figure 2. MiR-145 had the most significant effect on the expression of SOX11 protein. A. The heat map demonstrated significantly differentially expressed miRNAs in 23 pairs of EC and adjacent tissues. B. The Venn diagram illustrates the intersection of the TargetScan database, the microRNA database and the microarray results. There were 7 underexpressed mRNAs that could target SOX11 in EC. C. The expression of SOX11 was lowest in ECC-1 cells treated with miR-145. D. MiR-145 showed lower expression in EC tumor tissues compared with adjacent tissues according to RT-qPCR. * $P < 0.05$, compared with control group.

for the cell migration assay, except Matrigel was added to the chambers. Finally, the number of invasion cells were observed and counted under an optical microscope ($\times 200$) (Nikon, Tokyo, Japan).

Apoptosis assay

Cell apoptosis was evaluated using a FITC-Annexin V Apoptosis Detection Kit (BD Bioscience, San Jose, CA, USA) according to the manufacturer's instructions. Cells at the logarithmic growth phase were collected and placed in 24-well plates. After digestion using 0.25% trypsin, the collected cells were washed twice with ice-cold PBS and resuspended with 400 μ l

of $1 \times$ binding buffer solution. Next, 5 μ l of Annexin-V FITC and propidium iodide (PI) (Beyotime) was subsequently added to the cells, followed by incubation at 4°C in the dark for 30 min. Cell apoptosis was detected using a FC-500 flow cytometer (Beckman Coulter, Roissy, France).

3'UTR validation assay

Wild type (wt) and deletion-mutated SOX11 3'UTR sequences were inserted into the XhoI/NotI sites of the psiCHECK2 vector (Promega, Madison, USA). The 5 putative binding sites of miR-145 on SOX11 3'UTR were respectively deleted to generate 5 mutated SOX11 3'UTR

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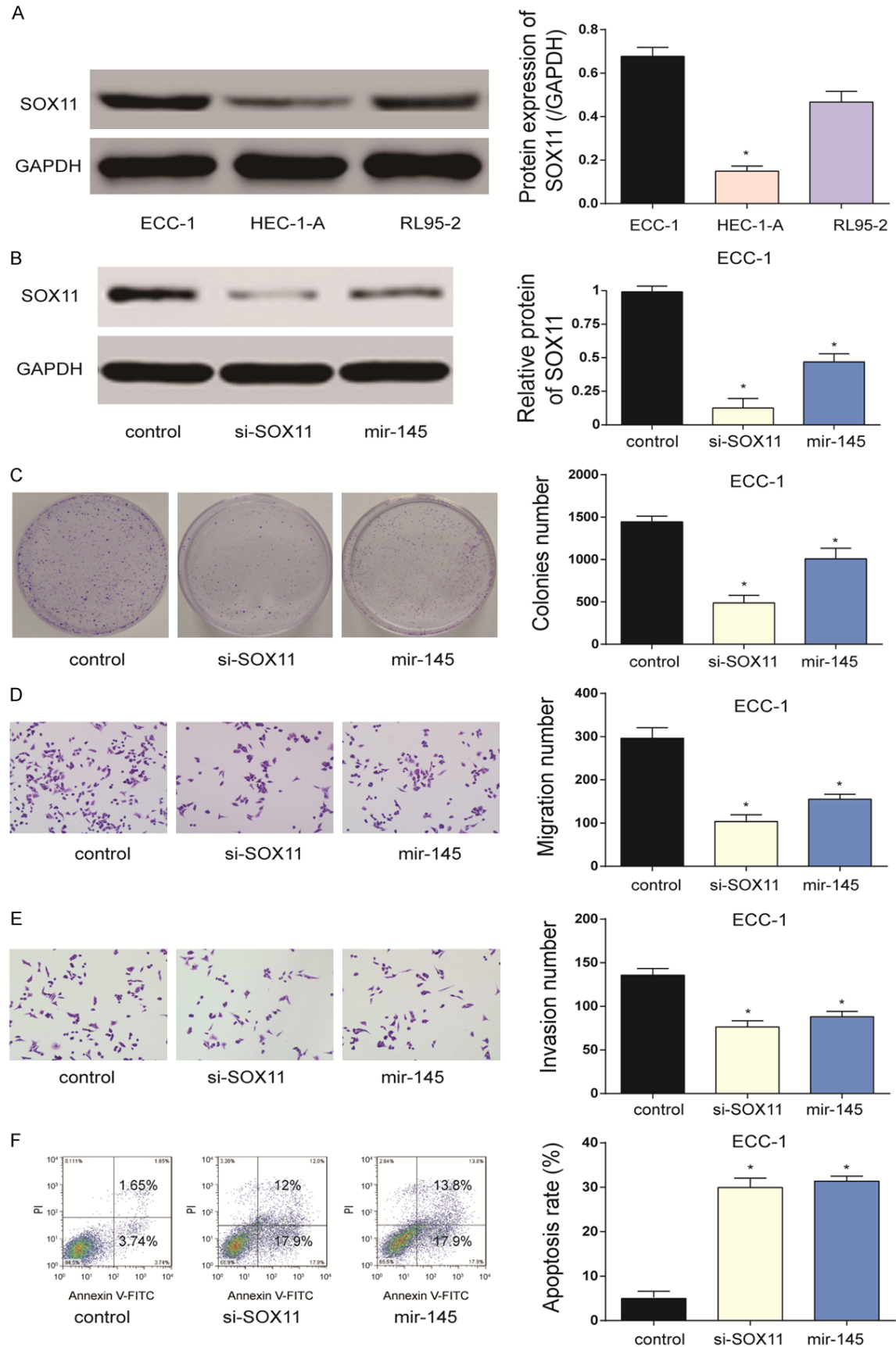


