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MCM2 promotes the stemness of endometrial cancer cells via the Akt/β-catenin pathway

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Minichromosome maintenance complex component 2 (MCM2) is a member of the MCM family and is involved in various cancers. However, the role of MCM2 in endometrial cancer (EC) remains unclear. In this study, we aim to determine the biological function of MCM2 in EC cells and identify the potential underlying mechanisms. MCM2 expression and prognostic significance were analyzed in TCGA-UCEC datasets. Combining bioinformatics analyses and experiments, stemness-related molecules and phenotypes were examined to evaluate the impact of MCM2 on stemness in EC cells. The major findings of these analyses are as follows: 1) MCM2 is expressed at higher levels in EC tissues than in normal endometrial tissues. High expression of MCM2 is related to the characteristics of poorly differentiated EC. High MCM2 expression is correlated with poor overall survival in EC patients; 2) MCM2 knockdown was found to decrease sphere formation ability, downregulate the expression of stemness-related molecules, and reduce the proportion of $CD133^+$ cells, while MCM2 overexpression elicited the opposite effect in EC cells; 3) MCM2-mediated stemness features are dependent on the activation of Akt/β-catenin signaling pathways; and 4) MCM2 knockdown increases cisplatin sensitivity in EC cells. MCM2 regulates stemness by regulating the Akt/β-catenin signaling pathway in EC cells.

Key words: minichromosome maintenance complex component 2; endometrial cancer; stemness; Akt/β-catenin pathway

Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide. In 2022, EC was the sixth most common cancer in women, and the associated mortality rate ranked sixth worldwide. It is estimated that in the United States, 65,950 new EC cases occurred, with 12,550 deaths resulting from the disease [1]. With the improvements in diagnosis and treatment, EC mortality rates have declined in the past decades. Most EC patients can be treated successfully via hysterectomy and post-operative therapy. The five-year overall survival (OS) rate is estimated to be more than 80% in patients in whom EC is confined to the uterine corpus at diagnosis [2]. However, the prognosis for recurrent EC is poor, with five-year OS reduced to 69% for pelvic recurrences and 18% for extra pelvic recurrences, respectively [1]. Platinum-based chemotherapy is recommended as the frontline chemotherapy for post-operative therapy or recurrent disease. However, drug resistance is common in EC therapy, and limits its efficacy [3]. Stemness has emerged as an important topic of interest due to its strong association with recurrence as well as chemoresistance in EC [4]. Several molecular markers involved in regulating the stemness of EC cells have been identified, such as BMI1, NANOG, and TRIB3 [5–7]. Despite the critical role of stemness in EC malignancy, the regulation of EC stemness remains unclear. The investigation of new molecular mechanisms in EC is necessary to identify new therapeutic targets.

The minichromosome maintenance complex component (MCM) family consists of MCM 2-9, which are highly conserved intracellular proteins [8]. The main function of MCMs is regulating DNA replication, cell cycle transition, and proliferation. Generally, MCM2–7 form a heterohexameric complex crucial for the initiation of DNA replication and limit the replication of chromosomes to only once per cell cycle [9]. MCM8–9 also form a complex that plays a role in homologous recombination repair and genomic integrity [10]. The overexpression of MCMs has been detected in several different types of cancer, indicating the importance of MCMs in tumorigenesis and malignancies. Among these MCMs, MCM2 is the most researched in cancers. MCM2 has been demonstrated as a sensitive biomarker for cancer

cell proliferation, such as in colon cancer [11], breast cancer [12], lung cancer [13]. In addition, MCM2 is also used as a predictive marker of chemotherapeutic response in several malignancies [14–15]. Recently, MCM2 has been identified as an important stemness regulator [16–18]. However, the expression and biological function of MCM2 in EC has not been fully studied so far.

In this study, we aimed to analyze MCM2 expression and biological function in EC. Furthermore, we investigated the impact of platinum sensitivity of MCM2 on EC cells. Our results may provide a theoretical and experimental basis for the potential targeting of MCM2 in the diagnosis and treatment of EC.

Materials and methods

Data from public databases and bioinformatics analysis. To explore the pattern of expression and prognostic implications of MCMs in EC, The Cancer Genome Atlas Uterine Corpus Endometrial Carcinoma (TCGA-UCEC) RNA-seq and clinical data were obtained through the official website of the National Cancer Institute (https://www.cancer.gov/ccg/research/genome-sequencing/tcga).

To characterize metastatic, stemness, and proliferative states for UCEC samples in the TCGA cohort, gene sets for metastasis and proliferation were obtained from MSigDB [19–20]. Based on metastasis and proliferation signatures, we calculated the metastatic potential score (MPS) and proliferation activity using the single-sample gene set enrichment (ssGSEA) method with the R GSVA package [21]. The mRNAsi of TCGA samples was obtained from Tathiane et al. [22]. The association of MCM2 expression with MPS, proliferation activity, and mRNAsi in UCEC samples was tested by Pearson correlation (Supplementary Table S1).

Cell lines. Human EC cell lines HEC1A, HEC1B, KLE, ISHIKAWA, and normal endometrial epithelial cells were obtained from laboratory preservation and cultured in DMEM/F12 medium (Gibco, USA) containing 10% fetal bovine serum (HyClone, Logan, USA). All cells were incubated in a humidified incubator at 5% CO_2 and 37 °C.

Cell transfection. Small interfering RNAs (siRNAs) targeting MCM2 were purchased from Biotend Biotechnology Co., Ltd. (Shanghai, China). A vector carrying the wild-type full-length human MCM2 cDNA was transfected into EC cells for MCM2 upregulation. The overexpression vector and negative control vector were manufactured by GeneChem (Shanghai, China). The PGMLV-CMV-MCS-3×Flag-EF1-ZsGreen1-T2A-Puro plasmid encoding the wild-type MCM2 was used for the transfection of tumor cells. Transfection of siRNAs and vectors was performed using Lipofectamine 3000 in accordance with the manufacturer's protocol.

RNA isolation and quantitative real-time PCR (qPCR). The total RNA was extracted from cells using TRIzol (Invitrogen, Carlsbad, USA). Then total RNA was transcribed into cDNA using the qPCR RT Kit (TaKaRa Bio, Shiga, Japan). Next, quantitative real-time PCR was performed using SYBR green Gene Expression Assay (TaKaRa Bio, Shiga, Japan). The following primer sequences (5'–3') were used: MCM2 forward, GGCGAGGAGGACGAGGAGATG; MCM2 reverse, AAGTTCTTGAAGCGGTGGTGGATC; β-actin: forward, GGCCAACCGCGAGAAGATGAC; β-actin reverse, GGATAGCACAGCCTGGATAGCAAC.

