Hsa-circ-0001860 promotes Smad7 to enhance MPA resistance in endometrial cancer via miR-520h

Shuang Yuan
International Peace Maternity and Child Health Hospital, Shanghai JiaoTong University School of Medicine https://orcid.org/0000-0002-1986-4090

Panchan Zheng
International Peace Maternity and Child Health Hospital, Shanghai JiaoTong University School of Medicine

Judan Zeng
International Peace Maternity and Child Health Hospital, Shanghai JiaoTong University School of Medicine

Xiao Sun
International Peace Maternity and Child Health Hospital, Shanghai JiaoTong University School of Medicine, Shanghai Key Laboratory of Embryo Original Disease, Shanghai Municipal Key Clinical Specialty

Lihua Wang (drwanglh0420@163.com)
International Peace Maternity and Child Health Hospital, Shanghai JiaoTong University School of Medicine https://orcid.org/0000-0002-1461-0438

Primary research

Keywords: circular RNA, MPA resistance, endometrial cancer, Smad7, Hsa_circ_0001860, miR-520h

Posted Date: June 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38066/v1

Abstract

Background

Medroxyprogesterone acetate (MPA) is one of the most commonly prescribed progestin for the treatment of endometrial cancer (EC). Despite initial benefits, many patients ultimately develop progesterone resistance. Circular RNA (circRNA) is a kind of noncoding RNA, contributing greatly to the development of human tumor. However, the role of circular RNA in MPA resistance is unknown.

Methods

We explored the expression profile of circRNAs in Ishikawa cells treated with (ISK/MPA) or without MPA (ISK) by RNA sequencing, and identified a key circRNA hsa_circ_0001860. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to verify its expression in MPA-resistant cell lines and tissues. CCK8, Transwell and flow cytometry were used to evaluate the functional roles of hsa_circ_0001860 in MPA resistance. The interaction between hsa_circ_0001860 and miR-520h was confirmed by bioinformatics analysis and luciferase reporter assay.

Results

The expression of hsa_circ_0001860 was significantly downregulated in MPA-resistant cell lines and tissues, and negatively correlated with lymph node metastasis and histological grade of EC. Functional analysis showed that hsa_circ_0001860 knockdown by shRNA promoted the proliferation, migration and invasion and inhibited the apoptosis of Ishikawa cells treated with MPA. Mechanistically, hsa_circ_0001860 promoted Smad7 expression by sponging miR-520h.

Conclusion

Hsa_circ_0001860 plays an important role in the development of MPA resistance in EC through miR-520h / Smad7 axis, and it could be targeted to reverse the MPA resistance in endometrial cancer.

Background

Endometrial cancer is one of the most common gynecological tumors in the United States[1]. In 2018, endometrial cancer affected 382,069 women worldwide and resulted in 89,929 deaths, and the incidence and mortality have been rapidly increasing in recent years[2–3]. More than 90% of endometrial cancers occurs in women over 45 years old, and about 7.1% of them are younger than 45 years old[4]. In order to preserve the fertility of young patients, progesterone such as medroxyprogesterone acetate (MPA) and megestrol acetate (MA), is regarded as the first-line drug for conservative treatment[5–7]. In addition, patients in advanced stages who can't tolerate surgery also receive conservative treatment. Although 70% of the patients respond to MPA initially, 30% – 40% of them would recur, and 63% of the patients do not respond when they receive MPA treatment again[8–9]. Using a stable MPA-resistant Ishikawa cell, it has

been shown that SIRT1 / FoxO1 / SREBP-1 as a pathway targeting PR is involved in the development of progesterone resistance in endometrial cancer cells[10], but the molecular mechanism still remains unclear. Therefore, it is of great significance to elucidate the mechanism and therapeutic target of MPA resistance in EC for individualized treatment of patients.

With the rapid development of RNA sequencing technology, a large number of previously known as "junk molecules" of non-coding RNA have been found to play important roles in human diseases, such as long non-coding RNA (lncRNA) and circRNA[11]. Characterized by covalent closed loop structure, circRNA has neither 5' end cap nor 3' end poly (A) tail. Therefore, it is difficult to be degraded by RNase R and relatively stable[12–13]. In addition, circRNA also has the characteristics of cell type and tissue specificity, spatio-temporal specificity, and evolutionary conservatism[14]. Therefore, these characteristics make it a potential and valuable biomarker for the prognosis and diagnosis in various carcinomas, such as hepatocellular carcinoma[15], oral squamous cell carcinoma[16] and gastric carcinoma[17].

In recent years, more and more studies have shown that circRNA plays important roles in the development of breast cancer[18] and gynecological cancer such as cervical cancer[19], ovarian cancer[20] and endometrial cancer[21]. Moreover, circRNA regulates biological functions in a variety of ways, such as serving as microRNA sponges, gene transcription regulators, and protein decoys, and directly translating into protein[22]. Among them, miRNA sponge is the most common mechanism. For example, in endometrial cancer, circ_PUM1 can increase Notch3 by sponging miR-13, thus promoting the development of endometrial cancer[23]. However, the function of circRNA as miRNA sponge in the resistance of EC to MPA has not been elucidated.

In this study, we validated the differentially expressed circRNA hsa_circ_0001860 in MPA-sensitive ISK and MPA-resistant KLE and ISK$^{PRB-/-}$ cells. Functional test and luciferase reporter assay confirmed that hsa_circ_0001860 downregulation enhanced EC resistance to MPA through miR-520 h / Smad7 axis. These findings may provide evidence to regulate MPA resistance of endometrial cancer by targeting circRNA hsa_circ_0001860 signaling pathway.

Methods

Patients and samples

Tissue samples and clinical data were collected from 113 endometrial cancer patients who received surgical treatment in the Shanghai International Peace Maternity and Child Health Hospital from December 2013 to December 2019. All patients were diagnosed according to histopathology report from biopsy after surgery, and none of them received chemotherapy or radiotherapy before operation. The tumor stages and histological grades were established in line with the criteria of Federation International of Gynecology and Obstetrics (FIGO) 2018 staging system. According to PR expression, patients were divided into MPA sensitive and MPA resistant groups. All tissue samples were stored at -80 °C until use.

The study was approved by the medical research ethics committee of the International Peace Maternal and Child Health Hospital, and the written informed consent of all patients was obtained when collecting specimens.

Cell Culture

Human EC cell lines including ISK and KLE were obtained from the American Type Culture Collection (ATCC, Manassas, vA, USA). MPA-resistant cell line $ISK^{PRB-/-}$ was established as previously described[10]. ISK, KLE and $ISK^{PRB-/-}$ were cultured in Dulbecco's modified Eagle medium (DMEM)/ F12 (Gibco, Auckland, New Zealand) containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 100 μg/ml penicillin and 100 U/ml streptomycin (Gibco) at 37 °C in a 5% CO2 humidified atmosphere.

