Am J Transl Res 2025;17(2):1480-1494
www.ajtr.org /ISSN:1943-8141/AJTR0156669

Original Article

RBM10 suppresses malignant transformation in endometrial cancer via the Hippo-YAP signaling pathway

Wei Shi[1], Mengfei Wang[1], Qin Lin[1], Zeheng Lv[1], Kun Gao[2], Xiaoping Wan[1]

[1]*Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal-Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai 200092, China;* [2]*Department of Clinical Laboratory, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, Shanghai 200092, China*

Received March 19, 2024; Accepted January 20, 2025; Epub February 25, 2025; Published February 28, 2025

Abstract: Objective: To explore the genetic alterations, pathobiological functions, and downstream molecular mediators of RBM10 in endometrial cancer (EC) cells. Methods: Targeted sequencing and The Cancer Genome Atlas (TCGA) dataset analysis were performed. Following knockout (KO) or exogenous overexpression of RBM10 in EC cell lines, the biological functions and underlying mechanism of RBM10 in EC cells were evaluated by Western blot, qRT-PCR, CCK-8, Transwell, RNA-sequencing (RNA-seq), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Results: RBM10 mutation was present in a subset of ECs. RBM10 KO EC cells showed increased growth, migration, and invasion, compared to parental cells. Conversely, RBM10 overexpression reduced EC cell growth, migration, and invasion. KEGG pathway enrichment analysis showed that the expression of the Hippo-YAP pathway downstream targets was markedly upregulated in RBM10 KO EC cells. Mechanistically, RBM10 suppressed Yes Kinase-associated Protein (YAP) activity by promoting YAP phosphorylation. Conclusion: RBM10 acts as tumor suppressor in EC by modulating the Hippo-YAP signaling pathway.

Keywords: Endometrial cancer, RNA-binding motif protein 10, growth, migration, invasion, Hippo-YAP signaling pathway

Introduction

Endometrial cancer (EC) is a prevalent malignancy in the female reproductive system, representing approximately 4.8% of all systemic tumors in women [1]. The most common symptom of the disease is irregular vaginal bleeding. The average age of onset is 60 years old. It is considered to mainly occur in postmenopausal women, with around 14% of cases occurring in premenopausal women, and 5% younger than 40 years old [1-3]. About 75% of the patients are diagnosed early in stage FIGO or stage II, and the 5-year survival rate is about 74-91% [4]. With the improvement of economic conditions and the prolongation of the average life span of the patients, the incidence rate of EC has been rising annually [5]. In 2020, there were 81,964 new cases of EC and 16,607 deaths reported in China [6]. Although the mortality of EC can be effectively controlled and reduced through early diagnosis, surgery and chemotherapy, middle and advanced EC or chemotherapy insensitive tumors still have a low five-year survival rate [7]. Given this low five-year survival rate, the development of new therapeutic strategies for EC is urgently required.

RNA-binding motif protein 10 (RBM10), a member of the RNA binding protein (RBP) family, plays a crucial role in RNA splicing and gene expression regulation [8]. RBM10 has a total length of nearly 3500 bp and is divided into 24 exons, which is translated into a protein containing 930 amino acids [8]. RBM10 is abnormally expressed in cancers such as lung cancer, breast cancer, colorectal cancer, EC, cervical cancer, and osteosarcoma [9]. Traditionally, RBM10 is considered a tumor suppressor, promoting apoptosis via p53, Bcl-2, Bax, and other proteins, and inhibiting proliferation through the Notch and Rap1a/Akt/CREB pathways [10, 11]. Recently, it was demonstrated that RBM10 can also inhibit colonization [12]. Mutations in

https://doi.org/10.62347/FHXK9469

RBM10 cause TARP syndrome, a rare X-linked syndrome often resulting in pre- or post-natal lethality in affected males, indicating that RBM10 plays an important role in fetal development [13]. RBM10 is reported to be mutated in many types of tumors. For example, RBM10 is mutated in nearly 7% of lung adenocarcinoma, and the mutation rate in advanced lung adenocarcinoma reaches 21% [14]. Mutations in RBM10 have also been found in pancreatic ductal papillary mucinous gland tumors, pancreatic ductal adenocarcinoma, rectal cancer, and undifferentiated thyroid cancer [10, 15-17]. The high occurrence of nonsense and frameshift mutations suggests RBM10's potential role as a tumor suppressor in EC. However, the mechanisms of RBM10 mutation-induced tumorigenesis remain largely unknown.

In the present study, RBM10 mutation in EC was examined by the targeted sequencing of EC specimens. The biological function and underlying molecular mechanisms of RBM10 in the proliferation, migration and invasion of EC cells were evaluated.

Materials and methods

Cell culture

Ishikawa, KLE, RL95-2, Hec-1A and Hec-1B were purchased from American Type Culture Collection or Shanghai Cell Bank. Ishikawa, KLE, and RL95-2 cells were cultured in RPMI 1640 medium, while Hec-1A and Hec-1B cells were cultured in Ham's F12 nutrient medium supplemented with 10% fetal bovine serum (FBS; Thermo), 1% penicillin, and 1% streptomycin at 37°C in a humidified incubator with 5% CO_2.

Construction of RBM10 knockout Ishikawa and KLE cell lines

CRISPR/Cas9-mediated gene knockout was conducted in Ishikawa and KLE cell lines. Guide oligos targeting RBM10 were subcloned into the pX459 plasmid (Addgene). The sgRNA construct was transfected into the cells for 24 hours, followed by culture in 1 μg/ml puromycin for 3 days. Surviving cells were isolated as monoclonal cell lines in a 96-well plate by limited dilution. Knockout (KO) cell clones were identified via Western blot and validated by Sanger sequencing. The sequences of sgRNA and primers for the amplification of the RBM10 gene's sgRNA-targeted sequence are listed in Supplementary Table 1.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RBM10 expression levels in EC cell lines were measured. Total RNA extracted using TRIzol reagent (Tiangen, China) was reverse-transcribed into cDNA using miRNA-specific RT primers (Thermo Fisher) and MultiScribe Reverse Transcriptase (Thermo Fisher). qRT-PCR was conducted with a TaqMan miRNA qRT-PCR assay (Applied Biosystem) using the ABI-Prism 7300 System (Applied Biosystem). GAPDH (Thermo) was the internal control, and RBM10 expression was analyzed using the 2-ΔΔCt method. Primers used in this study are shown in Supplementary Table 2.

Doxycycline inducible expression of exogenous RBM10

EC cell lines were infected with pGLV-FLAG-RBM10 lentivirus in the presence of polybrene (8 μg/ml) for 48 hours, and cells were selected with puromycin (1.5 μg/ml) for 2 weeks to generate stable cell lines. Inducible FLAG-RBM10 expression was achieved by adding doxycycline (DOX, 10 ng/ml) to the media for 24 hours.

Western blot

Total proteins were extracted from EC cell lines using Radio Immunoprecipitation Assay (RIPA) lysis buffer (Thermo). The supernatant was collected after centrifugation at 12,000×g for 10 minutes at 4°C. Protein concentration was measured using the Pierce BCA Protein Assay kit (Thermo), and 30 μg of protein were separated on a 12.5% SDS-PAGE gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Thermo). The membrane was blocked with 5% Bovine Serum Albumin (BSA) at 25°C for 2 hours, then incubated with primary antibodies: RBM10 (1:1,000), phosphorylated YAP (p-YAP; 1:1,000), and GAPDH (1:1,000) overnight at 4°C. Secondary antibody incubation (1:3,000) followed at 37°C for 2 hours. All antibodies were from Proteintech Group, Inc. The immunoreactivity was visualized using Enhanced Chemiluminescence (ECL) (Beyotime). Protein density was normalized to GAPDH and quantified using ImageJ software 4.6 (National Institutes of Health, Bethesda, MD, USA).

Cell proliferation assay

Cell proliferation was assessed using the CCK-8 Kit (Dojindo) following the manufacturer's instructions. Cells (1×10^3 cells/ml) were seeded in 96-well plates. At various time points, 10 µl CCK-8 solution was added to each well for 2 hours at 37°C. Optical density at 450 nm was recorded using a Multimode Plate Reader (Molecular Devices). Each treatment was performed in triplicate, and experiments were repeated three times.

Cell invasion and migration assays

Transwell chambers with and without Matrigel coating were used to assess cell migration and invasion. EC cell lines (1×10^5/ml) were placed into the upper chamber with a non-coated membrane for migration assays and in serum-free medium for invasion assays. Cells were transferred to the tops of BD BioCoat Matrigel Invasion Chambers (BD Biosciences) as per the manufacturer's instructions. Cells were stained with crystal violet solution (1%) for 30 minutes at room temperature. Tumor cell invasion and migration were counted in at least three randomly stained fields per membrane under a microscope.