Western blot. Cells were harvested and lysed in a radioimmunoprecipitation assay buffer with phenylmethanesulfonyl fluoride. Cellular lysates were electrophoresed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, USA). The membranes were blocked with 5% fat-free milk at room temperature for 1 h and then incubated with primary antibodies including anti-MCM2 antibody (1:1000; Cell Signaling Technology, Danvers, USA, 3619), anti-ALDH1A1 antibody (1:1000; Cell Signaling Technology, Danvers, USA, 54135), anti-CD133 (1:4000; Abcam, Cambridge, USA, ab222782) antibody, anti-AKT (1:4000; Cell Signaling Technology, Danvers, USA, 4691) antibody, anti-p-AKT (1:1000; Cell Signaling Technology, Danvers, USA, 4060) antibody, anti-p-GSK-3b (1:1000; Cell Signaling Technology, Danvers, USA, 5558) antibody, anti-c-Myc (1:4000; Cell Signaling Technology, Danvers, USA, 18583) antibody, anti-b-catenin (1:1000; Cell Signaling Technology, Danvers, USA, 8480) antibody, or anti-b-actin (1:4000; Cell Signaling Technology, Danvers, USA, 3700) antibody, at 4 °C overnight. After five washes for 5 min in phosphate-buffered saline supplemented with 0.1% Tween-20 (PBST), the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:4000; Cell Signaling Technology, Danvers, USA) for 2 h at room temperature. The enhanced chemiluminescence kit (Pierce, Rockford, USA) was used for detection.

Sphere formation assay. Sphere formation assay was carried out at 48 h post-transfection. The cells were harvested and counted. Then, 1,000 cells were plated in 24-well ultra-low attachment plates in DMEM/F12 medium containing 20 ng/ml EGF, 10 ng/ml bFGF, and 5 μg/ml insulin, and cultured for 10 d. The number of spheres was observed with an inverted microscope on day 5, after which they increased in size until day 10.

Flow cytometry. The proportion of $CD133^+$ cells was evaluated at 48 h after transfection. The cells were collected, counted, and resuspended in a sorting buffer. Then, 1×10^5 cells in 100 μl of buffer were stained with an Alexa Fluor 488-linked CD133 antibody (Abcam, Cambridge, USA, ab278053) on ice for 30 min. The cells were analyzed using a FACSVerse (BD) flow cytometer.

CCK-8 assay. At 48 h after transfection, the Cell Counting Kit-8 (CCK-8, Dojinda) was used to detect cell sensitivity to cisplatin. The cells were seeded into 96-well plates with

six replicates at a density of 1×10^3 cells/well. Then, a serial dilution of cisplatin was added and incubated with cells for 48 h or 72 h. Absorbance at 450 nm was detected in accordance with the manufacturer's instructions.

Statistical analysis. Comparisons between the two groups were performed by two-tailed Student's t-tests. Multiple-group comparisons were performed by two-way analysis of variance. The Kaplan-Meier method was used to conduct survival analysis with the log-rank test. Statistical analysis was performed with GraphPad Prism 7.02 (GraphPad Software, Inc.). Values of $p<0.05$ were considered to indicate statistically significant differences.

Results

The expression and the prognostic significance of MCM2-9 in TCGA-UCEC patient cohorts. We analyzed differences in the overall expression of MCM2-9 genes using TCGA-UCEC datasets. As shown in Figure 1A, the expression of MCM2, 4, 5, 7, and 8 genes, but not that of MCM3, 6, and 9 genes, in the UCEC tissues was significantly higher than in normal endometrial tissues ($p<0.01$). To explore the significance of MCM gene family members in clinical prognosis, UCEC patients were divided into two groups: a high- and low-expression group, based on the median expres-