Figure 3. MiR-145 inhibited the proliferation, migration and invasion of ECC-1 cells and promoted cell apoptosis by downregulating SOX11. A. The ECC-1 cell line had the highest protein expression of SOX11, whereas HEC-1-A cells had the lowest expression of SOX11 according to the western blot analysis. B. The expression of SOX11 in the miR-145 and si-SOX11 groups was lower than that in the control group. C-E. The number of colonies and migratory and invasive cells in the miR-145 and si-SOX11 groups was smaller than that in control group. F. Apoptosis rate in miR-145 and si-SOX11 groups was much higher than that in control group detected by flow cytometry. * $P < 0.05$, compared with control group.

constructs. The corresponding mutated 3'UTR of SOX11 was obtained using an overlapping PCR approach. The primers are displayed in **Table 2**. HEK293 cells were transfected with a 50 nM mixture of the pre-miR-145 (Ambion, TX, USA) and psiCHECK2-UTR vectors in a 48-well plate using Lipofectamine 2000 (Invitrogen). After transfection, the luciferase activity was analyzed using dual-fluorescein (Promega) and a FLUOstar photometer (BMG Labtech, Offenburg, Germany).

Statistical analysis and databases

Prognostic data for SOX11 and miRNA were downloaded from database of The Cancer Genome Atlas (TCGA, <https://cancergenome.nih.gov/>). Affymetrix Data Mining Tool version 3.1 (DMT 3.1) and scripts called within the R environment (<http://www.r-project.org/>) were used to screen differentially expressed mRNAs and miRNAs. The filtration criteria were Log2 (Fold Change) > 2 and $P < 0.001$. TargetScan was also used to identify miRNA putative binding sites. Predictions of SOX11 mRNA secondary structure were obtained using Mfold (RNA Folding Form (version 2.3 energies). SOX11 sequencing analysis was conducted according to the data of the nucleotide database affiliated with the National Center for Biotechnology Information (NCBI). All assays were repeated at least three times. SPSS 20.0 software (IBM, New York, USA) was applied for the statistical analysis. All experimental data are presented as the means \pm SD. Comparisons between groups were assessed using Student's unpaired *t*-test or one-way ANOVA. Kaplan-Meier analysis and the log-rank test were used for the analysis of the association between SOX11 expression and prognosis of EC patients. A P value < 0.05 was considered statistically significant.

Results

SOX11 was highly expressed in EC tissues

SOX family gene expression in of 23 pairs of EC and adjacent tissues was analyzed using a

microarray method. The results showed obviously high expression levels of SOX11 and SOX14 in tumor tissues (**Figure 1A, 1B**). We selected SOX11 for further study. The mRNA analysis showed that compared with normal tissues, SOX11 was highly expressed in tumor tissue samples ($P < 0.05$, **Figure 1C**). IHC staining validated the high expression of SOX11 in cancerous tissues (**Figure 1D**). Disease-free survival (DFS) and overall survival (OS) curves demonstrated that SOX11 was detrimental to the survival of patients with EC (**Figure 1E, 1F**).