Targeted sequencing of samples from EC patients

Endometrioid adenocarcinoma samples from 2015 to 2018 were collected. The primary tissue and blood samples were from EC patients who had not undergone radiotherapy or chemotherapy. Genomic DNA was extracted, and 99 formalin-fixed, paraffin-embedded (FFPE) EC specimens were collected for targeted sequencing. Targeted next-generation sequencing was conducted using a 520-gene panel (OncoScreen Plus, Burning Rock Biotech) to identify genetic alterations related to cancer development and targeted therapy. An average reading depth of 1000x was produced on a MiSeq platform (Illumina, USA). Sample collection was approved by the ethics committee of Tongji University.

Bioinformatics analyses

Bioinformatics analyses of the EC from TCGA dataset was performed. The potential prognostic value of RBM10 mutations in EC was evaluated. Total RNA of cell samples was extracted. Transcriptome libraries were constructed using the NEBNext Ultra RNA Library Prep Kit and sequenced on the Illumina NovaSeq platform with paired-end 150 bp reads, yielding approximately 50 million reads per sample. Raw data quality was assessed with FastQC, and low-quality reads (bases with quality scores <20 and reads shorter than 50 bp) as well as adapter sequences were removed using Trimmomatic. The cleaned reads were aligned to the human reference genome (GRCh38) using HISAT2, achieving alignment rates exceeding 95%. Gene expression levels were quantified using FeatureCounts, and differential expression analysis was performed with DESeq2 (Fold Change ≥2, FDR <0.05). Subsequently, KEGG enrichment analyses of the differentially expressed genes were conducted using clusterProfiler, and heatmaps were generated to visualize gene expression patterns. GSEA analysis of the hippo signal pathway-related gene signature in wild-type and RBM10 KO Ishikawa cells was performed as well.

Statistical analysis

Statistical Product and Service Solutions (SPSS) version 18.0 was used for data analysis. Counting data were expressed as percentages, and measurement data were described as mean ± standard deviation (SD). The t-test, one-way ANOVA or Repeated measures ANOVA followed by Bonferroni analysis were adopted for data analysis. A P value <0.05 was considered significant.

Results

RBM10 is mutated in EC

Firstly, 99 primary EC specimens of patients without chemotherapy in our hospital were collected and sequenced. By targeted sequencing, we obtained 312 genes of whole exome sequence and 208 genes of mutation sites in hotspot mutation regions. Particularly, we found the genetic alternation of RBM10 was observed in 9% of ECs, including 2 inframe mutations and 7 missense mutations (**Figure 1A**). Then, we performed bioinformatics analyses of the EC samples from the TCGA dataset. A total of 45 RBM10 mutations occurred in 546 EC tissues (8.2%), including 40 missense mutations (7.3%), 3 truncating mutations (0.5%), and 2 splice mutations (0.4%) (**Figure 1B**). We evalu-

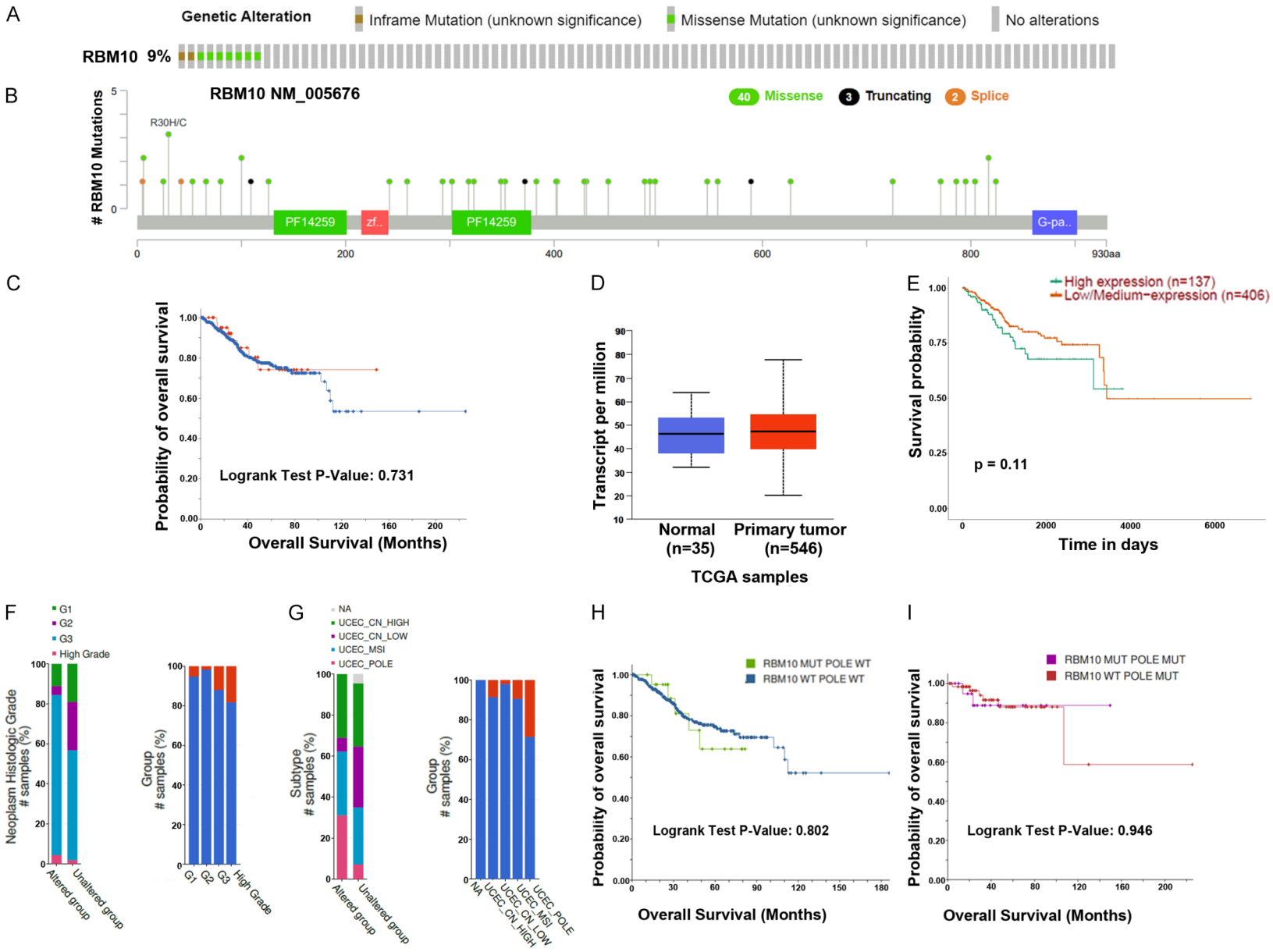
A
Genetic Alteration
Inframe Mutation (unknown significance)
Missense Mutation (unknown significance)
No alterations
RBM10 9%
B
RBM10 NM_005676
40 Missense
3 Truncating
2 Splice
RBM10 Mutations
R30H/C
PF14259
zf..
PF14259
G-pa..
930aa
C
Probability of overall survival
Logrank Test P-Value: 0.731
Overall Survival (Months)
D
Transcript per million
Normal (n=35)
Primary tumor (n=546)
TCGA samples
E
High expression (n=137)
Low/Medium-expression (n=406)
Survival probability
p = 0.11
Time in days
F
G1
G2
G3
High Grade
Neoplasm Histologic Grade # samples (%)
Altered group
Unaltered group
Group # samples (%)
G
NA
UCEC_CN_HIGH
UCEC_CN_LOW
UCEC_MSI
UCEC_POLE
Subtype # samples (%)
H
RBM10 MUT POLE WT
RBM10 WT POLE WT
Probability of overall survival
Logrank Test P-Value: 0.802
Overall Survival (Months)
I
RBM10 MUT POLE MUT
RBM10 WT POLE MUT
Probability of overall survival
Logrank Test P-Value: 0.946
Overall Survival (Months)

Figure 1. RBM10 mutation and prognostic value in the EC patients from TCGA database. A. Targeted next-generation sequencing (Illumina) was performed on 99 EC patients without chemotherapy, and RBM mutation on these patients was analyzed. B. Positions of individual somatic mutations of RBM10 in EC patients from TCGA database. C. KM survival curves of RBM10-mutated and wild-type EC patients. D. The mRNA expression of RBM10 in EC tumor tissues and its adjacent normal tissues. E. KM survival curves of RBM10-high and -low mRNA expressed EC patients. F. The co-occurrence of RBM10 mutation and EC histological grade in the EC patients from TCGA database. G. The co-occurrence of RBM10 mutation and POLE mutation in the EC patients from TCGA database. H. KM survival curves of RBM10-mutated and wild-type cohorts of EC patients with wild-type POLE. I. KM survival curves of RBM10-mutated and wild-type cohorts of EC patients with POLE mutation. Note: EC: Endometrial cancer; TCGA: The Cancer Genome Atlas.

ated the potential prognostic value of RBM10 mutations in EC and found that EC patients with RBM10 mutations appeared to have a better prognosis than patients without RBM10 mutations in their long-term survival cohorts, though statistics was not significant (P=0.731, **Figure 1C**). Meanwhile, there was no significant difference in the RBM10 mRNA level between EC and the adjacent normal tissues (**Figure 1D**). The mRNA expression level of RBM10 was not correlated with the overall survival of EC patients (**Figure 1E**). Moreover, RBM10 mutation was more frequently observed in grade 3 (G3) and high-grade EC patients, while patients without RBM10 mutations had a higher chance for diagnosis with ECs of grade 2 (G2) and grade 1 (G1) (**Figure 1F**). Previous study reported that survival benefit was found in EC tumors with POLE mutations when compared with other EC subtypes. We also observed the co-occurrence of RBM10 and POLE mutations in EC patients (**Figure 1G**). However, RBM10 mutation was not a favorable factor in both the POLE wild-type (**Figure 1H**, P=0.802) and POLE mutations (**Figure 1I**, P=0.945). Collectively, these data indicate that RBM10 is mutated in subset of ECs, and its clinical prognostic value in ECs requires further investigations.

Expression of RBM10 protein in EC cell lines

To explore the effect of RBM10 on the phenotype of EC cells, five EC cell lines including Ishikawa, KLE, RL95-2, Hec-1A and Hec-1B were collected. Then, protein expression of RBM10 was measured by Western blot. As shown in **Figure 2A**, RBM10 was ubiquitously expressed in all tested EC cell lines. The highest protein expression of RBM10 was found in Ishikawa and KLE cells, while lowest protein expression of RBM10 was found in Hec-1B cells. Therefore, we chose Ishikawa and KLE cells for RBM10 KO and Hec1-B cells for RBM10 overexpression.

Construction of RBM10 knockout Ishikawa and KLE cell lines

We constructed RBM10 KO Ishikawa (sg-RBM10#1 and sg-RBM10#2) and KLE cell lines (sg-RBM10#3) using the CRISPR/Cas9 methods. Western blot results showed that RBM10 protein was significantly reduced in KO cell lines (**Figure 2B**). The RBM10 mRNA levels were also significantly decreased in KO cell lines (**Figure 2C**). Then, we extracted the genomic DNA in the RBM10 KO Ishikawa cells, designed primers according to the target sequences of two RBM10 sgRNAs, and performed Sanger sequencing to confirm that we have knocked out the RBM10 fragment at the gene level. The results showed that the knockout strain sg-RBM10#1 lost 28 bases and sg-RBM10#2 lost 8 bases, all of which caused frameshift mutation (**Figure 2D**, **2E**). The above results indicated that we had successfully obtained two RBM10 KO Ishikawa cells. RBM10 KO KLE cell line (sg-RBM10#3) was also constructed. Western blot, qRT-PCR and Sanger sequencing confirmed RBM10 knockout at protein level (**Figure 3A**), mRNA level (**Figure 3B**), and genomic DNA level (**Figure 3C**, **3D**).

Exogenous RBM10 can be induced by DOX in EC cell lines

Exogenous RBM10 expression was induced by DOX in RBM10 KO Ishikawa cells and Hec-1B cells with low RBM10 expression. Western blot confirmed that the high expression level of exogenous RBM10 was induced by DOX in both RBM10 KO Ishikawa cells (**Figure 4A**) and l Hec-1B cells (**Figure 5A**).

Effect of RBM10 on EC cell growth

In order to explore the biological functions of RBM10 in EC cells, the effect of RBM10 on cell growth of EC cell lines was determined by

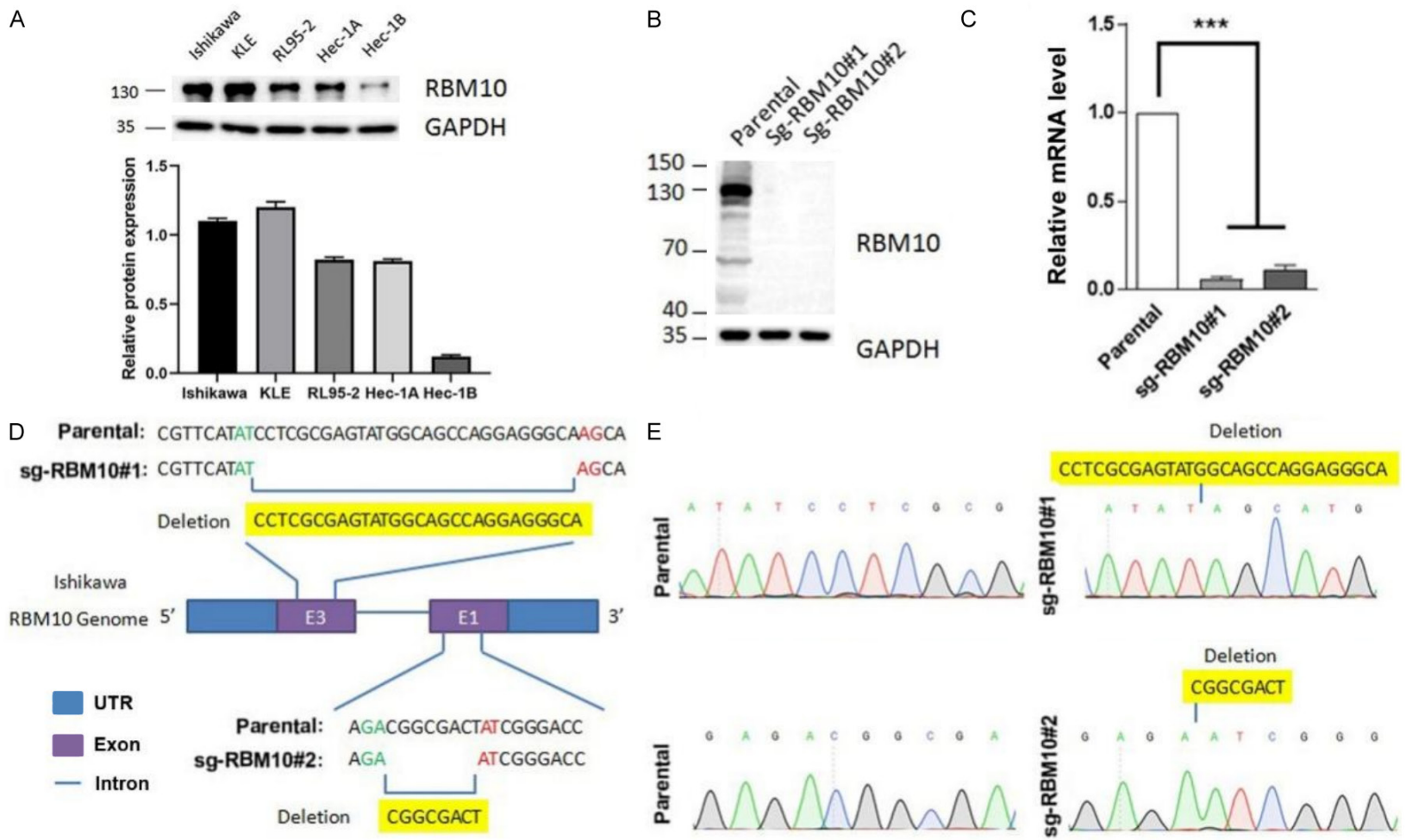

Figure 2. Identification of RBM10 knockout Ishikawa cell line. A. Expression of RBM10 protein in endometrial cancer cell lines, including Ishikawa, KLE, RL95-2, Hec-1A and Hec-1B (Western blot). B. Expression level of RBM10 protein in two RBM10 knockout Ishikawa cells (Western blot). C. The expression of RBM10 mRNA in two RBM10 knockout Ishikawa cells (qRT-PCR). D, E. DNA base deletion of two RBM10 knockout Ishikawa cells (Sanger sequencing). Note: Compared with parental group, ***P<0.001.