RNA Isolation And Quantitative Real-time PCR (qRT-PCR) Assays

Total RNA was isolated using Trizol reagent (Takara, Dalian, China) and the RNA concentration was determined by NanoDrop ND-2000 (NanoDrop, USA). To quantify the amounts of mRNA and circRNA, 500 ng of RNA was directly reverse transcribed using Prime Script RT Master Mix (Takara, Dalian, China). Reverse transcription of miRNA was performed using a miScript II RT Kit (Qiagen). cDNA was amplified using Hieff® qPCR SYBR Green Master Mix (Yeasen, Shanghai, China). Real-time PCR was conducted with Quant Studio 7 Flex system (Life Technologies, USA) in accordance with the manufacturer's instructions. Actin was used as the control for the detection of mRNA and circRNA expression levels, while U6 was used as the control for miRNA expression analysis. The primer sequences used for qRT-PCR were listed in Table S1. The ΔΔCt method was used for quantification.

Cell Transfection

The EC cells planted on a six-well plate with 70–80% confluence were transfected with siRNA and miRNA mimics or inhibitors synthesized by GenePharma (Shanghai, China) using Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's instructions. The sequences used are listed in Tables S2 and S3. Two siRNA sequences for the hsa_circ_0001860 were used in this study (si-circ_0001860-2 has the highest inhibition efficiency and si-circ_0001860 mentioned in the article refers to si-circ_0001860-2).

In Silico Target Prediction And Luciferase Reporter Assay

The potential targets of hsa_circ_0001860 were predicted based on online software including CircBank[24], CircInteractome[25], and StarBase[26]. Finally, miR-520 h was predicted as a target gene of hsa_circ_0001860.

ISK cells were seeded in 24-well plates and cotransfected with corresponding plasmids and miRNA mimics. At 48 h after transfection, luciferase reporter assays were conducted using a dual-luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions. Relative luciferase activity was normalized to Renilla luciferase activity.

Western Blot Analysis

Treated cells were lysed in RIPA buffer containing protease inhibitor phenylmethanesulfonyl fluoride (Beyotime, Nanjing, China). Protein samples were loaded into the 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel and subjected to electrophoresis at 120 V, and then transferred to polyvinylidenefluoride (PVDF) membrane (Millipore, Billerica, MA). The membranes were blocked with 5% BSA in TBST buffer and incubated with specific primary antibodies at 4 °C overnight. The next day, membranes were washed for 15 min 3 times in TBST and incubated with secondary antibodies for 1 h at room temperature. Immunoreactive bands were visualized by an enhanced chemiluminescence (ECL) system and imaged with Amersham Imager 600. GAPDH was used as loading control. Detailed information of antibodies used in this study was provided in Table S4.

Cell Proliferation And Cytotoxicity Assay

The cell proliferation and cytotoxicity was measured using Cell Counting Kit-8(CCK8) following the manufacturer's directions (Yeasen, Shanghai, China). The absorbance values were measured at 450 nm using a SpectraMax 190 microplate reader (Bio-Rad Model 680).

Migration And Invasion Assays

In the transwell migration and invasion assay, the upper transwell chambers (8-μm pore) were coated with 50 μl of Matrigel at a dilution of 1:6 (BD Biosciences, San Jose CA, USA). A total of 1×10^5 cells were seeded into the upper chamber of a 24-well chemotaxis chamber with polycarbonate filters (8-μm pore) (Corning Incorporated, Glendale, AZ, USA). DMEM/F12 supplemented with 10% FBS was added to the lower chamber. Then, cells were treated with MPA (10 μM) for 24 h or 48 h. After that the treatment, cells on the upper side of the chamber were removed, and cells on the lower side were fixed with 4% paraformaldehyde, stained with crystal violet, and photographed under a microscope at 100 × magnification. The number of crystal violet-stained cells was counted in five fields from each well.

Apoptosis Assay

Cell apoptosis was detected using a Annexin V-PE/7-AAD Detection Kit (Yeasen, Shanghai, China) according to the manufacturer's instructions. Briefly, after incubation with MPA (10 μM) or dimethyl sulfoxide (control) for 48 h, cells were trypsinized, washed and resuspended in binding buffer. Next, 5 μl

of Annexin V-PE and 10 μl of 7-AAD were added to the cell suspension and incubated in the dark at 4 °C for 15 min. A FACScan flow cytometer and FlowJo software (Tree Star Inc., Ashland, OR) were used to analyze the cells.

Statistical analysis

All experiments were performed in triplicate. Data were analyzed with SPSS software (version 19.0) (SPSS, Inc., Chicago, IL, USA) and presented as the mean ± SD. The statistical significance of the results was calculated using an unpaired Student's t-test. Clinicopathological features were analyzed by a $\chi 2$ test. A P value < 0.05 was considered statistically significant.

Results

Hsa_circ_0001860 is downregulated in MPA-resistant EC cells and tissues and negatively correlated with lymph node metastasis and histological grade

In our previous study, we explored the expression profile of circRNAs in Ishikawa cells treated with (ISK/MPA) or without MPA (ISK) by RNA sequencing, and identified 87 differentially expressed circRNAs. We further verified 20 dysregulated circRNAs by qRT-PCR and found that hsa_circ_0046843 and hsa_circ_0001860 were the most significant dysregulated circRNAs. Bioinformatics analysis such as Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and circRNA-miRNA interaction network analysis revealed that hsa_circ_0001860 was the most significantly upregulated circRNA and the key circRNA related to progesterone treatment in EC (data not shown).

To further study the role of hsa_circ_0001860 in progesterone resistance, we used qRT-PCR to verify its expression in MPA-resistant cell lines and tissues. Our data showed that Hsa_circ_0001860 was highly expressed in MPA-sensitive EC cell lines (ISK) compared with MPA-resistant EC cell lines ($ISK^{PRB-/-}$ and KLE). Its expression was dramatically increased in ISK by MPA treatment, however, it was not changed by MPA in $ISK^{PRB-/-}$ and KLE (Fig. 1A and 1B). Moreover, the expression of Hsa_circ_0001860 was significantly decreased in MPA-resistant EC tissue compared with MPA-sensitive EC tissue (Fig. 1C). To explore the correlation between hsa_circ_0001860 expression and clinicopathological parameters, the median hsa_circ_0001860 expression value was used as the cutoff threshold to categorize all patients with EC. The results showed that the level of hsa_circ_0001860 was negatively correlated with stage, histological grade and lymph node metastasis (Table 1).

Table 1
Correlation of relative hsa_circ_0001860 expression with the clinicopathological characteristics of 113 patients with endometrial cancer.

Variables	No. of patients	hsa_circ_0001860 expression		P-value
		Low	High	
Age(years)				
50	37	11	16	0.2936
≥ 50	86	45	41	
Stage				
+	92	39	53	0.0014
+	21	17	4	
histological grade				
G1 + G2	83	33	50	0.0005
G3	30	23	7	
Myometrial invasion				
1/2	78	36	42	0.28
≥ 1/2	35	20	15	
Lymoh node metastasis				
No	96	42	54	0.0033
Yes	17	14	3	

Downregulation of hsa_circ_0001860 promotes proliferation and inhibits apoptosis of endometrial cancer cells

Given that hsa_circ_0001860 was downregulated in MPA-resistant cell and tissue, we next examined the effect of hsa_circ_0001860 knockdown on EC cell lines, which were transfected with hairpin RNA (sh-circ_0001860) or the vector control (sh-NC). After transfection, the expression of hsa_circ_0001860 was dramatically decreased in sh-circ_0001860-transfected cells compared with sh-NC-transfected cells, indicating the successful knockdown by hsa-circ_0001860 (Fig. 2A). It was further revealed that the proliferation was increased and the apoptosis was inhibited when circ_0001860 was knocked down. In line with this, the overexpression of hsa_circ_0001860 in $ISK^{PRB-/-}$ and KLE cells decreased the proliferation and promoted apoptosis (Fig. 2B and 2D). However, little change in MPA-sensitivity was observed in these functional assays in ISK, $ISK^{PRB-/-}$ and KLE (Figure S1).