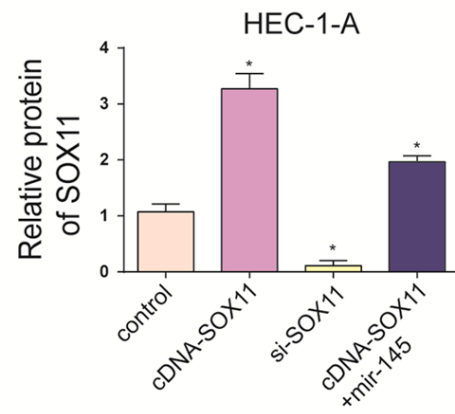
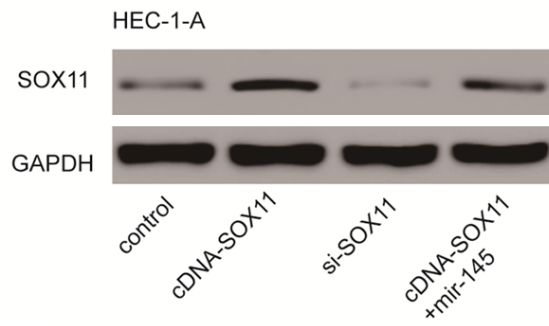
MiR-145 significantly reduced the expression of SOX11

To determine which mRNA had significantly low expression in EC, we conducted microarray analysis of 23 pairs of EC and adjacent tissue samples (**Figure 2A**). The intersection of the TargetScan database, the microRNA database and differentiated low expression RNA results showed that 7 miRNAs were downregulated and could target SOX11 (**Figure 2B**). Among these seven downregulated miRNAs, miR-145 had the most notable inhibitory effects on the expression of SOX11 compared with other miRNAs in the ECC-1 cell line ($P < 0.05$, **Figure 2C**). Additionally, comparison of the expression of miR-145 in 23 pairs of samples revealed that the expression of miR-145 in cancer tissues was higher than that in adjacent tissues ($P < 0.05$, **Figure 2D**).

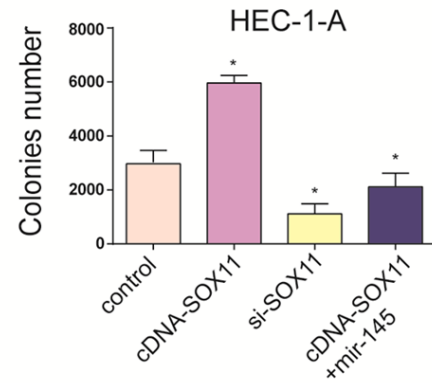
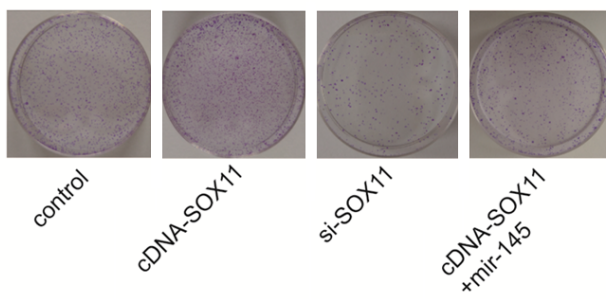
MiR-145 suppressed the proliferation, migration, and invasion of ECC-1 cells and promoted cell apoptosis by inhibiting SOX11

According to the results of western blotting and qRT-PCR, SOX11 had the highest expression in ECC-1 cells and the lowest expression in HEC-1-A cells (**Figure 3A**). Hence, we used the ECC-1 cell line to investigate the effects of miR-145 on the expression of SOX11, and the results indicated an obvious inhibitory effect of miR-145 on the expression of SOX11 in ECC-1 cells ($P < 0.05$, **Figure 3B**). Moreover, cells transfected with miR-145 showed fewer colonies and

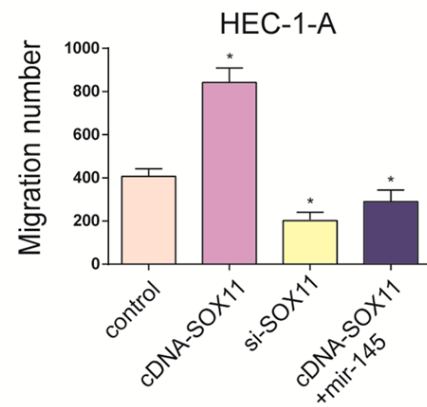
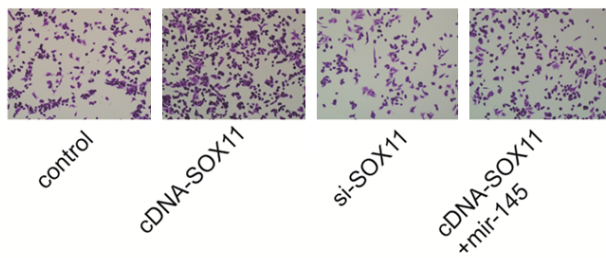
A