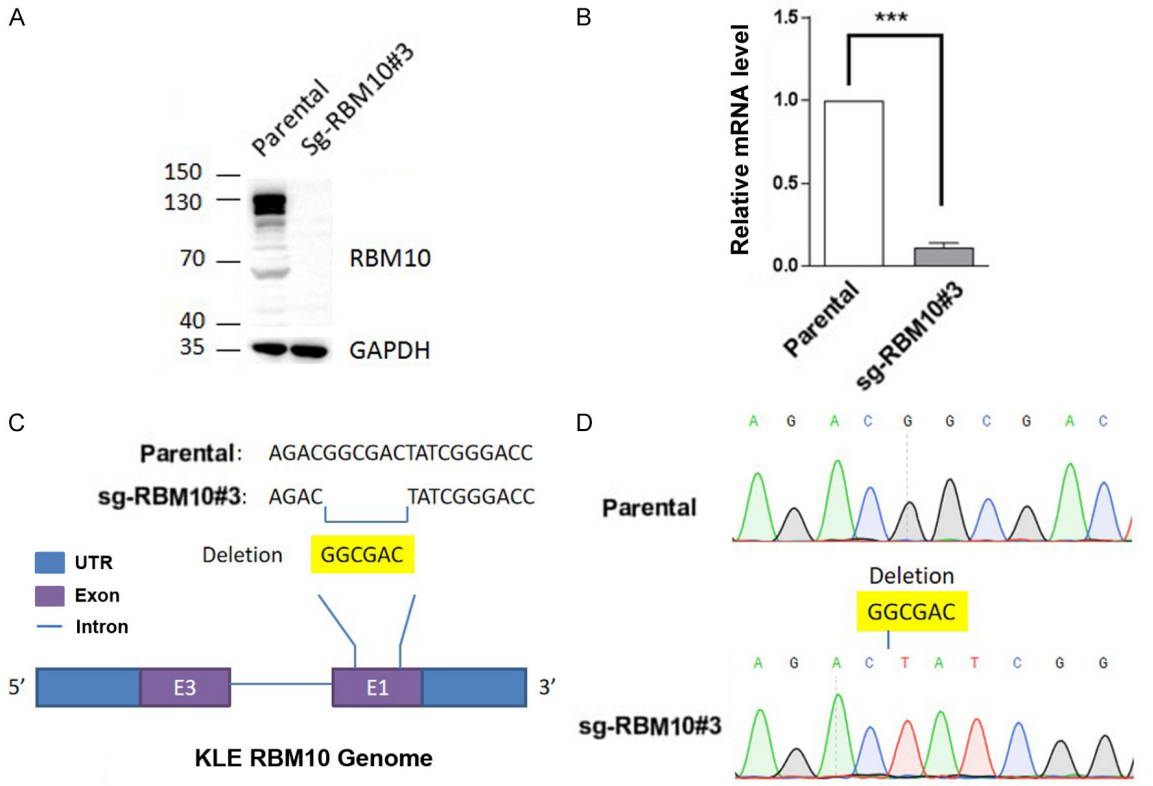

Figure 3. Identification of RBM10 knockout KLE cell line. A. The expression level of RBM10 protein in RBM10 knockout KLE cells (Western blot). B. The expression of RBM10 mRNA in RBM10 knockout KLE cells (qRT-PCR). C, D. DNA base deletion of RBM10 knockout KLE cells (Sanger sequencing). Note: Compared with parental group, ***P<0.001.

CCK-8 method and colony formation assays. Compared with wild-type EC cells, the proliferation ability (**Figure 4B**, **4C**) and the number of clones (**Figure 4F**, **4G**) was significantly enhanced in RBM10 KO Ishikawa and KLE cell lines (all P<0.01). In addition, we found that after exogenous induction of RBM10 expression in RBM10 KO Ishikawa and KLE cells by DOX induction, the proliferation ability (**Figure 4D**, **4E**) and the number of clones (**Figure 4F**, **4G**) were down-regulated, similar to those in wild-type cells (all P<0.01). Similarly, there were no significant differences in the proliferation ability (**Figure 5B**) and the number of clones (**Figure 5D**) between non-exogenous induction of RBM10 expression Hec-1B cells and wild-type cells. However, after exogenous induction of RBM10 expression by DOX, the proliferation ability (**Figure 5C**) and the number of clones (**Figure 5D**) were significantly down-regulated when compared to those in the wild-type cells (all P<0.01). Taken together, these data indicate that RBM10 negatively regulates EC cell growth.

Effect of RBM10 on migration and invasion of EC cell lines

The effect of RBM10 on the migration and invasion of EC cells was evaluated following RBM10 knockdown or overexpression. The results showed that compared with wild-type cells, the migration and invasion ability was markedly increased in RBM10 KO Ishikawa (**Figure 6A**, P<0.01) and KLE (**Figure 6B**, P<0.01) cells, while markedly decreased in RBM10-overexpressed Hec-1B cells (**Figure 6C**, P<0.01). Meanwhile, after being induced by DOX in RBM10 KO Ishikawa (**Figure 6A**) and KLE cells, the migration and invasion ability of cells was weakened, similar to wild-type cells (all P<0.01) (**Figure 6B**). Taken together, these data suggest that RBM10 suppresses EC cell migration and invasion.

Gene expression profile of RBM10 KO EC cell lines

In order to clarify the molecular mechanisms of malignant transformation of EC cells induced

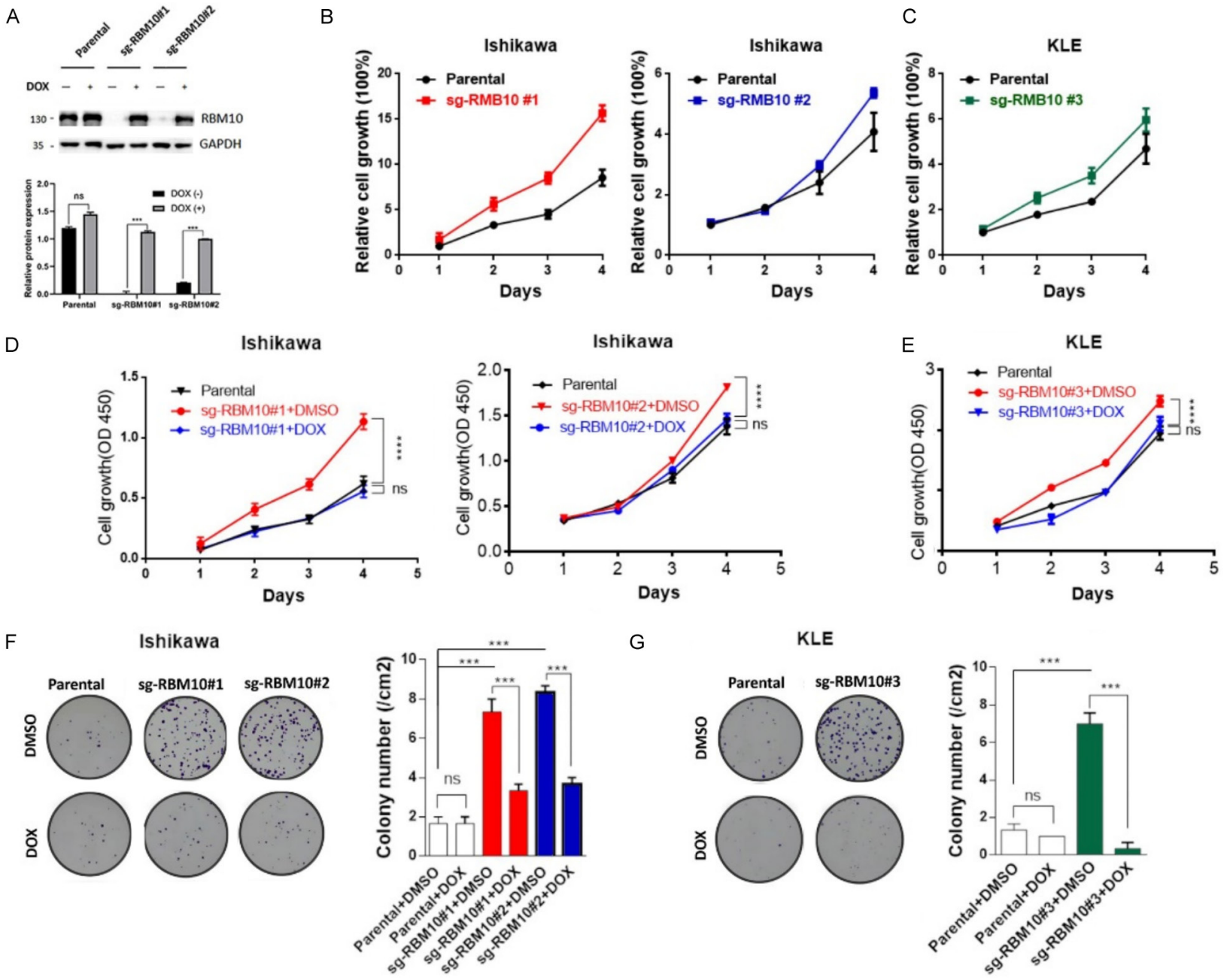
A
Parental
sg-RBM10#1
sg-RBM10#2
DOX
RBM10
GAPDH
Relative protein expression
DOX (-)
DOX (+)
ns
B
Ishikawa
Parental
sg-RMB10 #1
sg-RMB10 #2
Relative cell growth (100%)
Days
C
KLE
sg-RMB10 #3
D
Ishikawa
sg-RBM10#1+DMSO
sg-RBM10#1+DOX
sg-RBM10#2+DMSO
sg-RBM10#2+DOX
Cell growth(OD 450)
E
KLE
sg-RBM10#3+DMSO
sg-RBM10#3+DOX
F
Ishikawa
DMSO
Colony number (/cm2)
Parental+DMSO
Parental+DOX
sg-RBM10#1+DMSO
sg-RBM10#1+DOX
sg-RBM10#2+DMSO
sg-RBM10#2+DOX
G
KLE
sg-RBM10#3+DMSO
sg-RBM10#3+DOX

Figure 4. Effects of RBM10 on growth of endometrial cancer cell lines after RBM10 knockout and overexpression induced by doxycycline (DOX). A. RBM10 knockout Ishikawa cells were treated with virus and DOX (20 ng/mL) for 24 h, and the expression level of RBM10 protein (Western blot). B, C. The growth of wild-type and RBM10 knockout Ishikawa cells or KLE cells (CCK-8 assay). D, E. Overexpression of RBM10 of RBM10 knockout Ishikawa cells or KLE cells induced by DOX (cell growth was measured by CCK-8 assay). F, G. The colony number of cells RBM10 knockout Ishikawa and KLE cells with or without DOX induction. Note: ***P<0.001; ****P<0.0001; ns: no significance.