Cell migration and invasion experiments showed that MPA could significantly inhibit the migration and invasion of MPA-sensitive EC cell lines (ISK) compared with DMSO, whereas no effect was observed on MPA-resistant EC cell lines ($ISK^{PRB-/-}$ and KLE). In order to further explore the effect of hsa_circ_0001860 on MPA-sensitivity in the migration and invasion of EC cells, we downregulated hsa_circ_0001860 in ISK cells, and upregulated hsa_circ_0001860 in $ISK^{PRB-/-}$ and KLE cells. We found that the downregulation of hsa_circ_0001860 abolished MPA-induced reduction of the migration and invasion of ISK cells (Fig. 3A and 3B). On the other hand, overexpression of hsa_circ_0001860 in MPA-resistant $ISK^{PRB-/-}$ and KLE cells promoted MPA-induced migration and invasion (Fig. 3C and 3D). These results showed that EC cell sensitivity to MPA was mediated by hsa_circ_0001860.

Hsa_circ_0001860 regulates tumor progression and MPA sensitivity of EC Cells via binding to miR-520 h

We next tried to predict the potential targets of hsa_circ_0001860 using the CircBank, CircInteractome and StarBase (Fig. 4A) and identified miR-520 h as a potential target gene of hsa_circ_0001860, which has a binding site for miR-520 h (Fig. 4B). Luciferase reporter assay demonstrated that miR-520 h expression significantly reduced the luciferase activity of the reporter in ISK cells co-transfected with WT but not MUT, suggesting that hsa_circ_0001860 may function as a sponge for miR-520 h (Fig. 4C). Then we investigated the biological functions of miR-520 h by knocking down miR-520 h with miR-520 h inhibitor in ISK cells transfected with sh_circ_0001860. It was found that miR-520 h inhibitor can reverse the effects of sh_circ_0001860 on promoting ISK cell proliferation and inhibiting its apoptosis (Fig. 4D and 4E). Furthermore, knockdown of miR-520 h in sh_circ_0001860-transfected ISK cells rendered them sensitivity to MPA as evidenced by the inhibitory effect of MPA on the migration and invasion of miR-520 inhibitor-treated cells (Fig. 4F and 4G).

Hsa_circ_0001860 regulates Smad7 expression and activates the Smad7/EMT signaling pathway

It has been reported that miR-520 h enhances EOC cell dissemination and induces EMT in vivo by suppressing Smad7 expression[27]. We hypothesized that hsa_circ_0001860 could regulate tumorigenesis, migration and invasion of EC cells mediated by MPA by promoting Smad7 expression via acting as a sponge for miR-520 h. We examined the effect of hsa_circ_0001860 on the levels of downstream protein Smad7 of miR-520 h using Western blotting and found that knockdown of hsa_circ_0001860 decreased the levels of Smad7 and influenced EMT signaling pathway-related proteins such as phosphorylated Smad2/3, E-cadherin and N-cadherin. Meanwhile, concurrent knockdown of miR-520 h and hsa_circ_0001860 reversed hsa_circ_0001860 knockdown-induced decrease of Smad7 expression (Fig. 5A and 5B). These results suggest that circRNA hsa_circ_0001860 functions as a ceRNA to regulate Smad7 expression, activate the Smad7/EMT signaling cascade, and promote MPA sensitivity by targeting miR-520 h.

Discussion

EC is one of the most common gynecologic malignancies. Progestin therapy drugs including MPA and MA are often used to preserve fertility for young patients. However, almost a third of these patients eventually developed MPA resistance[8]. There are several mechanisms that underlie the acquired resistance to MPA, such as PR dysregulation[28], Immune system and inflammatory response[29] and the activation of lipid metabolism[30]. Recently, some studies show that ncRNAs such as miRNAs and long ncRNAs (lncRNAs) also play vital roles in MPA resistance. For example, HOTAIR and LSD1 collaboratively repress PRB expression and thus reduce progesterone sensitivity in endometrial carcinoma cells[31]. CHOP and Lnc-CETP-3 might be involved in progesterone-PRB pathway to activate ER stress and provide therapeutic targets for EC patients with negative PRB expression[32].

CircRNA has been considered to be essential in the carcinogenesis and tumor progression of EC[23]. Besides, some studies also suggest that it could be a biomarker candidate for diagnosis and treatment of EC[33,34]. However, the function of circRNAs in MPA resistance remains unknown. In this study, we demonstrated that circRNA expression is associated with MPA resistance in EC. We identified a novel circRNA hsa_circ_0001860 that was downregulated in tissue samples from MPA-resistant patients and in MPA-resistant cell lines (ISK$^{PRB-/-}$ and KLE). The expression of hsa_circ_0001860 was negatively correlated with histological grade and lymphatic metastasis, suggesting that hsa_circ_0001860 could serve as a diagnostic and therapeutic target for EC. Moreover, downregulation of hsa_circ_0001860 by siRNA accelerated proliferation, migration and invasion and decreased apoptosis, and promoted MPA-induced migration and invasion in ISK cells, whereas there was an opposite change in the KLE and ISK$^{PRB-/-}$ cells. Thus, hsa_circ_0001860 may serve as a tumor suppressor and important regulator in MPA-resistant and aggressive EC.

CircRNA can participate in biological functions in a variety of ways, and the most common way is acting as miRNA ‘sponges’ and regulate the expression and activity of the target genes[22]. In our study, we revealed that hsa_circ_0001860 acted as a miRNA sponge for miR-520 h. It is known that miRNAs participate in a majority of biological processes via regulating target gene expression[35]. MiR-520 h has also been studied in various cancers. A recent study shows that miR-520 h promotes the drug resistance of human breast cancer cells through protecting cells from paclitaxel-induced apoptosis by targeting Death-associated protein kinase 2 (DAPK2)[36]. In addition, miR-520 h also promotes EOC progression by downregulating Smad7 and activating the TGF-β signaling pathway[27]. Smad7 inhibits the TGF - β / Smad signal pathway by preventing the formation of Smad2 / 4 complex and nuclear translocation after phosphorylation of Smad2 and Smad3, thus inhibiting EMT[37].

In our present study, we demonstrated that downregulating miR-520 h reversed MPA resistance and promoted MPA-induced migration and invasion in ISK-sh-circ_0001860 cells. Moreover, downregulation of hsa_circ_0001860 suppressed Smad7 protein expression, which could be reversed by the concurrent downregulation of miR-520 h. Therefore, our study presented a model of EMT process in EC cells treated

with MPA, in which hsa_circ_0001860 may play a crucial role in EC metastasis and MPA resistance (Fig. 6).