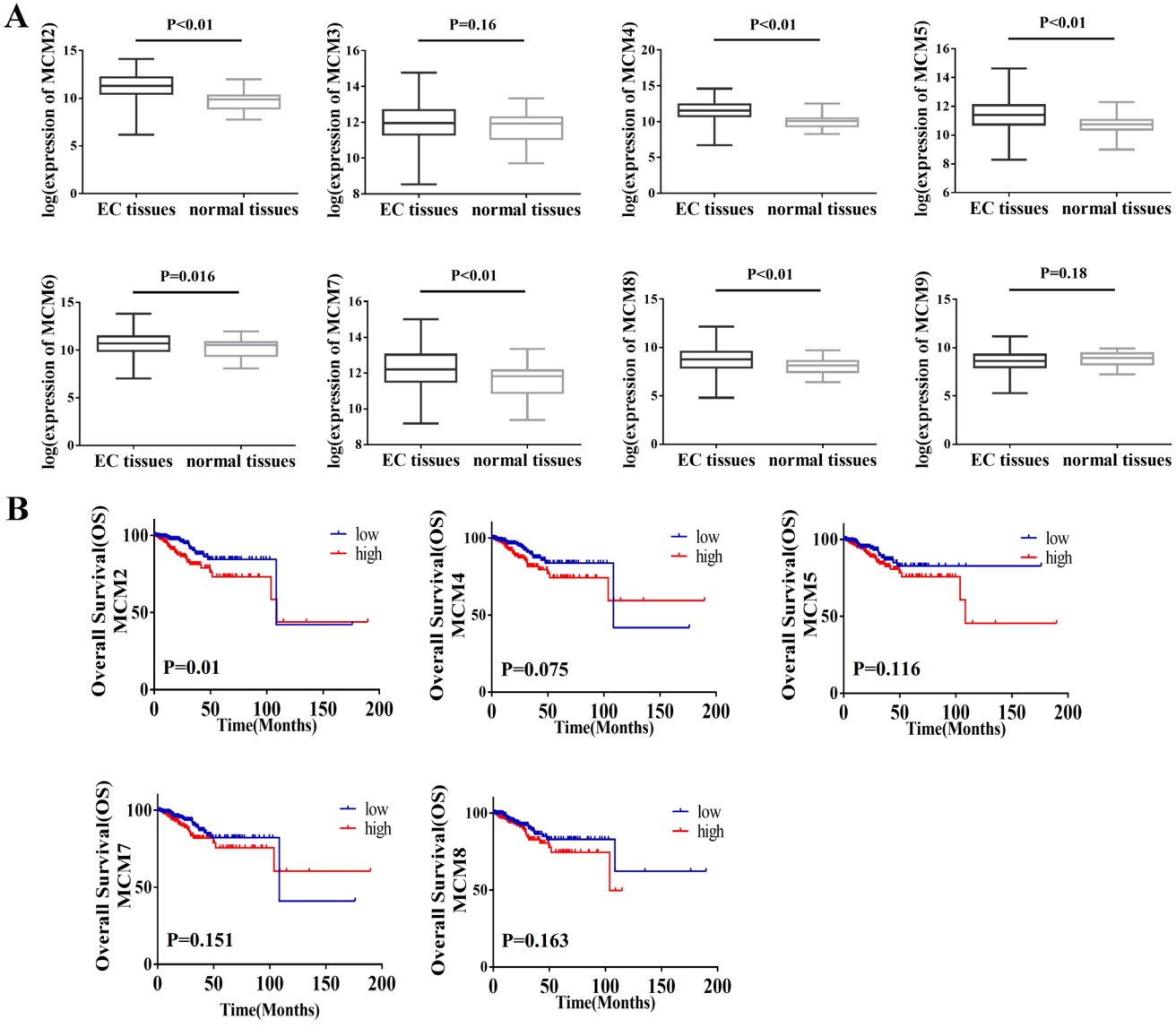

Figure 1. Expression and prognostic significance of MCM2-9 in TCGA-UCEC patient cohorts. A) The mRNA expression of MCM2-9 in EC from the TCGA-UCEC datasets. B) The prognostic value of the mRNA levels of MCM2, 4, 5, 7, and 8 in EC patients (Kaplan-Meier Plotter).

sion of MCMs. We used Kaplan-Meier survival analysis to determine OS for UCEC patients. The results indicated that high expression of MCM2, but not that of MCM4, MCM5, MCM7, and MCM8, was associated with poor prognosis in EC patients (HR=2.073, 95% CI 1.175–3.754, p<0.05) (Figure 1B).

The potential role of MCM2 in EC. To clarify the potential role of MCM2 in EC progression, clinical and pathological parameters of EC patients were compared with the expression levels of MCM2. The incidence of MCM2 detected was significantly higher for the advanced grade 3 than for grades 1 or 2, indicating that MCM2 could potentially regulate EC cell differentiation (Figure 2A). Additionally, there was no statistical difference between the different EC stages (p>0.05) (Figure 2A). Furthermore, we examined the expression of MCM2 protein in endometrial tissue through analysis of data from the HPA database. The results showed that MCM2 protein was not expressed, or expressed at low levels, in normal endometrial tissues, but moderately or highly expressed in cancer endometrium tissues. (Figure 2B). These results indicate that MCM2 is upregulated in EC at both the transcriptional and translational levels.

Proliferation, stemness, and distant metastases are major clinical manifestations in EC progression. Therefore, we explored whether MCM2 expression affects the metastatic, stemness, and proliferative phenotype of EC. We calculated

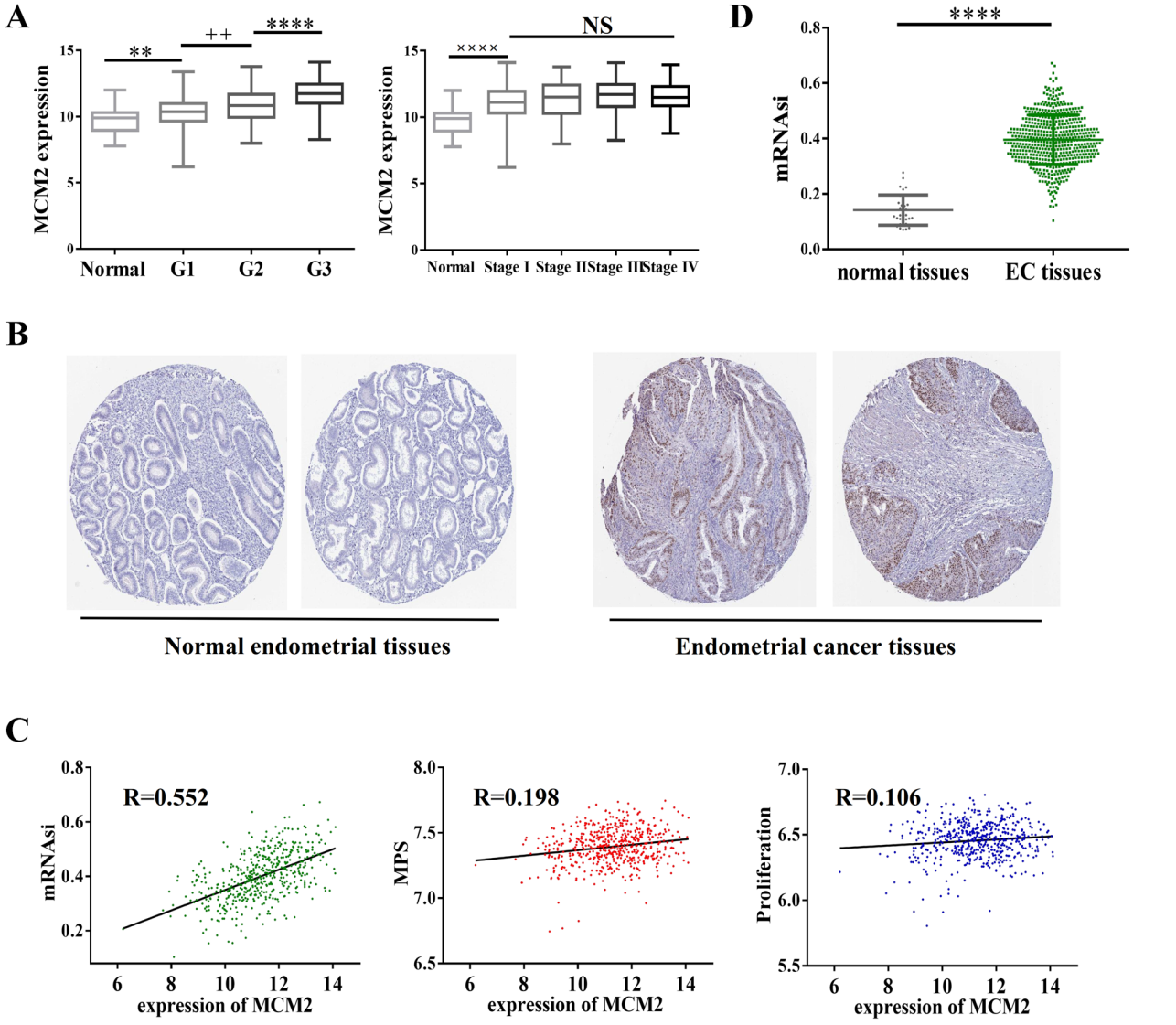

Figure 2. Association of MCM2 expression with EC stemness. A) The mRNA expression level of MCM2 in different grade (left) and stage (right) of EC tissues. **p<0.01 vs. normal; ++p<0.01 vs. G1; ****p<0.0001 vs. G2; ××××p<0.0001 vs. normal; NS means no significant difference. B) The protein expression of MCM2 in EC from the HPA database (IHC). C) Metastatic potential score (MPS), mRNA-based stemness index (mRNAsi), and proliferation activity were calculated for each EC patient by utilizing different scoring methods based on TCGA-UCEC RNA-seq data (Supplementary Table S1). D) Comparison of mRNAsi among the normal endometrium tissues and EC tissues; ****p<0.0001 vs. normal tissues.