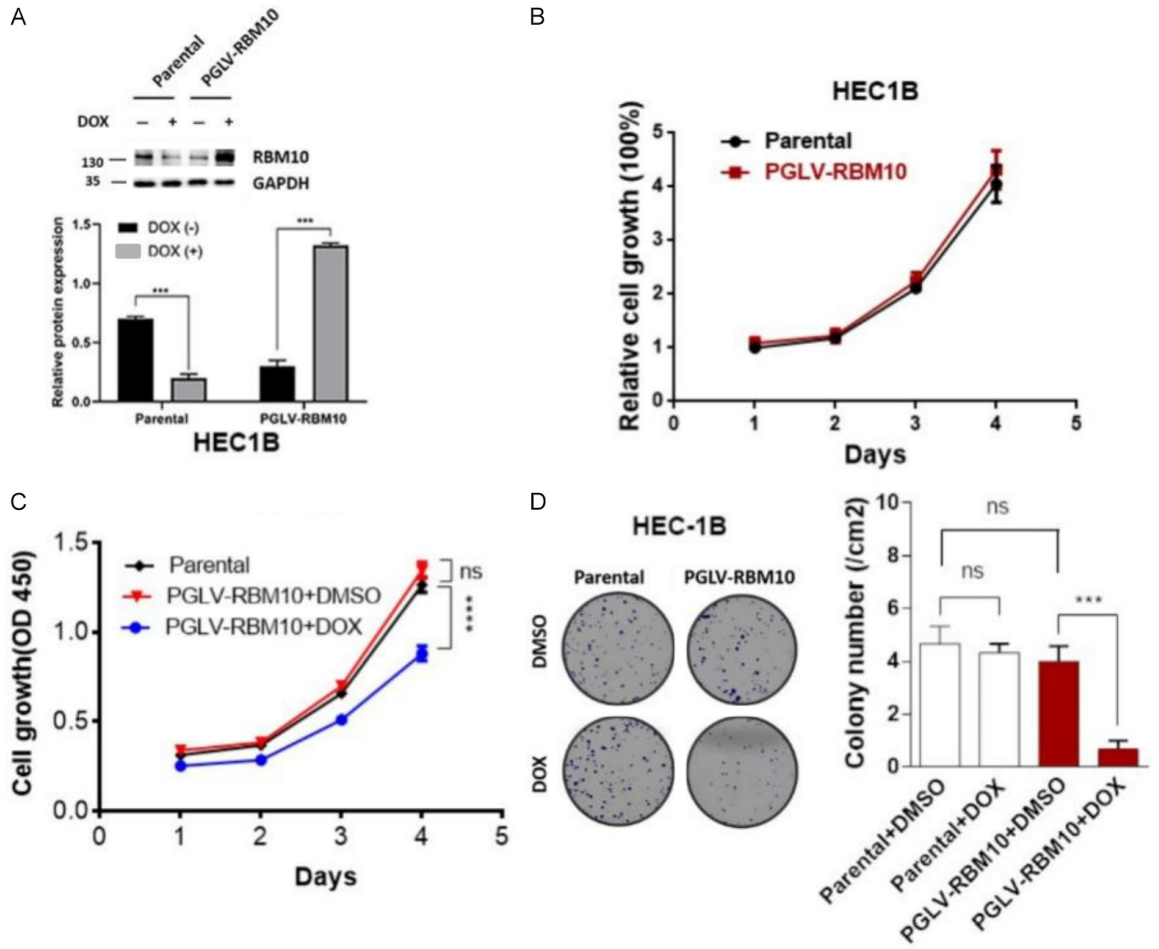

Figure 5. Effects of RBM10 on growth of Hec-1B cells after RBM10 overexpression induced by doxycycline (DOX). (A) Hec-1B cells were treated with virus and DOX (20 ng/mL) for 24 h, and the expression level of RBM10 protein was measured by Western blot. (B, C) The proliferation and (D) colony number of Hec-1B cells were measured after RBM10 overexpression induced by DOX. Note: ***P<0.001; ****P<0.0001; ns: no significance.

by RBM10 KO, we used the mixed cell pool of wild-type Ishikawa cells and two independent RBM10 knockout monoclones for transcriptome sequencing to analyze the changes of mRNA expression profile. Compared with wild-type cells, a total of 622 protein coding genes were differentially expressed in RBM10 knockout cells (**Figure 7A**). Among them, 341 genes were up-regulated (>2-fold) and 281 genes were down regulated (<2 times). Through cluster analysis of differential genes, we obtained the signal pathway and biological process affected by RBM10 knockout. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that a variety of signal pathways in RBM10 knockout cells had significant changes, including signaling pathways regulating pluripotency of stem cells, transcriptional misregulation in cancer, focal adhesion, and Hippo signal pathway (**Figure 7B**), suggesting that RBM10 was likely to interfere with these pathways. In this study, we aimed to explore the potential role of RBM10 in the Hippo/YAP signaling pathway which has an important

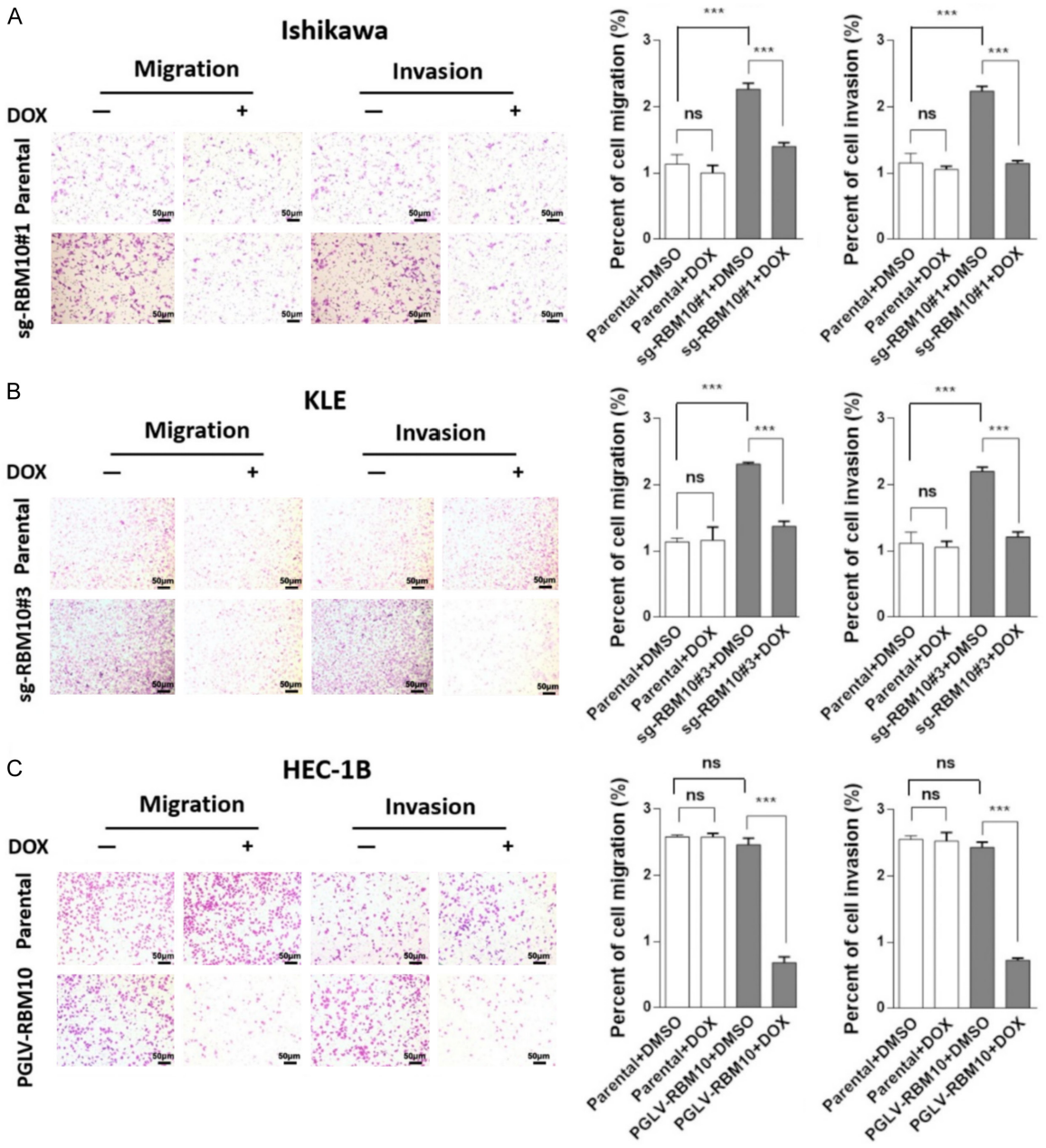

Figure 6. Effects of RBM10 on cell migration and invasion in endometrial cancer cell lines. The EC cell lines were treated with virus and DOX (20 ng/mL) for 24 h, and the migration and invasion of cells was determined in RBM10 knockout Ishikawa cells (A), KLE cells (B), and RBM10 overexpressed Hec-1B cells (C). Note: ***P<0.001; ns: no significance.

role in homeostasis and tumorigenesis. Gene set enrichment analysis (GSEA) suggested that RBM10 knockout upregulated 23 genes and downregulated 27 genes that are related to the hippo signal pathway in Ishikawa cells (**Figure 7C**, **7D**). Taken together, these data suggest that RBM10 regulates the transcriptional outputs of the Hippo/YAP pathway.

RBM10 KO affects the expression of YAP target genes

The mRNA expression of Hippo signal pathway-related genes after RBM10 KO was measured to confirm the above findings. The transcriptional targets of the Hippo signaling pathway, including ANKRD1, CTGF, CYR61, THBS1, AR-

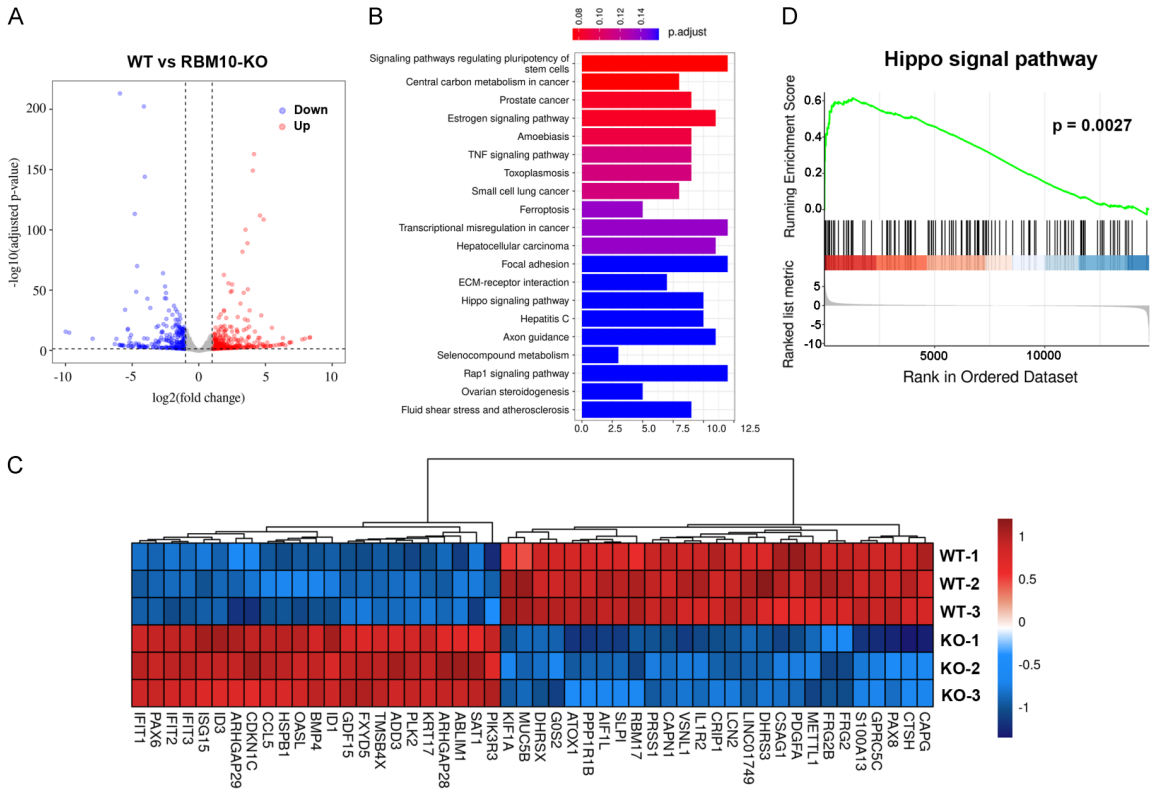

Figure 7. Gene expression profile of RBM10 knockout EC cell lines. A. RNA-seq analysis of differential genes between wild-type and RBM10 knockout Ishikawa cells. B. The signal pathway changes mediated by RBM10 knockout in Ishikawa cells (KEGG pathway enrichment). C. Heatmap showing 23 upregulated genes and 27 downregulated genes that were related to the hippo signal pathway in Ishikawa cells after RBM10 knockout. D. GSEA analysis of the hippo signal pathway-related gene signature in wild-type and RBM10 knockout (KO) Ishikawa cells. Note: WT: wild type; KEGG: Kyoto Encyclopedia of Genes and Genomes; GSEA: Gene Set Enrichment Analysis.

HGAP29, TAGLN, and CDH2, were significantly upregulated (**Figure 8A**). Accumulated evidence has proven the critical roles of the Hippo/YAP signaling pathway in tumor growth, angiogenesis, epithelial-mesenchymal transition (EMT), and metastatic dissemination. YAP Ser127 phosphorylation has been widely used as an indicator of YAP inactivation. We found that the expression of p-YAP in the RBM10 KO cell line was down-regulated, while the expression of p-YAP increased after exogenous induction of RBM10 expression (**Figure 8B**), indicating that RBM10 can promote YAP phosphorylation to inactivate YAP activity. **Figure 9** shows a schematic diagram of the effect and molecular mechanism of RBM10 on EC.

Discussion

Over the last two decades, research has been conducted to identify biomarkers to predict disease recurrence and targets in ECs [3-5]. RBM10, a member of the RBP family, is reported to be involved in different kinds of cancers [9]. In this study, we explored the biological function of RBM10, and suggests that RBM10 can suppress EC growth, migration, and invasion through the Hippo/YAP signaling pathway.

Functional studies have found that the expression of RBM10 is related to accelerating cell apoptosis, and reducing cell proliferation, clone formation, and tumor growth [18-20]. The first functional study on RBM10 found that the expression of RBM10 was related to the decreased proliferation and increased apoptosis of hypertrophic primary chondrocytes [21]. RBM10 was further found to inhibit cell proliferation through either the Notch signaling pathway or the RAP1/Akt/CREB signaling pathway [20, 22]. Overexpression of RBM10 reduces proliferation and colony formation of osteosarcoma cells, inhibits migration and invasion by downregulating Bcl-2 expression, and upregulating caspase-3 and TNF-α production [9]. Over-ex-

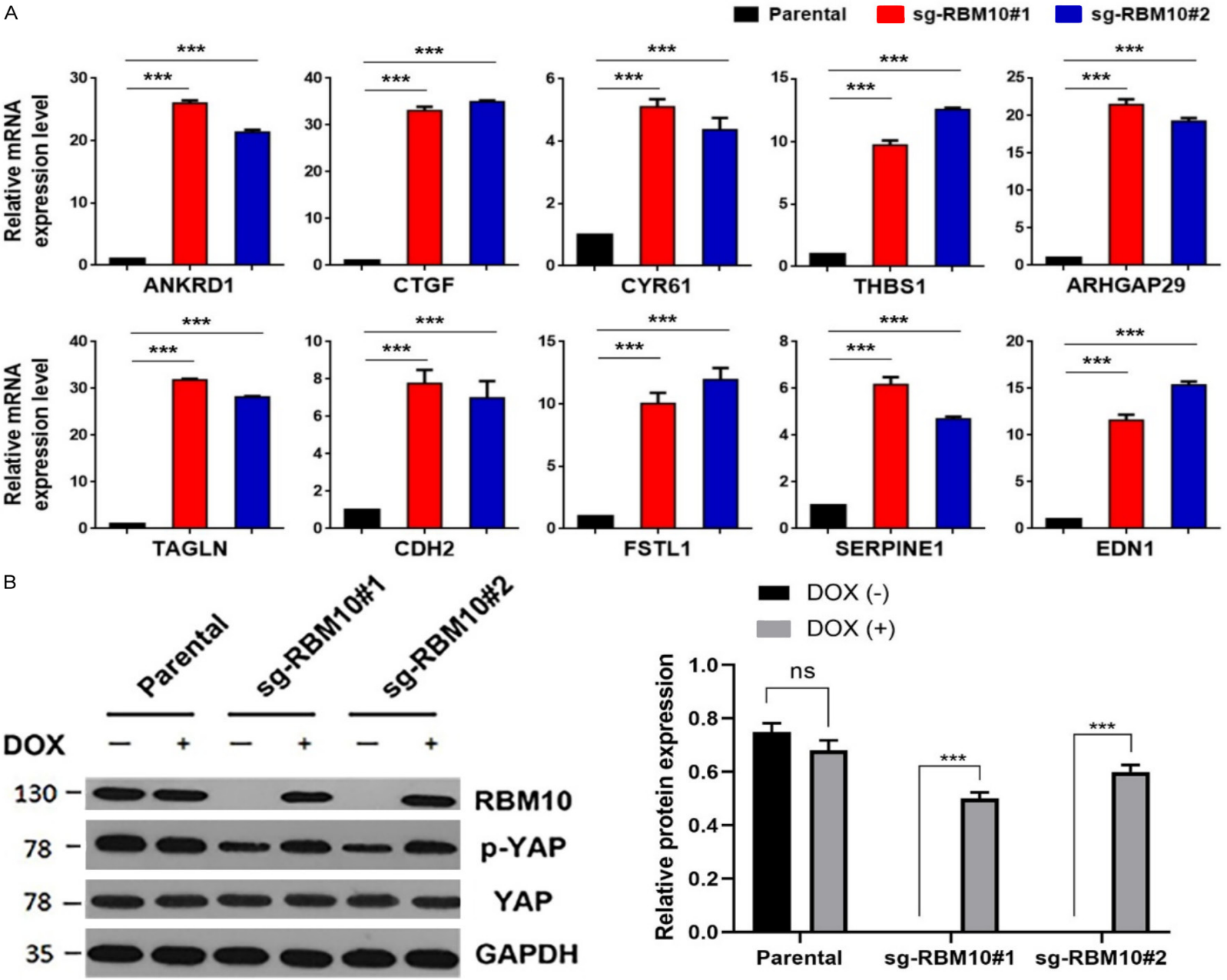
A
Parental
sg-RBM10#1
sg-RBM10#2
Relative mRNA expression level
ANKRD1
CTGF
CYR61
THBS1
ARHGAP29
TAGLN
CDH2
FSTL1
SERPINE1
EDN1
***
B
Parental
sg-RBM10#1
sg-RBM10#2
DOX
130
78
78
35
RBM10
p-YAP
YAP
GAPDH
DOX (-)
DOX (+)
Relative protein expression
ns
Parental
sg-RBM10#1
sg-RBM10#2