Conclusion

In conclusion, we show that hsa_circ_0001860 plays an important role in the resistance of EC to MPA through miR-520 h / Smad7 axis, and it could be developed into a novel marker and therapeutic target for MPA-resistant endometrial cancer.

Abbreviations

circRNA: circular RNA; miRNA: microRNA; lncRNA: long non-coding RNA; MPA: medroxyprogesterone acetate; EC: endometrial cancer; ISK: Ishikawa; qRT-PCR: quantitative reverse transcription polymerase chain reaction; FIGO: Federation International of Gynecology and Obstetrics; ATCC: American Type Culture Collection; PVDF: polyvinylidene fluoride membrane; ECL: enhanced chemiluminescence; CCK8: Cell Counting Kit-8; SD: standard deviation; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Declarations

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study was approved by the medical research ethics committee of the International Peace Maternal and Child Health Hospital, and the written informed consent of all patients was obtained when collecting specimens.

Consent for publication

All the authors agree to the publication clause.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81572547), Shanghai Municipal Key Clinical Specialty (No. shslczdzk06302) and the Clinical Research Fund of the

International Peace Maternity and Child Health Hospital (Grant No. GFY 1553).

Authors' contributions

Xiao Sun and Lihua Wang conceived and designed the experiments, Shuang Yuan, Panchan Zheng and Judan Zeng performed the experiments and analyzed the data. Each author conduced to this study and provided valuable advice for this manuscript. All authors read and approved the final manuscript.

Acknowledgments

We sincerely thank all involvers for their support to our research.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30. doi:10.3322/caac.21590.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.21492.
3. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–132. doi:10.3322/caac.21338.
4. Cancer of the endometrium-cancer stat facts. 2017. Available at: https://seer.cancer.gov/statfacts/html/corp.html. Accessed June 2, 2017.
5. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. Radiother Oncol. 2015;117(3):559–581. doi:10.1016/j.radonc.2015.11.013.
6. Rodolakis A, Biliatis I, Morice P, et al. European Society of Gynecological Oncology Task Force for Fertility Preservation: Clinical Recommendations for Fertility-Sparing Management in Young Endometrial Cancer Patients. Int J Gynecol Cancer. 2015;25(7):1258–1265. doi:10.1097/IGC.0000000000000493.
7. La Russa M, Zapardiel I, Halaska MJ, et al. Conservative management of endometrial cancer: a survey amongst European clinicians. Arch Gynecol Obstet. 2018;298(2):373–380. doi:10.1007/s00404-018-4820-7.
8. Chen M, Jin Y, Li Y, Bi Y, Shan Y, Pan L. Oncologic and reproductive outcomes after fertility-sparing management with oral progestin for women with complex endometrial hyperplasia and endometrial cancer. Int J Gynaecol Obstet. 2016;132(1):34–38. doi:10.1016/j.ijgo.2015.06.046.
9. Ushijima K, Yahata H, Yoshikawa H, et al. Multicenter phase II study of fertility-sparing treatment with medroxyprogesterone acetate for endometrial carcinoma and atypical hyperplasia in young women.

J Clin Oncol. 2007;25(19):2798–2803. doi:10.1200/JCO.2006.08.8344.

10. Wang Y, Zhang L, Che X, Li W, Liu Z, Jiang J. Roles of SIRT1/FoxO1/SREBP-1 in the development of progestin resistance in endometrial cancer. Arch Gynecol Obstet. 2018;298(5):961–969. doi:10.1007/s00404-018-4893-3.
11. Qu S, Yang X, Li X, et al. Circular RNA: A new star of noncoding RNAs. Cancer Lett. 2015;365(2):141–148. doi:10.1016/j.canlet.2015.06.003.
12. Chen LL, Yang L. Regulation of circRNA biogenesis. RNA Biol. 2015;12(4):381–388. doi:10.1080/15476286.2015.1020271.
13. Chen I, Chen CY, Chuang TJ. Biogenesis, identification, and function of exonic circular RNAs. Wiley Interdiscip Rev RNA. 2015;6(5):563–579. doi:10.1002/wrna.1294.
14. Shang Q, Yang Z, Jia R, Ge S. The novel roles of circRNAs in human cancer. Mol Cancer. 2019;18(1):6. Published 2019 Jan 9. doi:10.1186/s12943-018-0934-6.
15. Gong Y, Mao J, Wu D, et al. Circ-ZEB1.33 promotes the proliferation of human HCC by sponging miR-200a-3p and upregulating CDK6. Cancer Cell Int. 2018;18:116. Published 2018 Aug 13. doi:10.1186/s12935-018-0602-3.
16. Zhao SY, Wang J, Ouyang SB, Huang ZK, Liao L. Salivary Circular RNAs Hsa_Circ_0001874 and Hsa_Circ_0001971 as Novel Biomarkers for the Diagnosis of Oral Squamous Cell Carcinoma. Cell Physiol Biochem. 2018;47(6):2511-2521. doi:10.1159/000491624.
17. Sun H, Tang W, Rong D, et al. Hsa_circ_0000520, a potential new circular RNA biomarker, is involved in gastric carcinoma. Cancer Biomark. 2018;21(2):299-306. doi:10.3233/CBM-170379.
18. Sang Y, Chen B, Song X, et al. circRNA_0025202 Regulates Tamoxifen Sensitivity and Tumor Progression via Regulating the miR-182-5p/FOXO3a Axis in Breast Cancer. Mol Ther. 2019;27(9):1638–1652. doi:10.1016/j.ymthe.2019.05.011.
19. Ou R, Lv J, Zhang Q, et al. circAMOTL1 Motivates AMOTL1 Expression to Facilitate Cervical Cancer Growth. Mol Ther Nucleic Acids. 2020;19:50–60. doi:10.1016/j.omtn.2019.09.022.
20. Zhao Z, Ji M, Wang Q, He N, Li Y. Circular RNA Cdr1as Upregulates SCAI to Suppress Cisplatin Resistance in Ovarian Cancer via miR-1270 Suppression. Mol Ther Nucleic Acids. 2019;18:24–33. doi:10.1016/j.omtn.2019.07.012.
21. Chen BJ, Byrne FL, Takenaka K, et al. Analysis of the circular RNA transcriptome in endometrial cancer. Oncotarget. 2017;9(5):5786–5796. Published 2017 Dec 20. doi:10.18632/oncotarget.23534.
22. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: From the bench to the clinic. Pharmacol Ther. 2018;187:31–44. doi:10.1016/j.pharmthera.2018.01.010.
23. Zong ZH, Liu Y, Chen S, Zhao Y. Circ_PUM1 promotes the development of endometrial cancer by targeting the miR-136/NOTCH3 pathway [published online ahead of print, 2020 Feb 19]. J Cell Mol Med. 2020;10.1111/jcmm.15069. doi:10.1111/jcmm.15069.
24. Liu M, Wang Q, Shen J, Yang BB, Ding X. Circbank: a comprehensive database for circRNA with standard nomenclature. RNA Biol. 2019;16(7):899–905. doi:10.1080/15476286.2019.1600395.

25. Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M. CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol. 2016;13(1):34–42. doi:10.1080/15476286.2015.1128065.
26. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. 2014;42(Database issue):D92–D97. doi:10.1093/nar/gkt1248.
27. Zhang J, Liu W, Shen F, et al. The activation of microRNA-520h-associated TGF-β1/c-Myb/Smad7 axis promotes epithelial ovarian cancer progression. Cell Death Dis. 2018;9(9):884. Published 2018 Aug 29. doi:10.1038/s41419-018-0946-6.
28. Zhao S, Li G, Yang L, Li L, Li H. Response-specific progestin resistance in a newly characterized Ishikawa human endometrial cancer subcell line resulting from long-term exposure to medroxyprogesterone acetate. Oncol Lett. 2013;5(1):139–144. doi:10.3892/ol.2012.975.
29. Li W, Wang S, Qiu C, et al. Comprehensive bioinformatics analysis of acquired progesterone resistance in endometrial cancer cell line. J Transl Med. 2019;17(1):58. Published 2019 Feb 27. doi:10.1186/s12967-019-1814-6.
30. Qiu C, Dongol S, Lv QT, Gao X, Jiang J. Sterol regulatory element-binding protein-1/fatty acid synthase involvement in proliferation inhibition and apoptosis promotion induced by progesterone in endometrial cancer. Int J Gynecol Cancer. 2013;23(9):1629–1634. doi:10.1097/IGC.0000000000000004.
31. Chi S, Liu Y, Zhou X, et al. Knockdown of long non-coding HOTAIR enhances the sensitivity to progesterone in endometrial cancer by epigenetic regulation of progesterone receptor isoform B. Cancer Chemother Pharmacol. 2019;83(2):277–287. doi:10.1007/s00280-018-3727-0.
32. Cao W, Gao W, Zheng P, Sun X, Wang L. Medroxyprogesterone acetate causes the alterations of endoplasmic reticulum related mRNAs and lncRNAs in endometrial cancer cells. BMC Med Genomics. 2019;12(1):163. Published 2019 Nov 12. doi:10.1186/s12920-019-0601-9.
33. Xu H, Gong Z, Shen Y, Fang Y, Zhong S. Circular RNA expression in extracellular vesicles isolated from serum of patients with endometrial cancer. Epigenomics. 2018;10(2):187–197. doi:10.2217/epi-2017-0109.
34. Ye F, Tang QL, Ma F, et al. Analysis of the circular RNA transcriptome in the grade 3 endometrial cancer. Cancer Manag Res. 2019;11:6215–6227. Published 2019 Jul 5. doi:10.2147/CMAR.S197343.
35. Pu M, Chen J, Tao Z, et al. Regulatory network of miRNA on its target: coordination between transcriptional and post-transcriptional regulation of gene expression. Cell Mol Life Sci. 2019;76(3):441–451. doi:10.1007/s00018-018-2940-7.
36. Su CM, Wang MY, Hong CC, et al. miR-520h is crucial for DAPK2 regulation and breast cancer progression [published correction appears in Oncogene. 2017 Aug 21;:]. Oncogene. 2016;35(9):1134–1142. doi:10.1038/onc.2015.168.

37. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal. 2014;7(344):re8. Published 2014 Sep 23. doi:10.1126/scisignal.2005189.

Figures

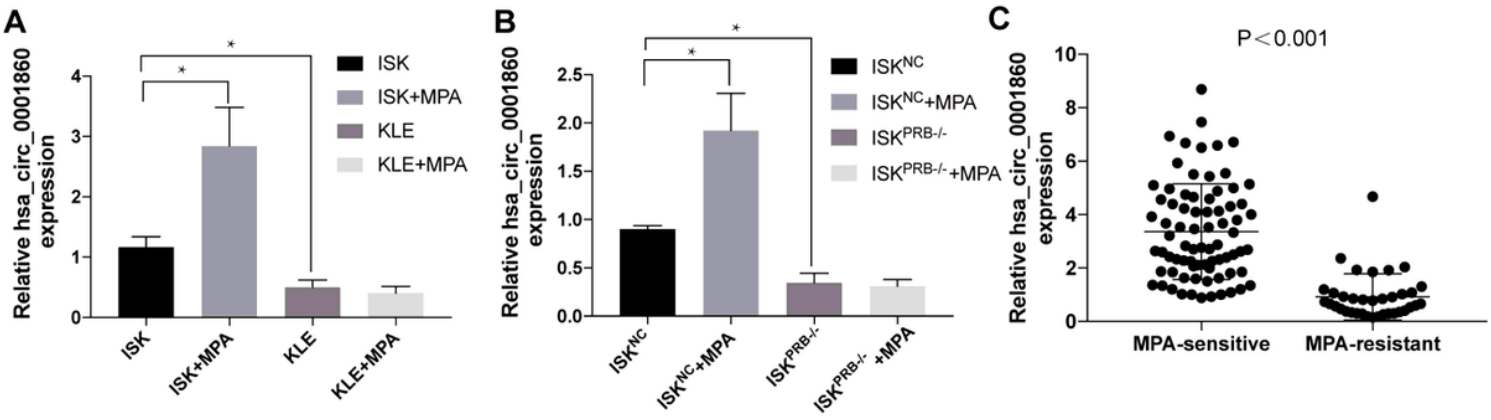

Figure 1

Hsa_circ_0001860 expression is decreased in MPA-resistant EC cells and tissues. Notes: (A, B) Expression levels of hsa_circ_0001860 in MPA-sensitive EC cell lines (ISK and ISKNC) and MPA-resistant EC cell lines (ISKPRB-/- and KLE) treated with vehicle or MPA. (C) qRT-PCR assay showed the expression level of hsa_circ_0001860 in tissue of patients from 36 MPA-resistant EC patients and 77 MPA-sensitive EC patients. *P < 0.05, ***P < 0.001