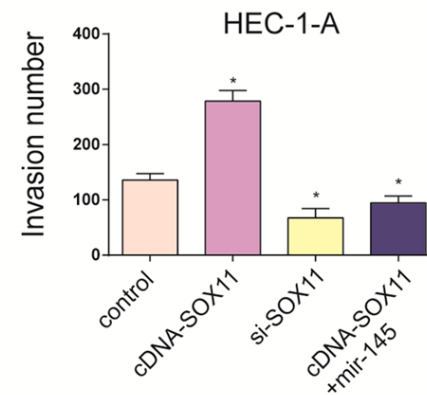
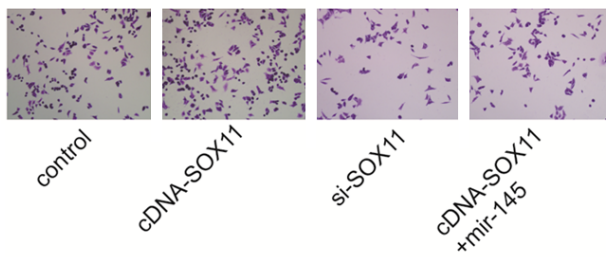
B



C



D



E

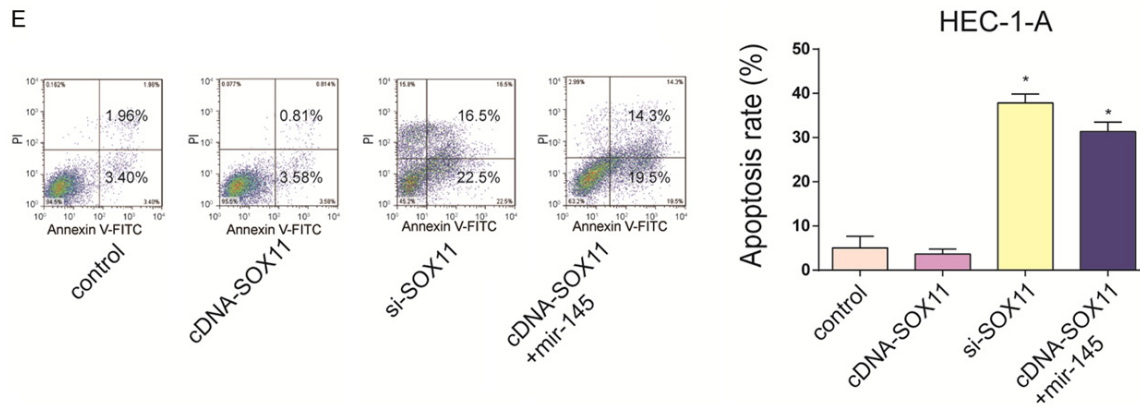


Figure 4. MiR-145 inhibited the proliferation, migration and invasion of HEC-1-A cells and promoted cell apoptosis by downregulating SOX11. A. The expression of SOX11 in cDNA-SOX11 and cDNA-SOX11+miR-145 groups was much higher than that in the control group, while that in the si-SOX11 group was lower compared with the control group. B-D. The number of colonies, migratory and invasive cells in cDNA-SOX11 group was higher than that in the control group. In the cDNA-SOX11+miR-145 and si-SOX11 groups, the number was smaller than in the control group. E. The apoptosis rate in the cDNA-SOX11+miR-145 and si-SOX11 groups was notably higher than that in the control group. No difference was observed between the control and cDNA-SOX11 groups. * $P < 0.05$, compared with the control group.

migratory and invasive cells than those in the control group. A higher apoptosis rate was also observed in the miR-145 group compared with the control group (Figure 3C-F, $P < 0.05$). Hence, miR-145 inhibited the expression of SOX11 to restrain the proliferation, migration and invasion of ECC-1 cells and promote cell apoptosis.

MiR-145 partially suppressed the proliferation, migration, and invasion of HEC-1-A cells and promoted cell apoptosis by inhibiting SOX11

To further study the effects of miR-145, we selected HEC-1-A cells for experiments. The results demonstrated that in HEC-1-A cells, miR-145 partially suppressed SOX11 expression ($P < 0.05$, Figure 4A). Furthermore, miR-145 inhibited the SOX11-induced proliferation, migration and invasion of HEC-1-A cells and stimulated cell apoptosis compared with the control group ($P < 0.05$, Figure 4B-E). Hence, by inhibiting SOX11, the proliferation, migration and invasion of HEC-1-A cells can be suppressed by miR-145, which also stimulated cell apoptosis.