Figure 8. Analysis of the expression of Hippo-YAP pathway target gene. A. The mRNA expression levels of Hippo-YAP pathway target genes in wild-type Ishikawa cells and RBM10 knockout cells (qRT-PCR). B. The expression of p-YAP in Ishikawa cells after RBM10 knockout (Western blot). Note: Compared with parental group, ***P<0.001.

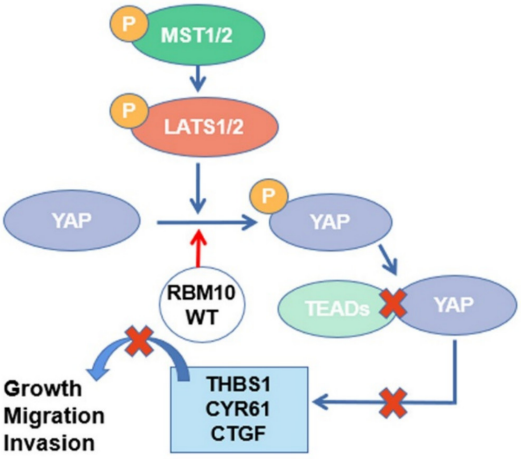

Figure 9. Schematic diagram of the effect and molecular mechanism of RBM10 on endometrial cancer.

pression of RBM10 can reduce the protein levels of EGFR and p-ERK, inhibit the proliferation and invasion of Hepatocellular carcinomas [23]. RBM10 is also a new p53 regulator. Overexpression of RBM10 can reduce the migration and EMT of tumor cells [24]. However, recent studies have found that RBM10 may play a cancer promoting role in some tumors because RBM10 is related to the increased expression of angiogenesis by promoting VEGF [25-27]. RBM5 regulated the expression of RBM10 by directly acting on specific RBM splice variants at the post transcriptional level in small cell lung cancer. RBM10 participates in transcription and hypoxia related processes in RBM5 KO small cell lung cancer cell lines, thus promoting tumor growth and metastasis. In particular, RBM10 KO can induce the reduction of glycolysis, EMT, and angiogenesis, which may directly regulate cell metabolism and oxidative phosphorylation [28]. In addition, RBM10 is found to be involved in inhibiting the invasion and metastasis of lung cancer and liver cancer cells [14, 29]. RBM10v1 and RBM10v2, sharing 49% and 54% identity respectively, bind different RNAs. The variants may exhibit divergent functions; for instance, RBM5-RBM10v2 regulates cell cycle progression and apoptosis, while RBM10v1 inhibits tumor apoptosis in breast cancer. The differential proportions of these variants in tumor cells warrant further investigation into upstream regulatory mechanisms of RBM10 expression. Our study found that RBM10 knockout can enhance EC cell growth, migration, and invasion, whereas RBM10 overexpression suppresses these activities.

The Hippo pathway, an evolutionarily conserved kinase signaling cascade involving MST and LATS kinases, phosphorylates and inhibits YAP/TAZ, promoting cytoplasmic retention [30]. Physiological or pathological inactivation of these kinases leads to YAP/TAZ dephosphorylation, nuclear accumulation, and activation of target gene transcription by binding TEA/ATTS domain transcription factors (TEAD1-4) [31]. The Hippo pathway regulates tissue homeostasis, cell proliferation, and apoptosis [32]. Considering the critical roles of the YAP/TAZ-TEAD complex in cancer initiation, progression, metastasis, and recurrence, these proteins and their upstream regulators can serve as potential therapeutic targets [33]. Regulation disorder of the Hippo-YAP/TEAD signal pathway is closely related to tumorigenesis and progression by affecting cell proliferation, EMT, migration, and invasion [34, 35]. In the current study, we showed that many YAP-targeted genes were up-regulated after RBM10 knockout, and phosphorylation level of YAP was positively correlated with RBM10 expression level, suggesting that RBM10 can promote Yap phosphorylation to activate the Hippo-YAP signal pathway to achieve growth inhibition. There were also some limitations in this study. For example, the underlying mechanisms are still not totally understood. Future studies are needed to further evaluate the mechanisms.

Conclusions

In conclusion, we found that RBM10 KO increased EC cells growth, migration, and invasion, while RBM10 overexpression decreased the growth, migration, and invasion ability of EC cells. We further clarified the regulatory roles of RBM10 in the Hippo/YAP signaling pathway. Our findings suggest that RBM10 can be used as a therapeutic target for precise treatment of EC patients in the future.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 919-

54106 and 81872109 to K.G., No. 81972438 and 82172975 to X.W.), the Natural Science Foundation of Shanghai (No. 22ZR1449200 to K.G.), Shanghai Science and Technology Commission Innovation Plan (No. 17411951600 to X.W.) and Shanghai Key Clinical Specialty Program (No. 2017ZZ02015 to X.W.).

Disclosure of conflict of interest

None.

Address correspondence to: Xiaoping Wan, Shanghai Key Laboratory of Maternal Fetal Medicine, Shanghai Institute of Maternal-Fetal Medicine and Gynecologic Oncology, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, No. 550 Hu'nan Road, Shanghai 200092, China. Tel: +86-02164070434; E-mail: xiaopingwan01@163.com; Kun Gao, Department of Clinical Laboratory, Shanghai First Maternity and Infant Hospital, School of Medicine, Tongji University, No. 550 Hu'nan Road, Shanghai 200092, China. Tel: +86-02164070434; E-mail: kungao@tongji.edu.cn

References

[1] Morice P, Leary A, Creutzberg C, Abu-Rustum N and Darai E. Endometrial cancer. Lancet 2016; 387: 1094-1108.

[2] Brooks RA, Fleming GF, Lastra RR, Lee NK, Moroney JW, Son CH, Tatebe K and Veneris JL. Current recommendations and recent progress in endometrial cancer. CA Cancer J Clin 2019; 69: 258-279.

[3] Son J, Carr C, Yao M, Radeva M, Priyadarshini A, Marquard J, Michener CM and AlHilli M. Endometrial cancer in young women: prognostic factors and treatment outcomes in women aged ≤40 years. Int J Gynecol Cancer 2020; 30: 631-639.

[4] Huvila J, Pors J, Thompson EF and Gilks CB. Endometrial carcinoma: molecular subtypes, precursors and the role of pathology in early diagnosis. J Pathol 2021; 253: 355-365.

[5] MacKintosh ML and Crosbie EJ. Prevention strategies in endometrial carcinoma. Curr Oncol Rep 2018; 20: 101.

[6] Sun P, Shen Y, Wang T, He Y, Zhang Y, Tian W, Yang B and Hu Y. Distinct clinical and genetic mutation characteristics in sporadic and Lynch syndrome-associated endometrial cancer in a Chinese population. Cancer Epidemiol 2021; 73: 101934.

[7] Wang Y, Zhou R and Wang J. Relationship between hypothyroidism and endometrial cancer. Aging Dis 2019; 10: 190-196.

[8] Inoue A. RBM10: structure, functions, and associated diseases. Gene 2021; 783: 145463.

[9] Han LP, Wang CP and Han SL. Overexpression of RBM10 induces osteosarcoma cell apoptosis and inhibits cell proliferation and migration. Med Sci (Paris) 2018; 34 Focus issue F1: 81-86.

[10] Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA and Knudsen ES. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015; 6: 6744.