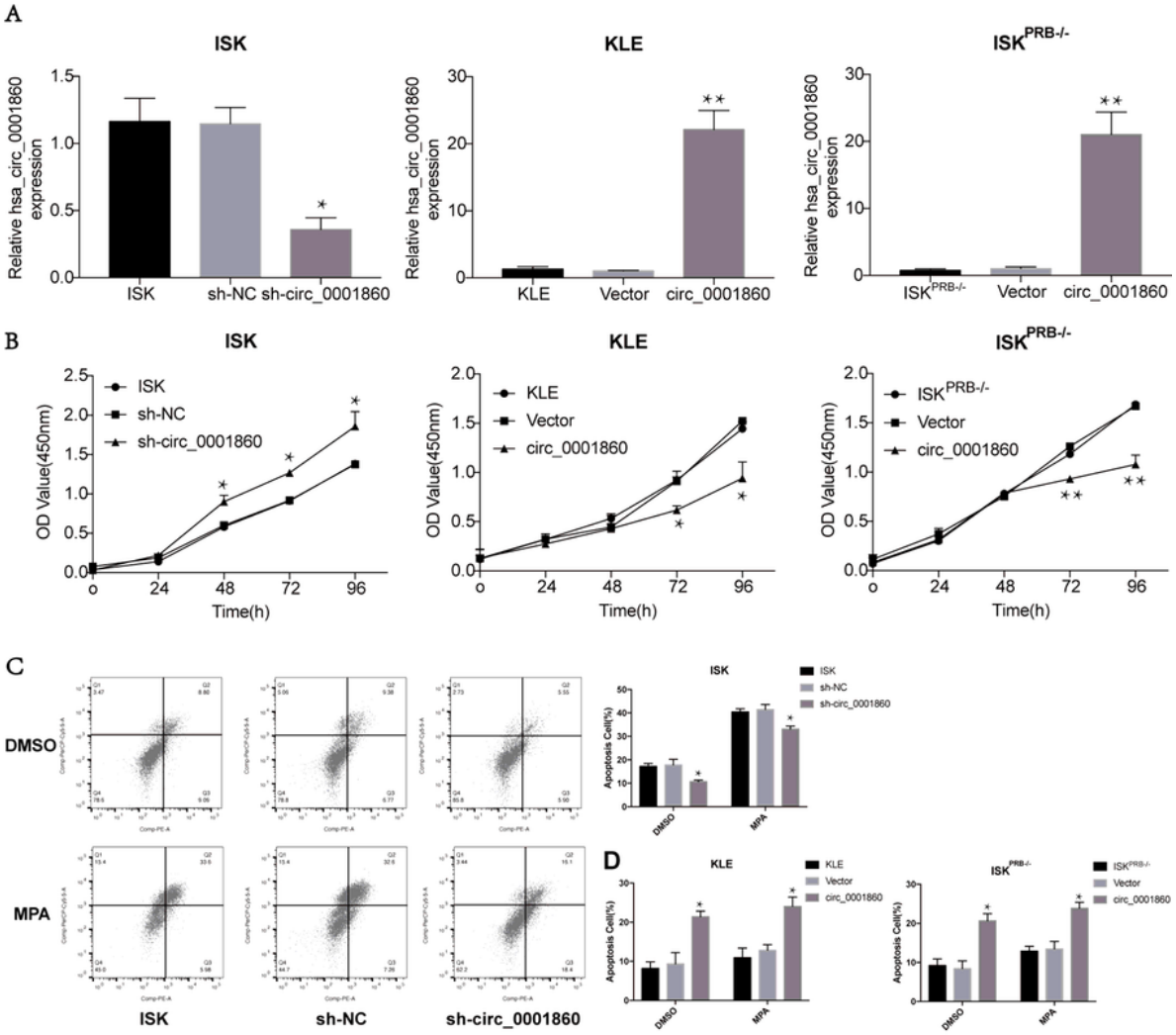

Figure 2

The effects of hsa_circ_0001860 on proliferation and apoptosis of endometrial cancer cells. Notes: (A) qRT-PCR analysis of hsa_circ_0001860 in ISK, ISKPRB-/- and KLE transfected with sh-NC, sh-circ_0001860, vector and circ_0001860. (B) CCK8 assay was conducted to evaluate cell proliferation. (C, D) Cells were treated with MPA (10 μM) or DMSO (control) and subjected to Annexin V-PE/7-AAD staining to detect apoptosis by flow cytometry. *P < 0.05, **P < 0.01

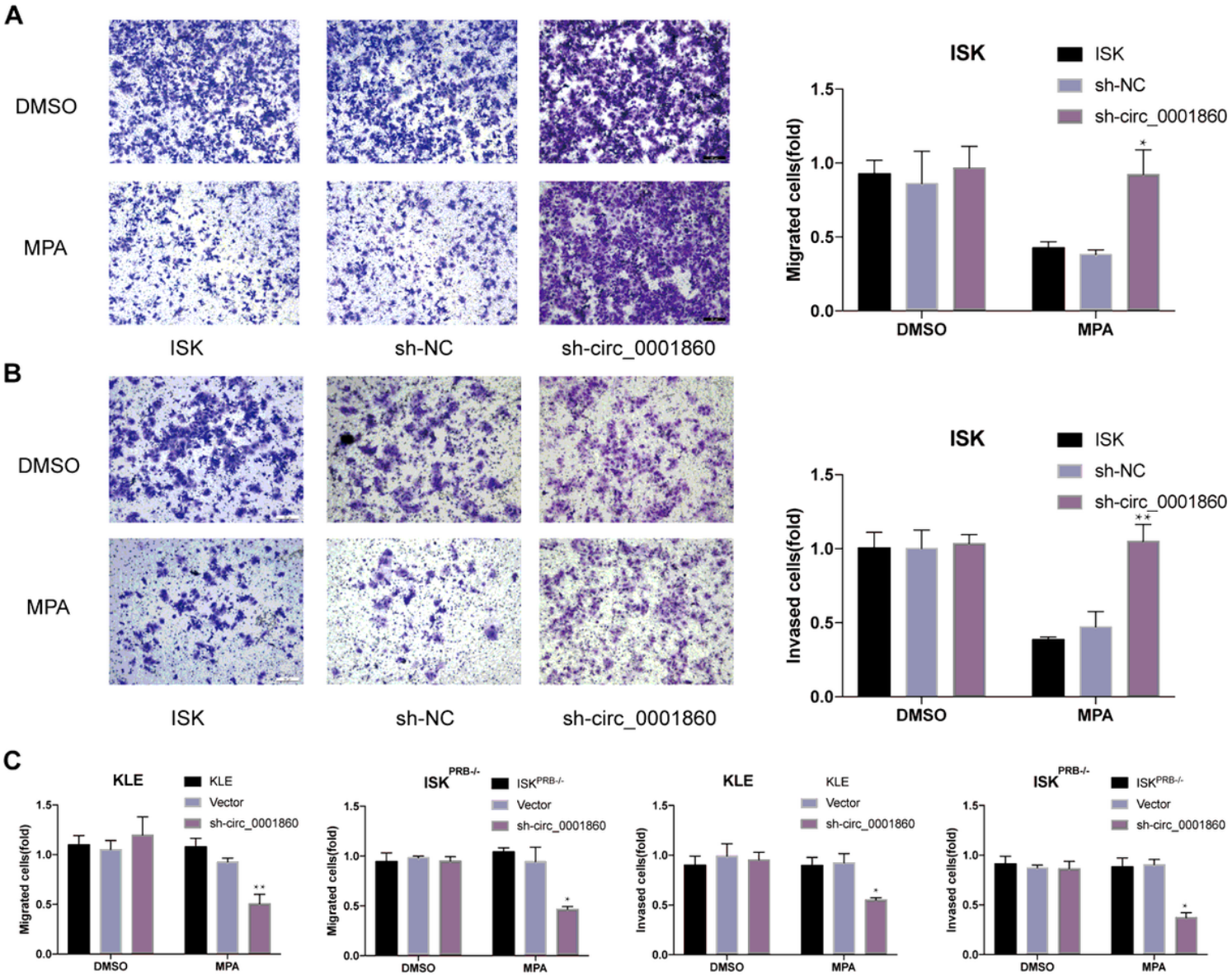

Figure 3

The effect of hsa_circ_0001860 on MPA sensitivity in migration and invasion of endometrial cancer cells. Notes: (A) Representative pictures and bar graphs showing the effect of hsa_circ_0001860 knockdown on MPA-induced alteration of ISK migration (magnification, 100*). (B) Representative pictures and bar graphs showing the effect of hsa_circ_0001860 knockdown on MPA-induced alteration of ISK invasion (magnification, 100*). (C, D) Overexpression of hsa_circ_0001860 in MPA-resistant ISKPRB-/- and KLE restored MPA sensitivity. *P < 0.05, **P < 0.01