MiR-145 suppressed the expression of SOX11 by targeting site 3 of the SOX11 3'UTR

To identify the molecular mechanism of miR-145 inhibition of SOX11, we identified 5 miR-145 binding sites in the SOX11 3'UTR using the TargetScan database. The sequence scheme is

presented in Figure 5A. The binding sequences are presented in Figure 5B. The results showed that the secondary structure of the SOX11 3'UTR changed the most after site 3 was deleted (Figure 5C). Dual luciferase reporter gene experiments verified that miR-145 repressed the expression of SOX11 by targeting site 3 in the SOX11 3'UTR ($P < 0.05$, Figure 5D).

Discussion

We investigated the expression of SOX11 in EC cells to identify its target miRNA and explore the effects of their interaction in EC cells in the present study. SOX11 showed a close correlation with the prognosis of EC patients and was demonstrated to downregulate SOX11 expression by targeting site 3 (3615) in the SOX11 3'UTR. miR-145 suppressed the propagation, migration and invasiveness of EC cells by inhibiting SOX11. MiR-145 and its target gene SOX11 could be considered as diagnostic markers in the treatment of EC.

The SOX family is a group of transcription factors that play a crucial role in the growth of cells [16]. In the present study, we measured the expression of the SOX family genes in EC tissue samples, showing the obviously higher expression of SOX11 in EC tissues than in adjacent tissues. The role of SOX11 in the promotion or inhibition of cancer progression has been thoroughly investigated. For example, Lei et al.

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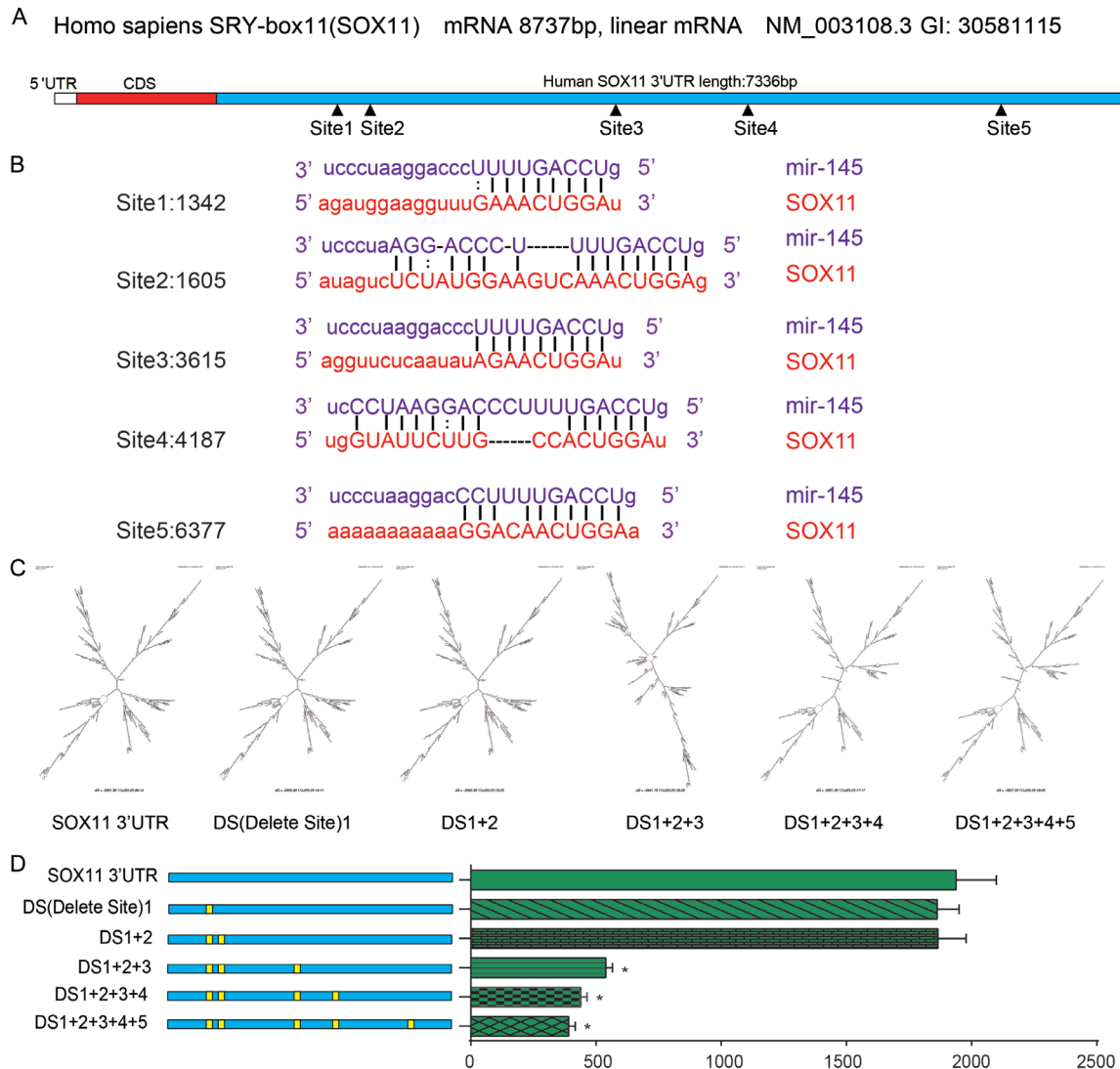