the MPS, mRNA-based stemness index (mRNAsi), and proliferation activity in TCGA-UCEC patient cohorts. The results showed that high MCM2 expression was most significantly associated with high mRNAsi and marginally associated with MPS and proliferation activity (Figure 2C). The mRNAsi in the tumor group was much higher than in the normal group (Figure 2D). Hence, we hypothesized that MCM2 expression mainly regulates the stemness phenotype of EC.

Impact of the regulation of MCM2 on the stemness of EC cells. To confirm the effects of MCM2 on the stemness of EC cells, we first examined the expression levels of MCM2 in several endometrial cell lines and normal endometrial cells. The MCM2 expression levels were the highest in HEC1A, while the lowest expression level, in terms of both mRNA and protein levels, was recorded in KLE (Figure 3A). Therefore, HEC1A and KLE cells were selected as the model for subsequent function studies. Complementary siRNAs were used to knock down the expression of MCM2 in HEC1A cells. Plasmids carrying the wild-type MCM2 sequences were used to achieve upregulation of MCM2 expression levels in the KLE cells (Figure 3B).

Next, changes in the expression levels of the stemness-related molecules were examined. The western blots confirmed that the suppression of MCM2 caused a decrease in the expression of ALDH1A1, c-Myc, and CD133 in the HEC1A cells, while MCM2 overexpression elicited the opposite effect in the KLE cells (Figure 4A). The proportion of $CD133^+$ cells was evaluated by flow cytometry. Results showed that MCM2 knockdown significantly reduced the proportion of $CD133^+$ cells in the HEC1A cells, while MCM2 overexpression had the opposite effect in KLE cells (Figures 4B, 4D). Similar effects were also observed in Ishikawa cells (Supplementary Figures S1A–S1C). Further, we analyzed how MCM2 affected the ability of EC cells to form spheres in a serum-free medium, an indication of stem cell-like behavior. Our results showed that MCM2 overexpression increased sphere formation ability in KLE cells, whereas MCM2 knockdown had the opposite effect in HEC1A cells (Figures 4B, 4C). Taken together, these results suggest that MCM2 is linked to stemness in EC cells.

MCM2 promotes cell stemness via the Akt/β-catenin pathway. The potential downstream signaling pathway of MCM2 was evaluated. Wnt, Notch, Hedgehog, and AKT signaling pathways are known to play decisive roles in EC stemness. To assess the potential influence of MCM2 on either of these pathways, GEPIA (http://gepia.cancer-pku.cn/) was used to explore the correlation between the expression of MCM2 and major molecules of these signaling pathways. Analysis based on GEPIA indicated that there is no correlation between the expression of MCM2 and several molecules of the Notch pathway namely, HES1, HES2, and HEY1, or of the HH signaling pathway, namely PTCH1, GLI,

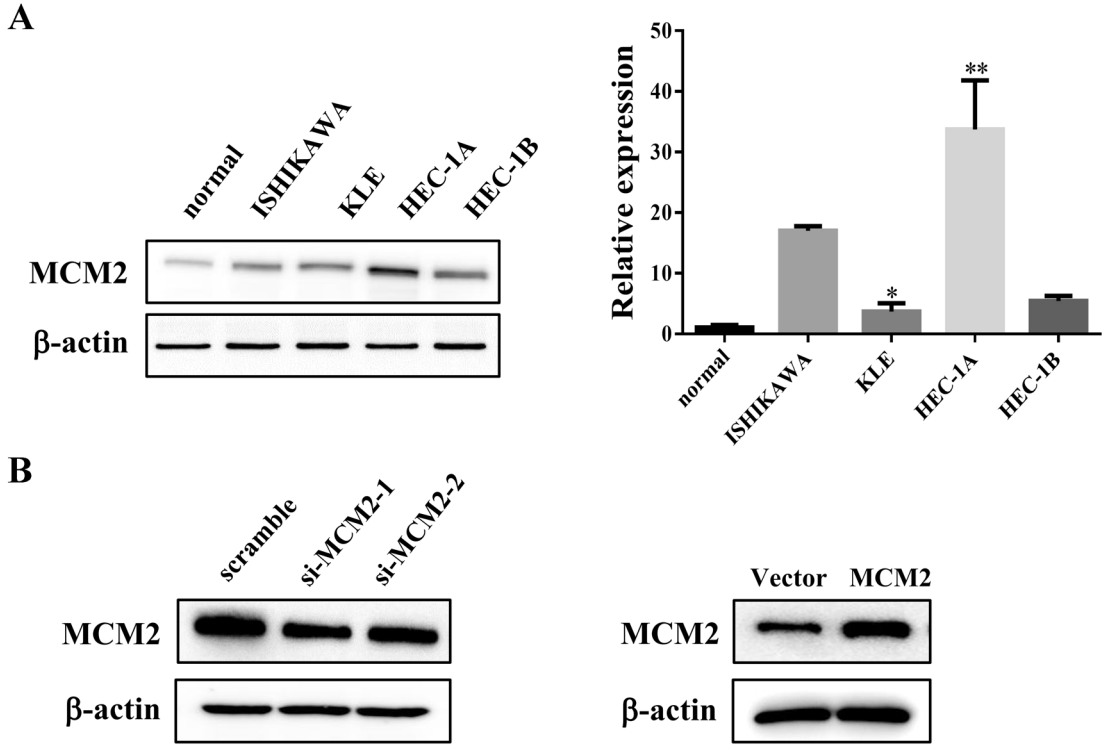

Figure 3. MCM2 is increased in EC cells. A) The mRNA (right) and protein (left) expression level of MCM2 were evaluated by qPCR and western blot assay in normal endometrial cells, Ishikawa, KLE, HEC-1A, and HEC-1B cell lines; $^{**}p<0.01$ vs. normal; $^{*}p<0.05$ vs. normal. B) The amplification effect and knockdown effect of MCM2 in EC cells were evaluated by performing a western blot assay.

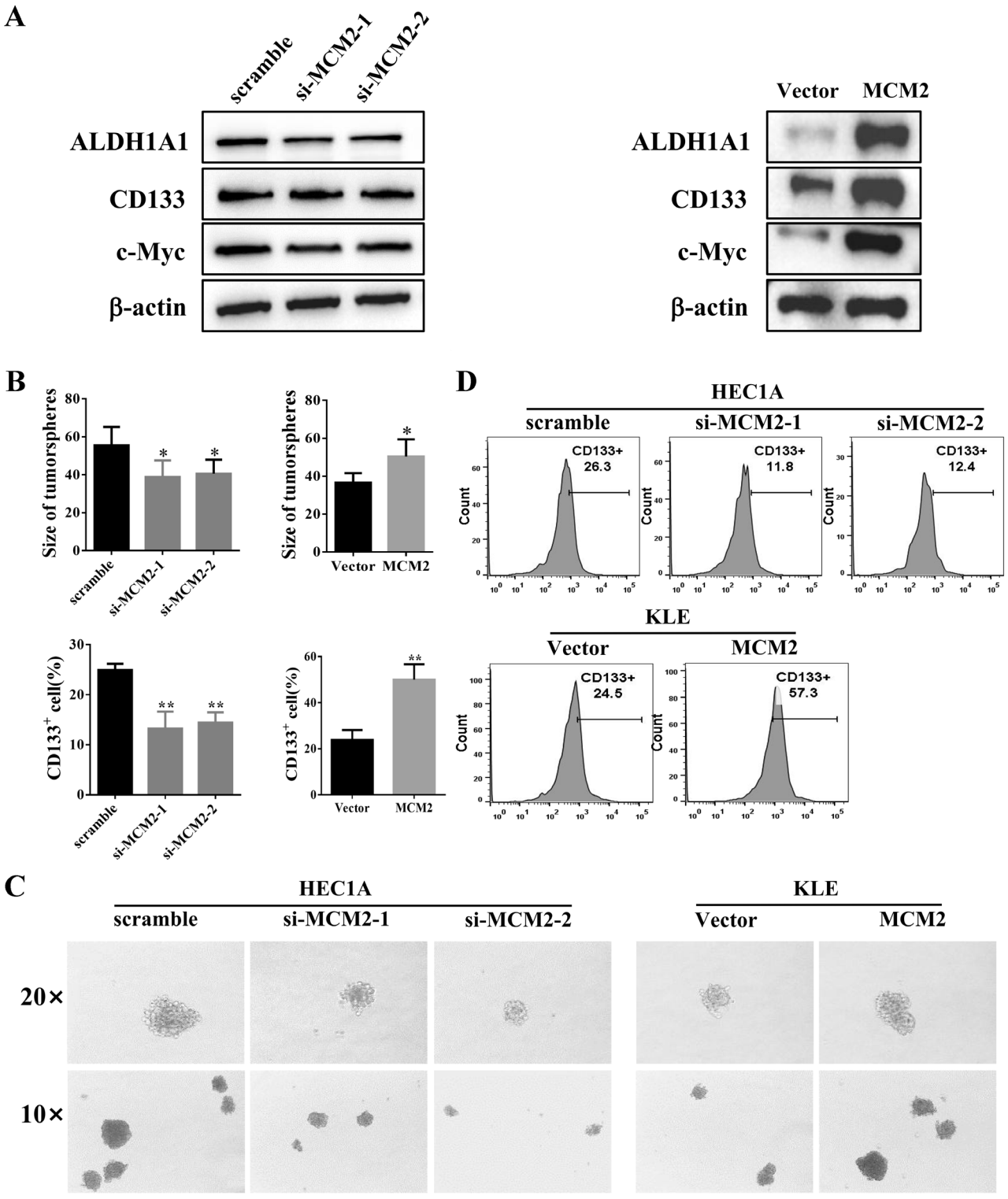

Figure 4. MCM2 promotes the stemness of EC cells *in vitro*. A) The expression of ALDH1A1, CD133, and c-Myc expression in the si-MCM2 and scramble groups determined by western blot assay (left); The expression of ALDH1A1, CD133, and c-Myc expression in the Vector and MCM2 groups determined by western blot assay (right). B) Comparison of sphere size (upper) and CD133⁺ cell proportion (lower) among groups after transfection in HEC1A or KLE cells; *p<0.05 vs. scramble or vs. vector; **p<0.01 vs. scramble or vs. vector. C) Representative images of sphere formation assay. D) Representative images of CD133⁺ cell proportion detection by flow cytometry assay.

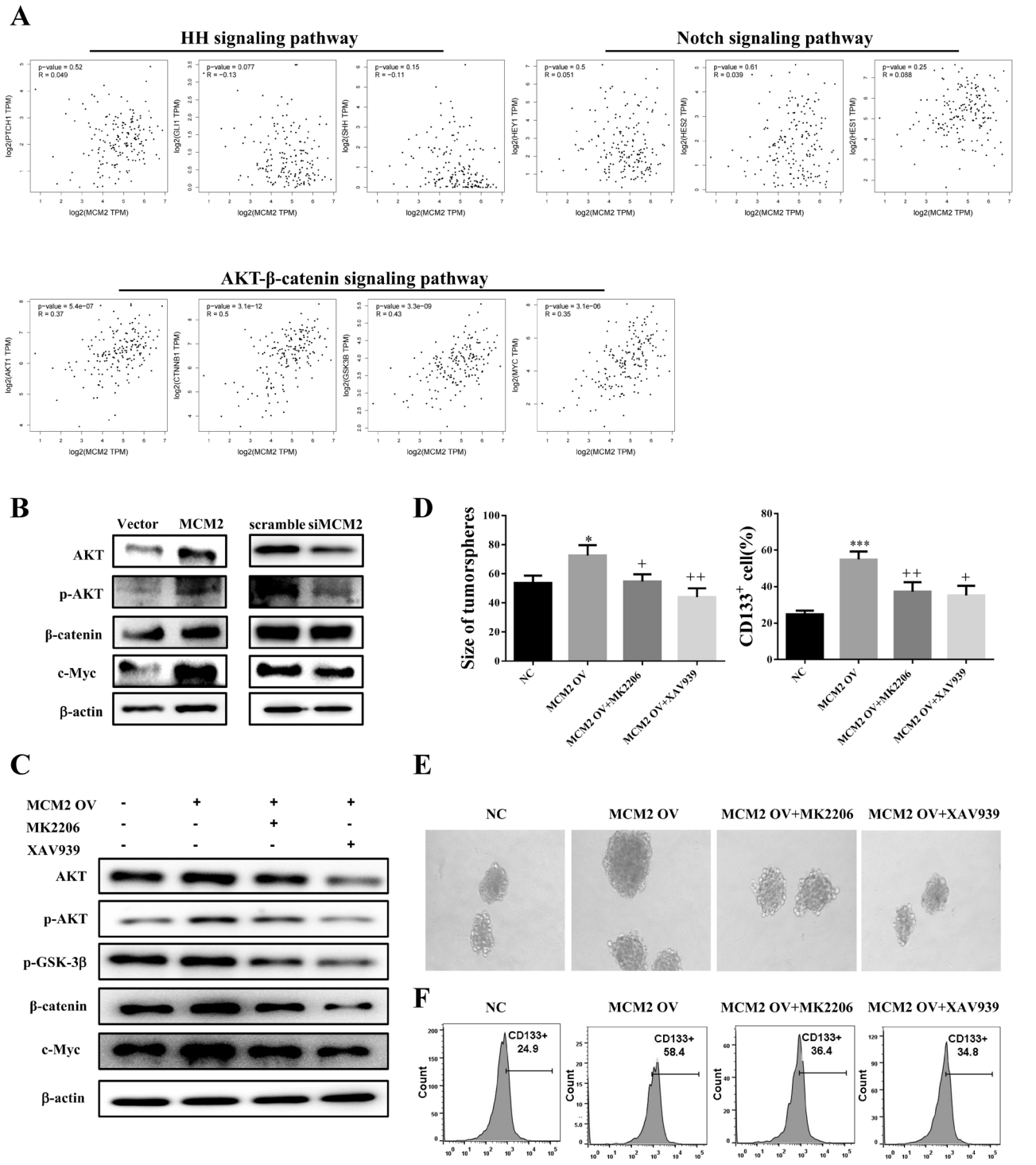

Figure 5. MCM2 regulates the stemness of EC cells through the Akt/β-catenin pathway. A) Scatter plots showing the correlation of MCM2 expression with PTCH1, GLI, SHH (HH signaling pathway), HES1, HES2, HEY1 (Notch signaling pathway), AKT1, CTNNB1, GSK3b, and MYC (AKT/β-catenin signaling pathway) based on the GEPIA dataset. B) Western blot assay was performed to detect the protein expression levels of AKT, p-AKT, β-catenin, and c-Myc after MCM2 overexpression (left) or knockdown (right). C) Western blot assay detected the protein expression levels of AKT, p-AKT, β-catenin, p-GSK-3β, and c-Myc after MCM2 overexpression and MK2206 (AKT inhibitor) or XAV939 (β-catenin inhibitor) treatment. OV means overexpression. D) Comparison of sphere size in the control group and MCM2 overexpression groups treated with MK2206 (AKT inhibitor) or XAV939 (β-catenin inhibitor) (left). Comparison of $CD133^+$ cell proportion in the control group and MCM2-overexpression groups treated with MK2206 or XAV939 (right); *p<0.05 vs. NC; ***p<0.001 vs. NC; +p<0.05 vs. MCM2 OV; ++p<0.01 vs. MCM2 OV. NC means negative control. E) Representative images of sphere formation assay. F) Representative images of $CD133^+$ cell proportion detection by flow cytometry assay.

and SHH. MCM2 significantly correlates with the expression of AKT1, CTNNB1, GSK3b, and MYC (AKT/β-catenin signaling pathways) (Figure 5A).

To determine whether Akt/β-catenin expression could be regulated by MCM2 in EC cells, western blot was used to detect the levels of Akt/β-catenin signaling pathway proteins in MCM2-modified EC cells. As expected, results revealed that MCM2 knockdown decreased the protein expression levels of total AKT, p-AKT, β-catenin, and c-Myc, while MCM2 overexpression had the opposite effect, suggesting that MCM2 activates the Akt/β-catenin signaling pathway (Figure 5B). Furthermore, to investigate whether MCM2 regulates stemness through Akt/β-catenin signaling, we changed the AKT or β-catenin levels by using inhibitor MK2206 (AKT inhibitor) or XAV939 (β-catenin inhibitor). Results showed that MK2206 and XAV939 downregulated the protein expression levels of AKT, p-AKT, and the downstream β-catenin, p-GSK-3b, and c-Myc (Figure 5C). In addition, AKT or β-catenin inhibition suppressed sphere formation ability and decreased the proportion of $CD133^+$ cells in MCM2-overexpressing cells (Figures 5D–5F). These results demonstrate that MCM2 promotes EC stemness, at least in part via the Akt/β-catenin signaling pathway.

Depletion of MCM2 increased cisplatin sensitivity in EC cells. Given the role of MCM2 in EC cell stemness, we investigated the effect of MCM2 on the anticancer properties of cisplatin. The cytotoxic effects of cisplatin on MCM2-knockdown cells and untreated cells were detected after 48 h and 72 h treatment with cisplatin, via CCK-8 assays (Figure 6A). Furthermore, we tested the viability of tumor cells treated with cisplatin at different concentrations for 72 h in both MCM2-knockdown cells and cells without MCM2 knockdown. The IC_{50} of cisplatin in tumor cells without MCM2 knockdown was 40.87 μM,

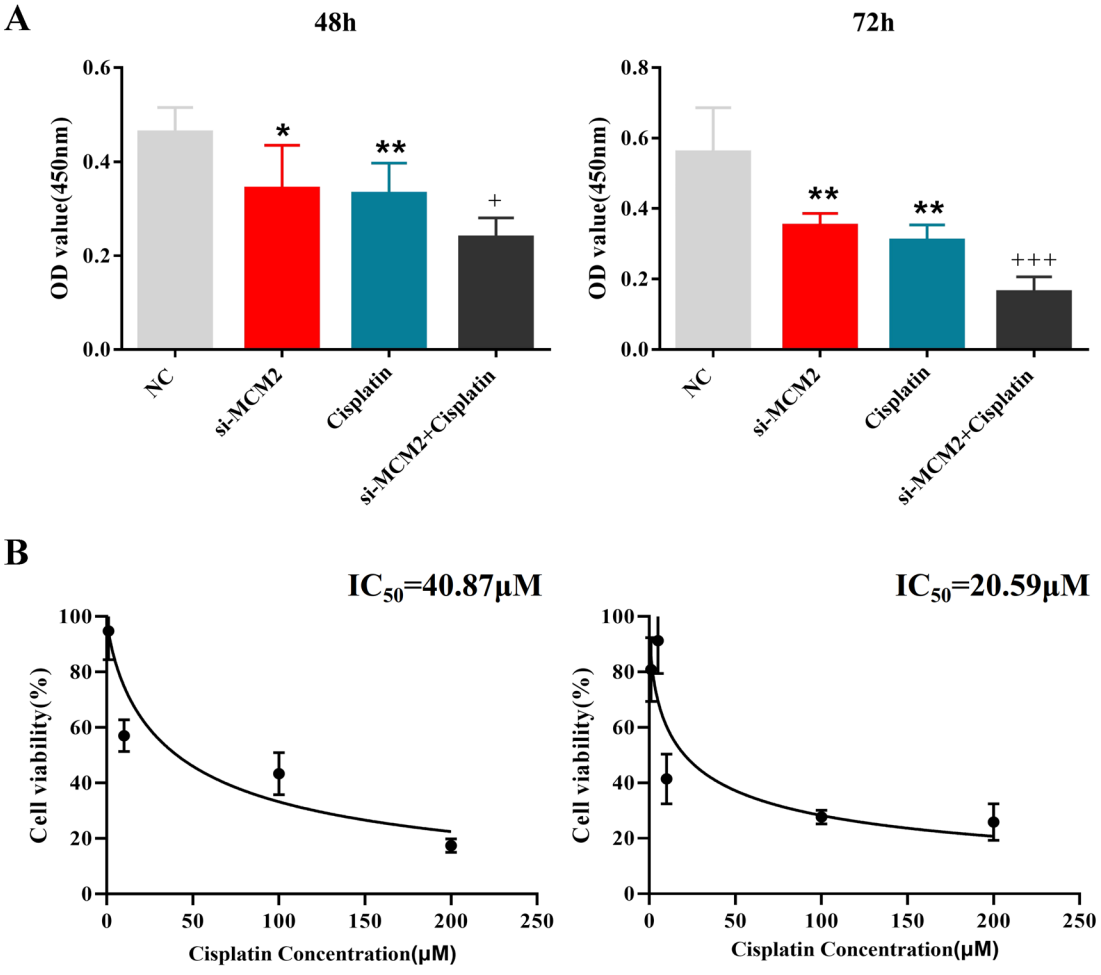

Figure 6. Influence of MCM2 on the anticancer effect of cisplatin. A) Cell viabilities were measured by CCK-8 assay among four groups: control group, two groups treated with MCM2 siRNA or cisplatin (40 μM) alone, and group treated with concurrently with MCM2 siRNA and cisplatin for 48 h and 72 h; *p<0.05 vs. NC; **p<0.01 vs. NC; +p<0.05 vs. cisplatin; +++p<0.001 vs. cisplatin. NC means negative control. B) The viability of tumor cells under treatment with different doses of cisplatin (1, 5, 10, 100, 200 μM) for 72 h among groups in MCM2-knockdown cells and cells without MCM2 knockdown.

whereas the IC_{50} of cisplatin in tumor cells that interfered with MCM2 expression with siRNA was 20.59 μM. These results showed that cisplatin further decreased cell viability in MCM2-knockdown cells compared with that in cells treated with cisplatin alone, indicating that MCM2 knockdown had increased sensitivity to cisplatin-induced growth inhibition (Figure 6B).

Discussion

Treatment of EC in the clinic remains challenging despite advances in recent decades. In particular, recurrence and drug resistance contribute to the poor prognosis of EC patients. It is well accepted that drug resistance and recurrence of EC are mainly caused by cancer cell stemness. Multiple genomic and transcriptomic features promote the ability of tumor cells to invade, self-renew, and grow [23]. Hence, it is important to develop methods that target cell stemness in order to improve the outcome of cancer therapy.

Under physiological conditions, MCM2 is involved in the regulation of DNA replication in cells [2]. However, abnormal expression of MCM2 has been implicated in various tumors, making it a novel molecular diagnostic and therapeutic target for cancers [24]. In this study, based on analysis of data from the TCGA cohort and HPA database, we found that the expression of MCM2 was upregulated at both mRNA and protein level in EC tissues. Moreover, the expression of MCM2 gradually increased with tumor grade, implying that the high expression of MCM2 is related to the characteristics of poorly differentiated EC. Furthermore, high MCM2 expression demonstrated a correlation with poor OS for EC patients. These findings suggest a tumor promoter role of MCM2 in EC.

The importance of MCM2 in the regulation of tumor stemness has been repeatedly emphasized in some other cancers. For example, MCM2 can induce cancer cells to acquire stem cell characteristics by activating the Hippo pathway in hepatocellular carcinoma cells [25]. In colon cancer, NF-kB maintains the stemness of colon cancer cells by downregulating miR-195-5p/497-5p and upregulating MCM2 [17]. These findings uncovered the relevance of MCM2 in cancer cell stemness. In regard to EC, Kato et al. [26] previously reported that, in contrast to the strong correlation between MCM2 and Ki-67 in normal endometrium, a weak correlation exists between MCM2 and Ki-67 in endometrial carcinoma, suggesting that other mechanisms are involved in EC development besides proliferation. In our study, based on bioinformatics assays, we found a positive correlation between MCM2 expression and mRNAsi score, a valid evaluation parameter to evaluate the degree of differentiation of a specific tumor. In subsequent cellular assays, we examined the expression of MCM2 in EC cells *in vitro*. This finding shows that cancer cells expressed MCM2 at higher levels than normal endometrial epithelial cells and MCM2 expression varied among several cancer lines. Following the sphere formation assay, the detection of stemness markers by western blot and flow cytometry revealed that MCM2 overexpression and knockdown promoted and reduced the stemness of EC cancer cells, respectively. These results suggest that MCM2 serves as an important molecule in EC cell stemness.