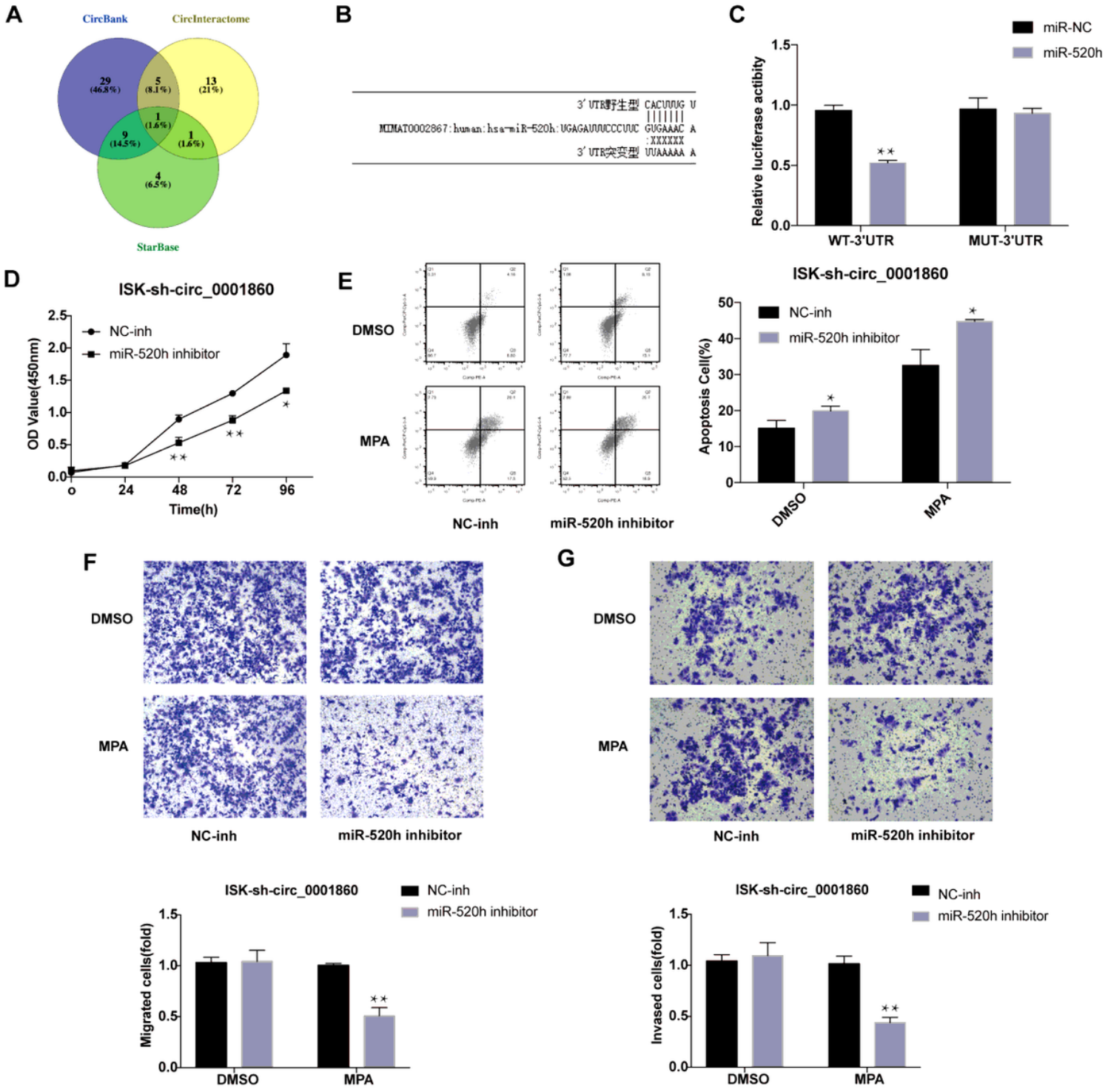

Figure 4

Hsa_circ_0001860 regulates tumor progression and MPA sensitivity of EC Cells via binding to miR-520h. Notes: (A) Venny diagram showing genes that are putative hsa_circ_0001860 targets computationally predicted by three algorithms (CircBank, CircInteractome and StarBase). (B) Bioinformatics analysis predicted the presence of a binding site for hsa_circ_0001860 on miR-520h. (C) The relative luciferase activities were analyzed in ISK cells co-transfected with miR-520h mimics or miR-NC and luciferase reporter WT or Mut. (D) Cell proliferation, (E) apoptosis, (F) migration and (G) invasion of ISK cells after

transfection with sh-circ_0001860 combined with miR-520h inhibitors or NC inhibitor. *P < 0.05, **P < 0.01