Figure 5. MiR-145 suppressed SOX11 expression by targeting site 3 in the SOX11 3'UTR. **A.** A total of five sites can be bound by miR-145 in the long SOX11 3'UTR. **B.** The sequences of the five predicted binding sites of miR-145 in SOX11 3'UTR. **C.** SOX11 3'UTR secondary structure changed the most after site 3 (3615) was deleted among the five mutations. **D.** Dual luciferase reporter system results showed significant differences in the DS1+2+3, DS1+2+3+4 and DS1+2+3+4+5 groups compared with the wild-type SOX11 3'UTR. * $P < 0.05$, compared with the SOX11 3'UTR group.

reported that the downregulation of SOX11 hindered the propagation, migration, and invasiveness of thyroid tumor cells [17]. However, Gang *et al.* revealed that the overexpression of SOX11 inhibited ovarian cancer cell proliferation and invasion [18]. Sandra *et al.* demonstrated that the reexpression of SOX11 in an epithelial ovarian cancer cell line reduced cell proliferation, indicating that the induction of SOX11 overexpression may result in a significant decrease in epithelial ovarian cancer cell propagation [19]. A single miRNA may demon-

strate different expression trends. Thus, aberrant miRNA expression could be a useful carcinogenesis biomarker. Moreover, the DFS and OS rates of patients with lower SOX11 expression showed better prognosis outcomes than those of patients with high SOX11 levels. Consistent with our research, Verónica *et al.* also asserted that SOX11-negative mantle cell lymphoma patients exhibited better 5-year overall survival rates (78%) compared with SOX11-positive mantle cell lymphoma patients (36%) [20]. Additionally, Navarro *et al.* reported

similar findings that *SOX11* expression predicted a poor OS for mantle cell lymphoma patients [21].

MiRNAs have been demonstrated to play an important role in tumor initiation, development and progression. Among miRNAs, miR-145 has been implicated in various types of malignancies, and its function is realized through targeting specific genes. In the present study, miR-145 showed low expression in EC samples and exhibited binding with *SOX11*. Similarly, Sheng *et al.* reported the downregulation of miR-145 expression in colorectal cancer cells, and demonstrated that the restored expression of this miRNA significantly suppressed the migration and invasion of colorectal cancer cells [22]. The aberrant expression of miR-145 was frequently reported to have effects on cell propagation, migration and aggression. For example, Yan *et al.* reported that miR-145 induced the apoptosis of glioma cells by targeting *BNIP3* and notch signaling [23]. Gotte *et al.* verified that the upregulation of miR-145 inhibited breast cancer cell motility and invasiveness [24]. Moreover, the effects of miR-145 on EC were verified. Wu *et al.* confirmed that the upregulation of miR-145 promoted the differentiation of human endometrial adenocarcinoma cells [25]. Bai *et al.* also showed that miR-145 suppressed EC cell propagation by targeting *OCT4* [15]. Similarly, in the present study, miR-145 suppressed the propagation and metastasis of ECC-1 cells by inhibiting *SOX11*. Thus, not only can the ectopic expression of miR-145 be used as an EC biomarker, but the forced reexpression of miR-145 could also be used as a novel treatment.

However, the present study was subject to some limitations. Only data from experiments *in vitro* were considered in the present study, and *in vivo* experiments are needed to confirm these conclusions. Sophisticated signaling always participates in cancer development; thus, further studies on specific signaling pathways are also needed.

Conclusion

In summary, the high expression of *SOX11* in EC tissue samples indicated the involvement of this molecule in the progression of EC. The results of data analysis and green fluorescent protein reporter system experiments showed

that miR-145 could regulate the protein expression of *SOX11* by targeting site 3 in the *SOX11* 3'UTR. Additionally, miR-145 significantly repressed the proliferation, migration, and invasion of EC cells by lowering the expression of *SOX11*. These findings indicated that miR-145 and *SOX11* might serve as therapeutic agents for the treatment of EC.

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Disclosure of conflict of interest

None.

Abbreviations

EC, Endometrial cancer; IHC, immunohistochemistry; miRNAs, MicroRNAs; 3'UTRs, 3'-untranslated regions; BNCC, BeNa Culture Collection; PBC, phosphate-buffered saline; BSA, bovine serum albumin; BCA, bicinchoninic acid; PVDF, polyvinylidene difluoride; TBST, Tris-buffered saline-Tween 20; ECL, Enhanced chemiluminescence; OD, optical density; wt, Wild type; NCBI, National Center for Biotechnology Information; OS, overall survival.

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References

- [1] Eritja N, Chen BJ, Rodriguez-Barrueco R, Santacana M, Gatus S, Vidal A, Marti MD, Ponce J, Bergada L, Yeramian A, Encinas M, Ribera J, Reventos J, Boyd J, Villanueva A, Matias-Guiu X, Dolcet X and Llobet-Navas D. Autophagy orchestrates adaptive responses to targeted therapy in endometrial cancer. *Autophagy* 2017; 13: 608-624.
- [2] Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, Marth C, Nout R, Querleu D, Mirza MR, Sessa C; ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up. *Ann Oncol* 2016; 27: 16-41.