[11] Cao Y, Di X, Zhang Q, Li R and Wang K. RBM10 regulates tumor apoptosis, proliferation, and metastasis. Front Oncol 2021; 11: 603932.

[12] Sun X, Jia M, Sun W, Feng L, Gu C and Wu T. Functional role of RBM10 in lung adenocarcinoma proliferation. Int J Oncol 2019; 54: 467-478.

[13] Gripp KW, Hopkins E, Johnston JJ, Krause C, Dobyns WB and Biesecker LG. Long-term survival in TARP syndrome and confirmation of RBM10 as the disease-causing gene. Am J Med Genet A 2011; 155A: 2516-2520.

[14] Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, Cho J, Suh J, Capelletti M, Sivachenko A, Sougnez C, Auclair D, Lawrence MS, Stojanov P, Cibulskis K, Choi K, de Waal L, Sharifnia T, Brooks A, Greulich H, Banerji S, Zander T, Seidel D, Leenders F, Ansén S, Ludwig C, Engel-Riedel W, Stoelben E, Wolf J, Goparju C, Thompson K, Winckler W, Kwiatkowski D, Johnson BE, Jänne PA, Miller VA, Pao W, Travis WD, Pass HI, Gabriel SB, Lander ES, Thomas RK, Garraway LA, Getz G and Meyerson M. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012; 150: 1107-1120.

[15] Giannakis M, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, Bahl S, Cao Y, Amin-Mansour A, Yamauchi M, Sukawa Y, Stewart C, Rosenberg M, Mima K, Inamura K, Nosho K, Nowak JA, Lawrence MS, Giovannucci EL, Chan AT, Ng K, Meyerhardt JA, Van Allen EM, Getz G, Gabriel SB, Lander ES, Wu CJ, Fuchs CS, Ogino S and Garraway LA. Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Rep 2016; 15: 857-865.

[16] Furukawa T, Kuboki Y, Tanji E, Yoshida S, Hatori T, Yamamoto M, Shibata N, Shimizu K, Kamatani N and Shiratori K. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep 2011; 1: 161.

[17] Ibrahimpasic T, Xu B, Landa I, Dogan S, Middha S, Seshan V, Deraje S, Carlson DL, Migliacci J, Knauf JA, Untch B, Berger MF, Morris L,

Tuttle RM, Chan T, Fagin JA, Ghossein R and Ganly I. Genomic alterations in fatal forms of non-anaplastic thyroid cancer: identification of MED12 and RBM10 as novel thyroid cancer genes associated with tumor virulence. Clin Cancer Res 2017; 23: 5970-5980.

[18] Rintala-Maki ND, Goard CA, Langdon CE, Wall VE, Traulsen KE, Morin CD, Bonin M and Sutherland LC. Expression of RBM5-related factors in primary breast tissue. J Cell Biochem 2007; 100: 1440-1458.

[19] Bechara EG, Sebestyén E, Bernardis I, Eyras E and Valcárcel J. RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. Mol Cell 2013; 52: 720-733.

[20] Hernández J, Bechara E, Schlesinger D, Delgado J, Serrano L and Valcárcel J. Tumor suppressor properties of the splicing regulatory factor RBM10. RNA Biol 2016; 13: 466-472.

[21] James CG, Ulici V, Tuckermann J, Underhill TM and Beier F. Expression profiling of Dexamethasone-treated primary chondrocytes identifies targets of glucocorticoid signalling in endochondral bone development. BMC Genomics 2007; 8: 205.

[22] Jin X, Di X, Wang R, Ma H, Tian C, Zhao M, Cong S, Liu J, Li R and Wang K. RBM10 inhibits cell proliferation of lung adenocarcinoma via RAP1/AKT/CREB signalling pathway. J Cell Mol Med 2019; 23: 3897-3904.

[23] Zhao Z, Li J and Shen F. Protective effect of the RNA-binding protein RBM10 in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2020; 24: 6005-6013.

[24] Martínez-Arribas F, Agudo D, Pollán M, Gómez-Esquer F, Díaz-Gil G, Lucas R and Schneider J. Positive correlation between the expression of X-chromosome RBM genes (RBMX, RBM3, RBM10) and the proapoptotic Bax gene in human breast cancer. J Cell Biochem 2006; 97: 1275-1282.

[25] Jung JH, Lee H, Cao B, Liao P, Zeng SX and Lu H. RNA-binding motif protein 10 induces apoptosis and suppresses proliferation by activating p53. Oncogene 2020; 39: 1031-1040.

[26] Garrisi VM, Strippoli S, De Summa S, Pinto R, Perrone A, Guida G, Azzariti A, Guida M and Tommasi S. Proteomic profile and in silico analysis in metastatic melanoma with and without BRAF mutation. PLoS One 2014; 9: e112025.

[27] Jackson TC, Du L, Janesko-Feldman K, Vagni VA, Dezfulian C, Poloyac SM, Jackson EK, Clark RS and Kochanek PM. The nuclear splicing factor RNA binding motif 5 promotes caspase activation in human neuronal cells, and increases after traumatic brain injury in mice. J Cereb Blood Flow Metab 2015; 35: 655-666.

[28] Loiselle JJ, Roy JG and Sutherland LC. RBM10 promotes transformation-associated processes in small cell lung cancer and is directly - regulated by RBM5. PLoS One 2017; 12: e0180258.

[29] Yamada H, Tsutsumi K, Nakazawa Y, Shibagaki Y, Hattori S and Ohta Y. Src family tyrosine kinase signaling regulates FilGAP through association with RBM10. PLoS One 2016; 11: e0146593.

[30] Ma S, Meng Z, Chen R and Guan K L. The hippo pathway: biology and pathophysiology. Annu Rev Biochem 2019; 88: 577-604.

[31] Liu CY, Zha ZY, Zhou X, Zhang H, Huang W, Zhao D, Li T, Chan SW, Lim CJ, Hong W, Zhao S, Xiong Y, Lei QY and Guan KL. The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCF{beta}-TrCP E3 ligase. J Biol Chem 2010; 285: 37159-37169.

[32] Moya IM and Halder G. Hippo-YAP/TAZ signalling in organ regeneration and regenerative medicine. Nat Rev Mol Cell Biol 2019; 20: 211-226.

[33] Piccolo S, Dupont S and Cordenonsi M. The biology of YAP/TAZ: hippo signaling and beyond. Physiol Rev 2014; 94: 1287-1312.

[34] Sebé-Pedrós A, Zheng Y, Ruiz-Trillo I and Pan D. Premetazoan origin of the hippo signaling pathway. Cell Rep 2012; 1: 13-20.

[35] Poernbacher I, Baumgartner R, Marada SK, Edwards K and Stocker H. Drosophila Pez acts in Hippo signaling to restrict intestinal stem cell proliferation. Curr Biol 2012; 22: 389-396.

Supplementary Table 1. The sequences of sgRNA and primers for amplification of the sgRNA-targeted sequence of the RBM10 gene

Name	Sequences (5' to 3')
RBM10-sg-1-F	CACCGGGTCCCGATAGTCGCCGTCT
RBM10-sg-1-R	AAACAGACGGCGACTATCGGGACCC
RBM10-sg-2-F	CACCGGCAGCCGAGACCACGACTAC
RBM10-sg-2-R	AAACGTAGTCGTGGTCTCGGCTGCC
RBM10-sg-3-F	CACCGCGTTCATATCCTCGCGAGTA
RBM10-sg-3-R	AAACTACTCGCGAGGATATGAACGC
RBM10-PCMV-FLag-F	GCCATGGAGGCCCGAATTCGGATGGAGTATGAAAGACGTACG
RBM10-PCMV-Flag-R	GTCTGGATCCCCGCGGCCGCTCACTGGGCCTCGTTGAAGCG
RBM10-PGLV-E-FLAG-F	CCTACCCTCGTAAAGAATTCATGGACTACAAGGACGACGATGGAGTATGAAAGACGTACG
RBM10-PGLV-B-R	CAGCGAGCTCTAGGGATCCTCACTGGGCCTCGTTGAAGCG

Supplementary Table 2. The primers used in this study

Name	Sequences (5' to 3')
RBM10-F	CTCTACTATGACCCCAACTCCCA
RBM10-R	GTCCGCCTCTCCCCATCCCA
hmGAPDH-F	ATGACATCAAGAAGGTGGTG
hmGAPDH-R	CATACCAGGAAATGAGCTTG
CYR61-F	ATGAATTGATTGCAGTTGGAAA
CYR61-R	TAAAGGGTTGTATAGGATGCGA
ARHGAP29-F	GGAATCAGAACGCAAGCAAAATGCG
ARHGAP29-R	GGGATGCTGATTCAGCCTCTTGG
CTGF-F	CCCAAGGACCCAAACCGTG
CTGF-R	CTAATCATAGTTGGGTCTGGGC
THBS1-F	AGCGTCTTCACCAGAGACCT
THBS1-R	CATTCACCACGTTGTTGTCA
ANKRD1-F	ACGCCAAAGACAGAGAAGGA
ANKRD1-R	TTCTGCCAGTGTAGCACCAG