Several signaling pathways have been reported to be functionally associated with the induction of cancer stemness in EC, including Notch, Wnt, Akt, and Hedgehog pathways [27]. MCM2 can activate or inhibit intracellular signaling pathways to regulate tumor biological behavior. In ovarian cancer, MCM2 inhibits the apoptosis of ovarian cancer cells by regulating the p53 pathway, thereby increasing the sensitivity of ovarian cancer cells to carboplatin [15]. In melanoma cells, MCM2 promotes cell proliferation by regulating the Akt signaling pathway [28]. MCM2 in hepatocellular carcinoma activates the Hippo pathway to induce the acquisition of stem cell characteristics and sorafenib resistance in tumor cells [25]. Dysregulation of AKT signaling was found in all subtypes of EC, and associated with more aggressive disease [29]. Effective blocking of the AKT pathway may be therapeutically valuable in the treatment of EC. Previous studies have revealed that MCM2 regulates AKT expression, although it remains unclear whether MCM2 exerts effects in EC cells. The present study shows that MCM2 directly targets upregulated AKT and thereby increases the expression of p-AKT, β-catenin, and c-Myc. Inhibition of AKT and β-catenin with specific inhibitors reduced the stemness feature of EC cells, suggesting the involvement of AKT/β-catenin pathways in MCM2-mediated cancer cell stemness.

Stemness is a vital contributor to drug resistance. The effect of MCM2 on sensitivity to cisplatin, which is generally recommended for the treatment of advanced and recurrent EC, was also investigated. Data revealed that MCM2 knockdown increased the sensitivity to cisplatin. How MCM2 regulates sensitivity to platinum in cancers is unclear so far. Deng et al. reported that the significantly enhanced chemoresistance of ovarian cancer to carboplatin following MCM2 knockdown may be caused by the accumulation of damaged DNA [15]. In this study, we propose a different mechanism, namely, that MCM2 may mediate the chemoresistance of EC to platinum via regulation of cancer cell stemness.

Collectively, our study revealed the upregulated expression of MCM2 in EC tissues. High MCM2 expression is correlated with poor OS for EC patients. We verified that MCM2 regulates EC cell stemness by regulating the Akt/β-catenin signaling pathway *in vitro*. The identification of MCM2 as a critical stemness regulator of EC may provide evidence for the development of novel molecular therapies for EC treatment.

Supplementary information is available in the online version of the paper.

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MCM2 promotes the stemness of endometrial cancer cells via the Akt/β-catenin pathway

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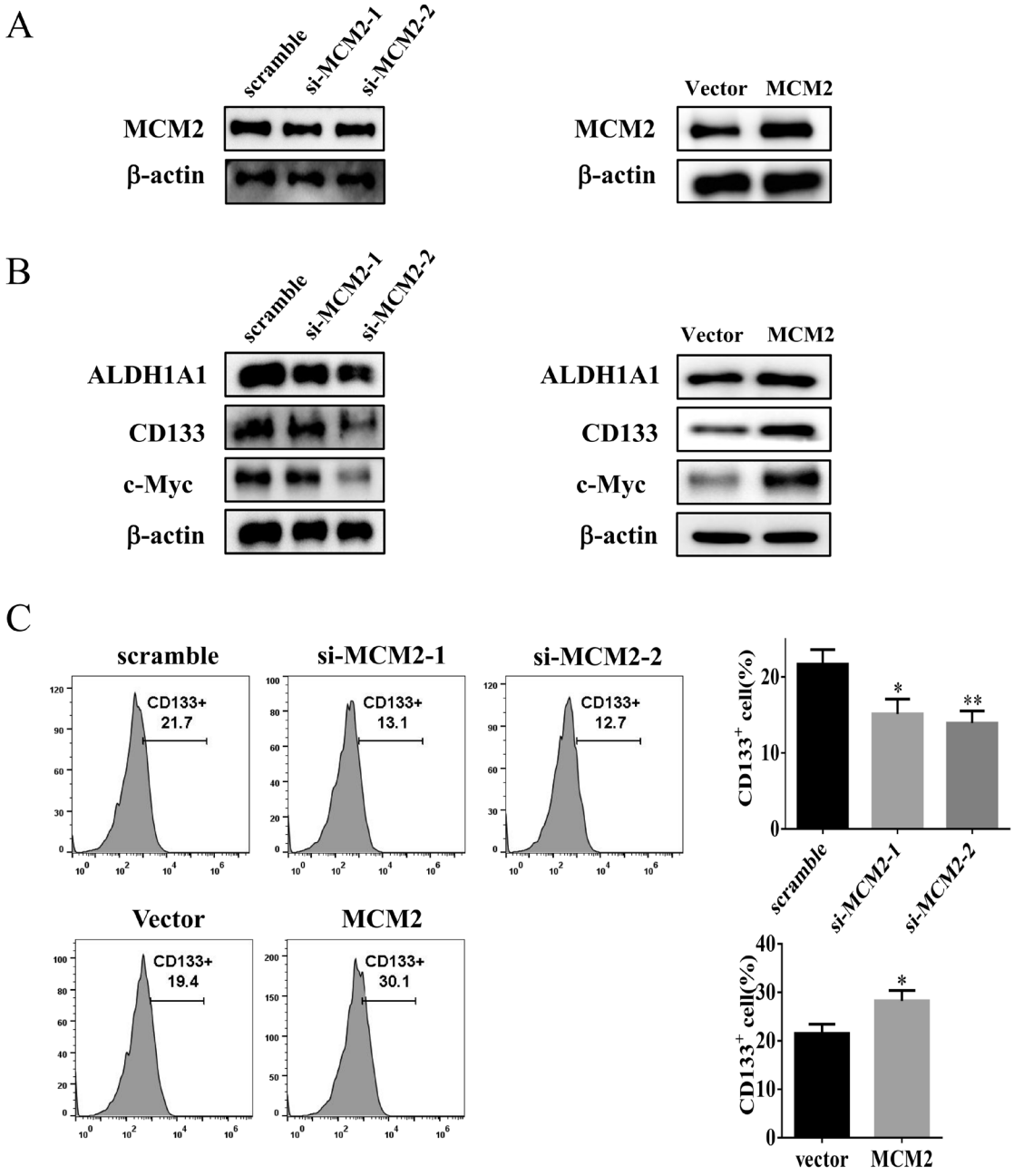
Supplementary Information



Supplementary Figure S1. The effect of MCM2 on Ishikawa cells. A) The amplification and knockdown effects of MCM2 in Ishikawa cells were evaluated by performing western blot assay. B) The expression of ALDH1A1, CD133, and c-Myc in the si-MCM2 and scramble groups determined by western blot assay (left); The expression of ALDH1A1, CD133, and c-Myc in the vector and MCM2 groups determined by western blot assay (right). C) Comparison of CD133+ cell proportion among groups after transfection in Ishikawa cells; *p<0.05 vs. scramble or vs. vector; **p<0.01 vs. scramble or vs. vector