- [3] Trabert B, Wentzensen N, Felix AS, Yang HP, Sherman ME and Brinton LA. Metabolic syndrome and risk of endometrial cancer in the united states: a study in the SEER-medicare linked database. *Cancer Epidemiol Biomarkers Prev* 2015; 24: 261-267.
- [4] Haenisch S, Zhao Y, Chhibber A, Kaiboriboon K, Do LV, Vogelgesang S, Barbaro NM, Alldredge BK, Lowenstein DH, Cascorbi I and Kroetz DL. SOX11 identified by target gene evaluation of miRNAs differentially expressed in focal and non-focal brain tissue of therapy-resistant epilepsy patients. *Neurobiol Dis* 2015; 77: 127-140.
- [5] Kuo PY, Leshchenko VV, Fazzari MJ, Perumal D, Gellen T, He T, Iqbal J, Baumgartner-Wennerholm S, Nygren L, Zhang F, Zhang W, Suh KS, Goy A, Yang DT, Chan WC, Kahl BS, Verma AK, Gascoyne RD, Kimby E, Sander B, Ye BH, Melnick AM and Parekh S. High-resolution chromatin immunoprecipitation (ChIP) sequencing reveals novel binding targets and prognostic role for SOX11 in mantle cell lymphoma. *Oncogene* 2015; 34: 1231-1240.
- [6] Lord M, Wasik AM, Christensson B and Sander B. The utility of mRNA analysis in defining SOX11 expression levels in mantle cell lymphoma and reactive lymph nodes. *Haematologica* 2015; 100: e369-372.
- [7] Palomero J, Vegliante MC, Eguileor A, Rodriguez ML, Balsas P, Martinez D, Campo E and Amador V. SOX11 defines two different subtypes of mantle cell lymphoma through transcriptional regulation of BCL6. *Leukemia* 2016; 30: 1596-1599.
- [8] Holler E, Kolb HJ, Mittermuller J, Kaul M, Lederose G, Duell T, Seeber B, Schleuning M, Hintermeier-Knabe R, Ertl B, et al. Modulation of acute graft-versus-host-disease after allogeneic bone marrow transplantation by tumor necrosis factor alpha (TNF alpha) release in the course of pretransplant conditioning: role of conditioning regimens and prophylactic application of a monoclonal antibody neutralizing human TNF alpha (MAK 195F). *Blood* 1995; 86: 890-899.
- [9] Kompass KS and Witte JS. Co-regulatory expression quantitative trait loci mapping: method and application to endometrial cancer. *BMC Med Genomics* 2011; 4: 6.
- [10] Dweep H and Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods* 2015; 12: 697.
- [11] Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, Brock JE, Richardson AL and Weinberg RA. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. *Cell* 2009; 137: 1032-1046.
- [12] Madhavan B, Yue S, Galli U, Rana S, Gross W, Muller M, Giese NA, Kalthoff H, Becker T, Buchler MW and Zoller M. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. *Int J Cancer* 2015; 136: 2616-2627.
- [13] Li Z, Yu X, Shen J and Jiang Y. MicroRNA dysregulation in uveal melanoma: a new player enters the game. *Oncotarget* 2015; 6: 4562-4568.
- [14] Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, Richardson JA, Bassel-Duby R and Olson EN. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev* 2009; 23: 2166-2178.
- [15] Bai M, Yuan M, Liao H, Chen J, Xie B, Yan D, Xi X, Xu X, Zhang Z and Feng Y. OCT4 pseudogene 5 upregulates OCT4 expression to promote proliferation by competing with miR-145 in endometrial carcinoma. *Oncol Rep* 2015; 33: 1745-1752.
- [16] Renjie W and Haiqian L. MiR-132, miR-15a and miR-16 synergistically inhibit pituitary tumor cell proliferation, invasion and migration by targeting Sox5. *Cancer Lett* 2015; 356: 568-578.
- [17] Wang L, Shen YF, Shi ZM, Shang XJ, Jin DL and Xi F. Overexpression miR-211-5p hinders the proliferation, migration, and invasion of thyroid tumor cells by downregulating SOX11. *J Clin Lab Anal* 2017; [Epub ahead of print].
- [18] Fang G, Liu J, Wang Q, Huang X, Yang R, Pang Y and Yang M. MicroRNA-223-3p regulates ovarian cancer cell proliferation and invasion by targeting SOX11 expression. *Int J Mol Sci* 2017; 18.
- [19] Sernbo S, Gustavsson E, Brennan DJ, Gallagher WM, Rexhepaj E, Rydnert F, Jirstrom K, Borrebaeck CA and Ek S. The tumour suppressor SOX11 is associated with improved survival among high grade epithelial ovarian cancers and is regulated by reversible promoter methylation. *BMC Cancer* 2011; 11: 405.
- [20] Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, Camacho F, Bea S, Hartmann E, Amador V, Hernandez L, Agostinelli C, Sargent RL, Rozman M, Aymerich M, Colomer D, Villamor N, Swerdlow SH, Pileri SA, Bosch F, Piris MA, Montserrat E, Ott G, Rosenwald A, Lopez-Guillermo A, Jares P, Serrano S and Campo E. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res* 2010; 70: 1408-1418.
- [21] Navarro A, Clot G, Royo C, Jares P, Hadzidimitriou A, Agathangelidis A, Bikos V, Darzentas N, Papadaki T, Salaverria I, Pinyol M, Puig X,

- Palomero J, Vegliante MC, Amador V, Martinez-Trillos A, Stefancikova L, Wiestner A, Wilson W, Pott C, Calasanz MJ, Trim N, Erber W, Sander B, Ott G, Rosenwald A, Colomer D, Gine E, Siebert R, Lopez-Guillermo A, Stamatopoulos K, Bea S and Campo E. Molecular subsets of mantle cell lymphoma defined by the IGHV mutational status and SOX11 expression have distinct biologic and clinical features. *Cancer Res* 2012; 72: 5307-5316.
- [22] Sheng N, Tan G, You W, Chen H, Gong J, Chen D, Zhang H and Wang Z. MiR-145 inhibits human colorectal cancer cell migration and invasion via PAK4-dependent pathway. *Cancer Medicine* 2017; 6: 1331-1340.
- [23] Du Y, Li J, Xu T, Zhou DD, Zhang L and Wang X. MicroRNA-145 induces apoptosis of glioma cells by targeting BNIP3 and Notch signaling. *Oncotarget* 2017; 8: 61510-61527.
- [24] Gotte M, Mohr C, Koo CY, Stock C, Vaske AK, Viola M, Ibrahim SA, Peddibhotla S, Teng YH, Low JY, Ebnet K, Kiesel L and Yip GW. miR-145-dependent targeting of junctional adhesion molecule A and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness. *Oncogene* 2010; 29: 6569-6580.
- [25] Wu Y, Liu S, Xin H, Jiang J, Younglai E, Sun S and Wang H. Up-regulation of microRNA-145 promotes differentiation by repressing OCT4 in human endometrial adenocarcinoma cells. *Cancer* 2011; 117: 3989-3998.