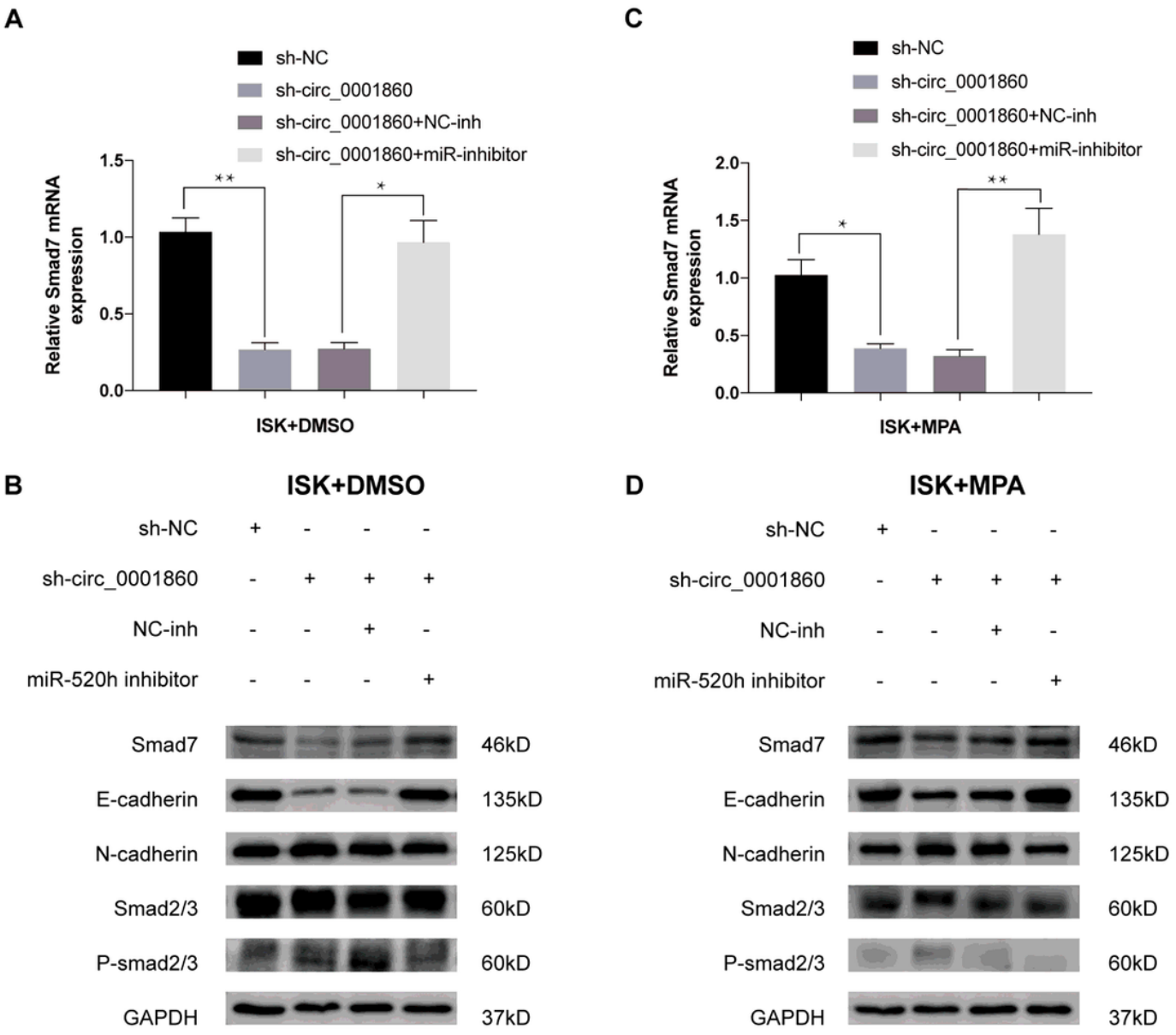

Figure 5

Hsa_circ_0001860 regulates Smad7 expression and activates the Smad7/EMT signaling pathway. *P < 0.05, **P < 0.01, ***P < 0.001 Notes: (A, C) The expression levels of Smad7 were analyzed using RT-qPCR. ISK cells were transfected with indicated vectors alone or co-transfected with inhibitors treated with DMSO (control) or MPA (10 μM). (B, D) The expression levels of Smad7, Smad7/EMT signaling molecules such as phosphorylated Smad2/3, E-cadherin and N-cadherin were determined using western blotting in ISK cells transfected with the indicated vectors alone or co-transfected with inhibitors treated with DMSO (control) or MPA (10 μM).

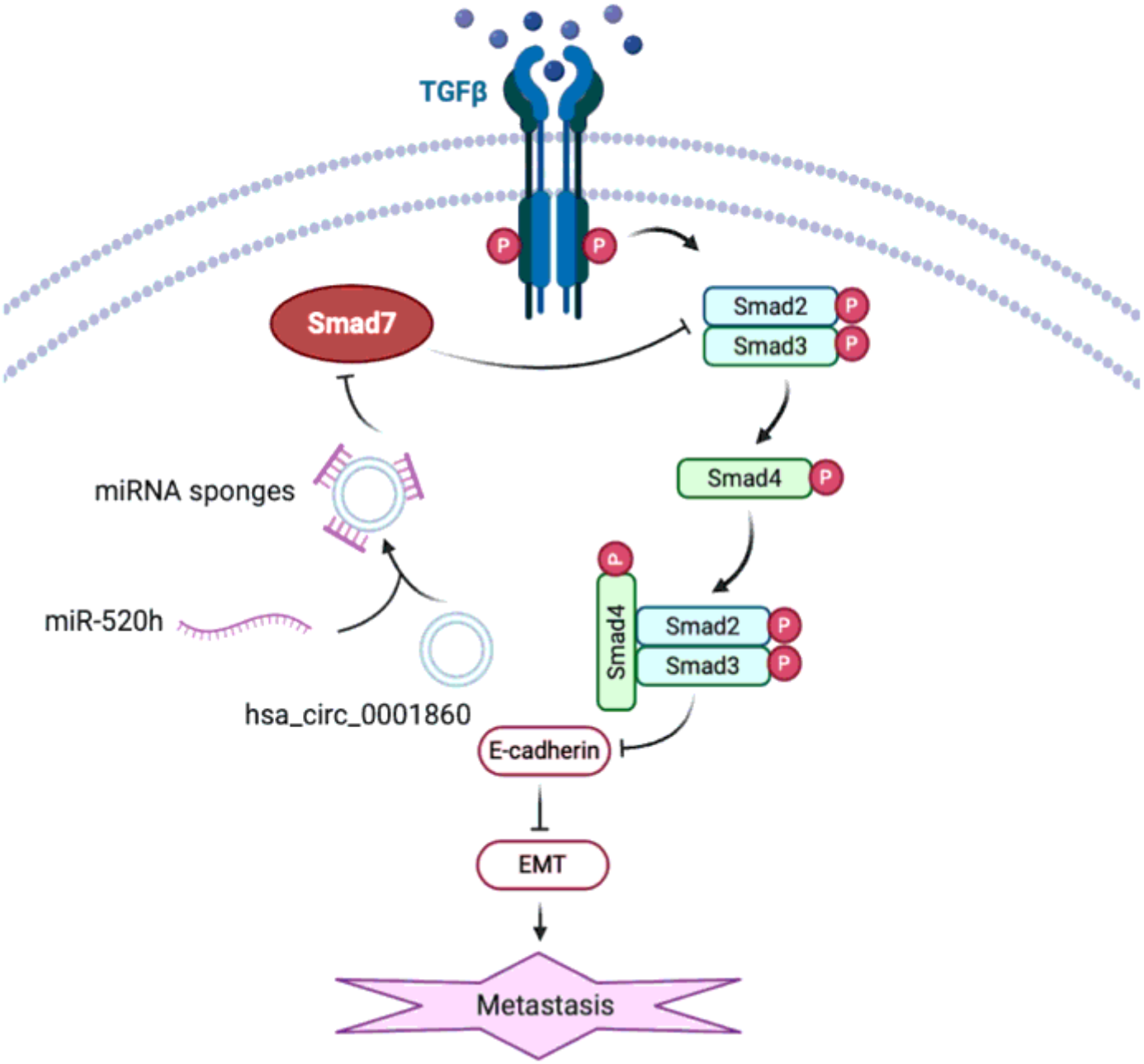

Figure 6

A working model for the role of hsa_circ_0001860 in EMT of EC cells treated with MPA. Notes: The expression of up-regulates in EC cells treated with MPA treatment induced increase of hsa_circ_0001860, which functions as a ceRNA for miR-520h and regulates the expression and activity of Smad7, thus inhibits phosphorylation of Smad2 / 3, leading to the formation of Smad2 / 4 complex and nuclear translocation and enhanced E-cadherin expression. Then, increased E-cadherin inhibits the migration and invasion and EC progression through EMT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx