

# CDC20 promotes the progression of hepatocellular carcinoma by regulating epithelial-mesenchymal transition

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**Abstract.** Hepatocellular carcinoma (HCC) is a type of primary liver cancer, which is associated with high mortality. HCC is one of the most common malignant tumors worldwide. Cell division cycle 20 (CDC20) has been reported to be associated with the development of various malignant tumors and epithelial-mesenchymal transition (EMT) has been reported to be involved in the malignant metastasis of HCC. Therefore, the present study hypothesized that CDC20 may participate in the malignant biological behavior of HCC via EMT. The present study analyzed the expression levels of CDC20 in HCC and the association between CDC20 and poor prognosis. Furthermore, the effects of CDC20 on the proliferation, invasion and migration of HCC cells were examined using proliferation, migration and invasion assays. Finally, alterations in EMT were analyzed. The results revealed that CDC20 was highly expressed in HCC and HCC cell lines ( $P<0.05$ ), and its high expression level was significantly associated with poor prognosis in patients with HCC ( $P<0.05$ ). CDC20 silencing inhibited the proliferation, migration and invasion of HCC cells. Furthermore, CDC20 silencing increased the expression levels of E-cadherin, and decreased the expression levels of N-cadherin, vimentin and Ki-67. In conclusion, the present study reported that CDC20 may be a novel therapeutic target in HCC and CDC20 could promote the progression of HCC by regulating EMT.

## Introduction

Hepatocellular carcinoma (HCC) is a common type of cancer that originates from hepatocytes and accounts for 70-90% of primary liver cancer cases worldwide (1). Approximately 500,000 cases of HCC are diagnosed globally every year, and HCC is the fifth most common type of cancer in men and the eighth most common in women (2). If HCC is not treated, patients succumb quickly, and the global 5-year survival rate is only 5% (3-5). Therefore, it is necessary to elucidate the specific mechanism underlying the occurrence and development of HCC, and to provide a scientific basis for the diagnosis and treatment of HCC.

Cell division cycle 20 (CDC20) is a cell cycle checkpoint regulator (6). CDC20 and another regulator E-cadherin can directly bind and activate the anaphase-promoting complex (APC), which has an important role in the process of cells entering and exiting mitosis (7). Recently, an increasing number of studies have shown that CDC20 is upregulated in various types of human malignant tumor, including pancreatic ductal adenocarcinoma, breast cancer, glioblastoma and gastric cancer; in addition, CDC20 may be closely associated with the poor prognosis of various types of cancer (8-11). A previous study reported that overexpression of CDC20 may promote the resistance of castration-resistant prostate cancer cell lines to docetaxel in a Bim-dependent manner (12). Zhang *et al* (13) reported that reducing the expression of CDC20 may inhibit the expression of CD44<sup>+</sup> prostate cancer stem cells. Moreover, CDC20 has been shown to promote the degradation of AXIN1, reduce the phosphorylation of  $\beta$ -catenin and promote the nuclear translocation of  $\beta$ -catenin, thus enhancing the self-renewal ability of CD44<sup>+</sup> prostate stem cells (13). However, to the best of our knowledge, the association of CDC20 with HCC has only been determined through bioinformatics analysis, and no research has been conducted on the association of CDC20 with cell function and related molecular signaling pathways in HCC (13). Therefore, the present study aimed to assess the specific mechanism underlying the effects of CDC20 on HCC.

Epithelial-mesenchymal transition (EMT) refers to the process by which cells transition from an epithelial phenotype to a mesenchymal phenotype under specific physiological or pathological conditions. Notably, EMT has been confirmed to

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play an important role in HCC (14). The process of EMT causes loss of expression of E-cadherin, claudin, occludin and other connecting molecules in epithelial cells, destroys cell polarity and promotes some lytic enzymes that are involved in degradation of the extracellular matrix and basement membrane (15). Increased expression of matrix metalloproteinases, which occurs during EMT, destroys the histological barrier of tumor cell invasion, facilitating the separation of tumor cells from the primary tumor, invasion and metastasis (16). These findings indicated that EMT may serve an important role in promoting HCC metastasis.

The present study aimed to investigate the effect of CDC20 on the poor prognosis of patients with HCC and the biological functions of HCC cells. In addition, the molecular mechanism by which CDC20 affects malignant progression of HCC through EMT was investigated, in order to provide potential strategies for the prognosis and treatment of patients with HCC.

## Materials and methods

**Patients and tumor specimens.** A total of 71 HCC and paired adjacent tissue specimens were collected from Department of Hepatocellular Surgery, Affiliated Hospital of North Sichuan Medical College (Nanchong, China) between January 2010 and May 2013. The mean age of the patients was 61 years (age range, 30-77 years), and the cohort consisted of 30 men and 41 women (Table I). Patients with HCC were followed up for 90 months after surgery, in order to evaluate survival rate (patients were followed up by phone every 4 months, and the total follow-up period was 90 months). All procedures involving human participants were approved by the Ethics Committee of Affiliated Hospital of North Sichuan Medical College and were conducted in accordance with the Declaration of Helsinki. The corresponding clinicopathological data were obtained from Affiliated Hospital of North Sichuan Medical College. All patients who participated in the experiment provided written informed consent.

The detailed inclusion criteria were as follows: i) HCC was confirmed by postoperative pathology, and the histopathological diagnosis was clear; ii) patients were diagnosed with HCC for the first time without distant metastasis; iii) patients had not received any treatment prior to surgery; iv) no other serious malignant disease had been diagnosed; v) the clinical, pathological and surgical data were complete; vi) the follow-up information was complete and available.

**Immunohistochemical staining.** A total of 71 HCC tissues were fixed in 10% formalin for 12 h at room temperature, embedded in paraffin and cut into 4- $\mu$ m sections. The tissue sections were then used to generate tissue microarray cores (1.5-mm diameter). The microarrays were deparaffinized in xylene I for 15 min and xylene II for 15 min at room temperature, and were rehydrated in a graded ethanol series (100, 95, 80 and 75% ethanol, 5 min each). Subsequently, the microarrays were incubated with 3% H<sub>2</sub>O<sub>2</sub> for 30 min at 37°C and with 5% goat serum (Origene Technologies, Inc.) for 15 min at 37°C to block non-specific binding. The microarrays were then incubated with a monoclonal anti-CDC20 antibody (1:2,000; cat. no. ab215908; Abcam) at 4°C overnight. Subsequently, the

sections were incubated with a secondary biotin-labeled IgG antibody (1:100; cat. no. SAP-9100; Origene Technologies, Inc.) at 37°C for 30 min. The visualization signal was detected using 3,3'-diaminobenzidine (Boster Biological Technology) at room temperature for 10 sec. Slides were visualized using a light microscope (magnification, x100; Zeiss AG).

The immunostaining results were analyzed using the following scoring parameters: Staining intensity [according to the color development degree of the positive marker (range, 0-3): No staining, negative (score, 0); light yellow staining, weak (score, 1); brown-yellow staining, moderate (score, 2); and brown black staining, strong (score, 3)] and percentage of positive cells (range, 0-4: 0, <5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; and 4, 76-100%). Total score was determined by adding the staining intensity score to the percentage of positive cells score. Slides with a total score <4 were defined as low CDC20 expression, whereas slides with a score  $\geq$ 4 were defined as high CDC20 expression (17).

**Cell lines and transfection.** Hep3B, MHCC-97H, MHCC-97L and Huh-7 cell lines, and the THLE2 cell line were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The HCC cell lines (Hep3B, MHCC-97H, MHCC-97L and Huh-7) were cultured in DMEM supplemented with 10% FBS, whereas the normal liver cell line (THLE2) was cultured in Bronchial Epithelial Cell Growth Medium supplemented with 10% FBS (all from Gibco; Thermo Fisher Scientific, Inc.); all cells were maintained in a humidified incubator containing 5% CO<sub>2</sub> at 37°C.

Hep3B and Huh-7 cell lines (2x10<sup>5</sup>) were transfected with 50 nM small interfering (si)RNA against CDC20 (si-CDC20) (Guangzhou RiboBio Co., Ltd.). The sequences for the siRNAs were as follows: si-CDC20 forward, 5'-GGAUUGGAGUUCUGGGAAUTT-3' and reverse, 5'-AUUCCCAGAACUCCAUCCTT-3'; and siRNA-negative control (si-NC; scrambled siRNA) forward, 5'-UUCUUCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGAATT-3'. The cells were transiently transfected using Lipofectamine<sup>®</sup> 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc) and the transfection was maintained for >24 h. Subsequent experiments were performed 72 h after transfection.

**RNA extraction and reverse transcription-quantitative PCR (RT-qPCR).** Briefly, total RNA was extracted from HCC tissues, adjacent tissues and cell lines using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RT to cDNA was performed using a PrimeScript RT reagent (Takara Bio, Inc.), and qPCR was performed using SYBR Premix Ex Taq II (Takara Bio, Inc.) and a LightCycler system (Roche Diagnostics). The temperature protocol for RT was as follows: 25°C for 10 min, followed by 45°C for 30 min and 80°C for 30 min. The primer sequences were as follows: CDC20 forward, 5'-GACCACTCCTAGCAAACCTGG-3' and reverse, 5'-GGGCGTCTGGCTGTTTTC-3';  $\beta$ -actin forward, 5'-GGATGCAGAAGGAGATCATCTG-3' and reverse, 5'-CGATCCACACGGAGTACTTG-3'. The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 10 min, followed by 40 cycles at 90°C for 30 sec and 60°C for 30 sec, and a final extension step at 72°C for 10 min.  $\beta$ -actin was used as the internal

Table I. Association between CDC20 expression and clinico-pathological features of patients with hepatocellular carcinoma.

Variables	CDC20 expression			P-value
	Cases	Low (n=29)	High (n=42)	
Age, years				0.801
<50	33	14	19	
≥50	38	15	23	
Sex				0.901
Male	30	12	18	
Female	41	17	24	
TNM stage				0.163
I/II	37	18	19	
III/IV	34	11	23	
Tumor size, cm				0.003
≤5	29	18	11	
>5	42	11	31	
HBsAg				0.233
Positive	45	16	29	
Negative	26	13	13	
AFP, ng/ml (17)				0.629
≤20	27	12	15	
>20	44	17	27	
Liver cirrhosis				0.541
Present	47	18	29	
Absent	24	11	13	
Number of tumors				0.002
Single	36	21	15	
Multiple (≥2)	35	8	27	
Vascular invasion				0.030
Present	33	9	24	
Absent	38	20	18	

AFP,  $\alpha$ -fetoprotein; CDC20, cell division cycle 20; HBsAg, hepatitis B surface antigen.

reference gene, and the mRNA expression levels of CDC20 were analyzed using the  $2^{-\Delta\Delta C_q}$  method (18).

**Western blotting.** Total protein was extracted from cell lines ( $1 \times 10^6$ ) and tissues using a 100:1 mixed solution of RIPA buffer (Beyotime Institute of Biotechnology) and PMSF (Beyotime Institute of Biotechnology). The protein concentrations were quantified using the Bradford protein assay (Bio-Rad Laboratories, Inc.). Equal concentrations of protein ( $30 \mu\text{g}/\text{lane}$ ) were separated by SDS-PAGE on a 10% gel and transferred to PVDF membranes. The membranes were then blocked with 5% nonfat dried milk at room temperature for 1 h and were probed at  $4^\circ\text{C}$  overnight with primary antibodies against CDC20 (1:2,000; cat. no. ab183479;), N-cadherin (1:5,000; cat. no. ab76011), vimentin (1:5,000; cat. no. ab92547), E-cadherin (1:100; cat. no. ab40772), Ki-67 (1:5,000; cat. no. ab92742) and  $\beta$ -actin (1:5,000; cat. no. ab8227) (all

from Abcam). After washing, the membranes were incubated with the appropriate HRP-conjugated secondary antibodies (1:5,000; cat. no. ab6721; Abcam) at room temperature for 1 h. Finally, immunoreactive protein bands were visualized using an enhanced chemiluminescence solution (EMD Millipore) and a ChemiDoc Imaging system (Bio-Rad Laboratories, Inc.).  $\beta$ -actin was used as the internal control. Protein expression was semi-quantified using Quantity One version 4.6 software (Bio-Rad Laboratories, Inc.).

**Bioinformatics analysis.** The Gene Expression Profiling Interactive Analysis (GEPIA) (19) database (<http://gepia.cancer-pku.cn/index.html>) was used to analyze the expression levels of CDC20 in unpaired non-tumor liver tissue samples and HCC tissue samples. The threshold settings were set to  $P < 0.01$ , fold change  $\geq 1$  (19).

**Cell immunofluorescence.** Hep3B and Huh-7 cells were seeded ( $1 \times 10^5$  cells) and cultured on coverslips. After si-CDC20 transfection, Hep3B and Huh-7 cells were fixed with 4% paraformaldehyde at room temperature for 30 min, and permeabilized with 0.25% Triton X-100 solution at room temperature for 15 min. Subsequently, Hep3B and Huh-7 cells were washed with PBS and then blocked with 5% bovine serum albumin (OriGene Technologies, Inc.) at room temperature for 1 h. The coverslips were incubated with anti-CDC20 (1:500; cat. no. ab215908; Abcam) overnight at  $4^\circ\text{C}$ . After washing with PBS, cells were incubated with an appropriate goat anti-rabbit secondary antibody (1:100; cat. no. ZF-0311; OriGene Technologies, Inc.) at  $37^\circ\text{C}$  for 30 min and DAPI. The slides were imaged using an inverted fluorescence microscope and results were recorded.

**Cell Counting Kit-8 (CCK-8) assay.** Hep3B and Huh-7 cells ( $2 \times 10^3$  cells/well) were transfected with si-CDC20 or si-NC for 48 h and cultured in a 96-well plate for 24, 48 and 72 h. The CCK-8 assay (Dojindo Molecular Technologies, Inc.) was used to detect cell proliferation according to the manufacturer's protocol.

**5-Ethynyl-2'-deoxyuridine (EdU) assay.** Hep3B and Huh-7 cells ( $1 \times 10^3$ /well) were transfected with si-CDC20 or si-NC for 48 h and cultured in a 96-well plate. Hep3B and Huh-7 cells were incubated with  $50 \mu\text{M}$  EdU,  $100 \mu\text{l}$  1X ApolloR reaction cocktail (cat. no. 100T; Guangzhou RiboBio Co., Ltd.) and  $100 \mu\text{l}$  1X Hoechst 33342 for 30 min at  $37^\circ\text{C}$ . Hep3B and Huh-7 cell proliferation was analyzed by counting the mean number of cells in three fields for each sample using a fluorescence microscope (magnification,  $\times 100$ ).

**Cell migration and invasion assays.** After si-CDC20 transfection, Hep3B and Huh-7 cells were resuspended in high-glucose DMEM containing 1% FBS and seeded into the upper Transwell chamber at a density of  $1 \times 10^5$  cells/well. Transwell chambers (Corning, Inc.) were used to detect cell invasion and migration. For migration assays,  $500 \mu\text{l}$  high-glucose DMEM containing 10% FBS was added to the lower chamber. After incubation for 12 h at  $37^\circ\text{C}$ , the Transwell chambers were fixed with 4% methanol at room temperature for 30 min and stained with 0.1% crystal violet for 20 min at room temperature. For

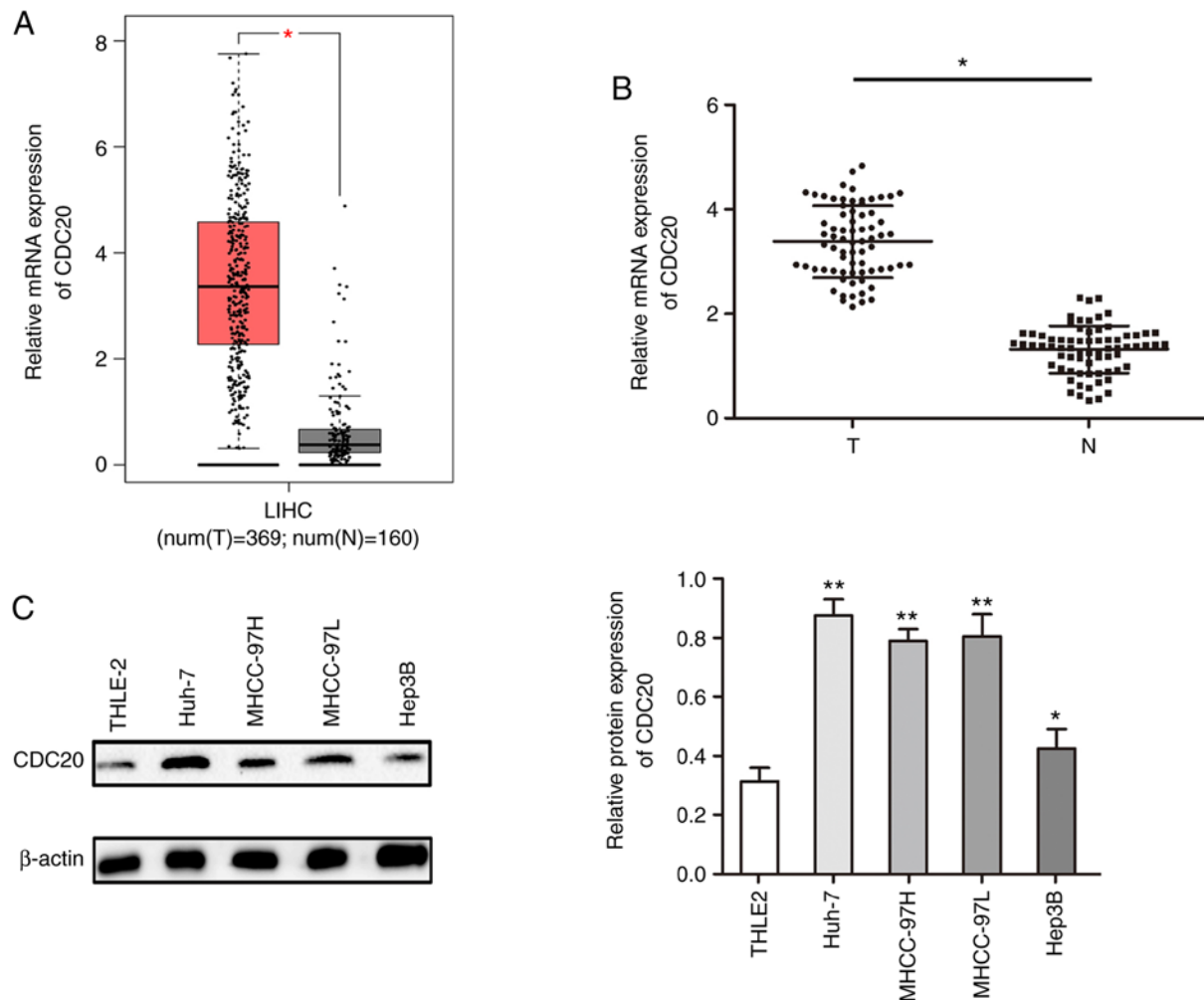


Figure 1. CDC20 expression in HCC tissues and HCC cell lines. (A) mRNA expression levels of CDC20 in HCC and adjacent noncancerous tissues were analyzed by Gene Expression Profiling Interactive Analysis. (B) mRNA expression levels of CDC20 in HCC and adjacent noncancerous tissues were analyzed in 71 clinical HCC samples. (C) Protein expression levels of CDC20 in HCC cells. \* $P < 0.05$ , \*\* $P < 0.01$  vs. THLE2 or as indicated. CDC20, cell division cycle 20; HCC, hepatocellular carcinoma; N, normal tissue; T, tumor tissue; LIHC, liver hepatocellular carcinoma.

the invasion assay, the inserts were precoated with Matrigel (1 mg/ml). After incubation for 16 h at 37°C, the Transwell chambers were fixed with 4% paraformaldehyde at room temperature for 30 min and stained with 0.5% crystal violet at room temperature for 20 min. Finally, invasive and migratory cells were counted under an inverted optical microscope (Olympus DP70 light microscope; Olympus Corporation; magnification, x200).

**Statistical analysis.** All experiments were performed in triplicate. Statistical analyses were performed using SPSS version 22.0 software (IBM Corp.) and GraphPad Prism version 8.0 software (GraphPad Software, Inc.). The significant difference in CDC20 expression between HCC tissues and adjacent normal tissues was assessed using paired t-test. The relationship between CDC20 expression and clinicopathological characteristics was analyzed by  $\chi^2$  test. Overall survival analysis was performed using the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression analyses were used to analyze the prognostic significance of CDC20. Statistical differences among multiple groups were analyzed by one-way ANOVA, followed by Tukey's test.

$P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**CDC20 is highly expressed in HCC.** The present study analyzed the expression levels of CDC20 in HCC using the GEPIA database; the results demonstrated that the mRNA expression levels of CDC20 were significantly higher in HCC tissues compared with those in paired adjacent tissues ( $P < 0.05$ ; Fig. 1A). This finding was confirmed in collected HCC samples; the mRNA expression levels of CDC20 were higher in the HCC samples compared with those in the paired adjacent tissues ( $P < 0.05$ ; Fig. 1B). Furthermore, the expression levels of CDC20 were higher in HCC cell lines compared with those in a normal liver cell line (THLE2) ( $P < 0.05$ ; Fig. 1C).

**CDC20 is associated with clinicopathological features of patients with HCC.** Immunohistochemistry was used to detect the protein expression levels of CDC20 in HCC; the results indicated that high CDC20 expression was observed in 59.2% of HCC samples (41/71) (Fig. 2A). Furthermore, the association

Table II. Univariate and multivariate analyses of risk factors for overall survival in patients with hepatocellular carcinoma.

Variables	n	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years		0.769 (0.423-1.304)	0.534		
<50	33				
≥50	38				
Sex		0.506 (0.347-0.863)	0.364		
Male	30				
Female	41				
TNM stage		1.338 (0.450-4.338)	0.673		
I/II	37				
III/IV	34				
Tumor size, cm		1.863 (1.035-3.713)	0.011	1.903 (1.156-3.066)	0.017
≤5	29				
>5	42				
HBsAg		1.304 (0.408-4.368)	0.364		
Positive	45				
Negative	26				
AFP, ng/ml (17)		0.783 (0.425-1.557)	0.403		
≤20	27				
>20	44				
Liver cirrhosis		0.933 (0.489-1.806)	0.869		
Presence	47				
Absence	24				
Number of tumors		1.366 (1.108-3.761)	0.036	1.997 (1.297-6.330)	0.031
Single	36				
Multiple (≥2)	35				
Vascular invasion		0.673 (0.339-1.208)	0.574		
Present	33				
Absent	38				
CDC20 expression		1.735 (1.196-3.667)	0.011	2.036 (1.296-6.647)	0.007
High	29				
Low	42				

AFP, α-fetoprotein; CDC20, cell division cycle 20; CI, confidence interval; HBsAg, hepatitis B surface antigen; HR, hazard ratio.

between CDC20 expression and the clinicopathological characteristics of patients with HCC was assessed. High CDC20 expression was revealed to be positively associated with tumor size ( $P=0.003$ ), number of tumors ( $P=0.002$ ) and vascular invasion ( $P=0.030$ ) (Table I).

*CDC20 overexpression is associated with poor prognosis in HCC.* The association between CDC20 expression and the poor prognosis of patients with HCC was analyzed using the 71 collected clinical HCC samples; the results revealed that high CDC20 expression was associated with significantly shorter overall patient survival compared with low CDC20 expression ( $P<0.01$ ; Fig. 2B). In addition, univariate analysis indicated that tumor size ( $HR=1.863$ ;  $P=0.011$ ), multiplicity ( $HR=1.366$ ;  $P=0.036$ ) and CDC20 expression ( $HR=1.735$ ;  $P=0.011$ ) were significantly associated with overall survival

in HCC (Table II). Multivariate analysis also suggested that tumor size ( $HR=1.903$ ;  $P=0.017$ ), multiplicity ( $HR=1.997$ ;  $P=0.031$ ) and CDC20 expression level ( $HR=2.036$ ;  $P=0.007$ ) were independent prognostic factors for overall survival in HCC (Table II).

*Knockdown of CDC20 inhibits the proliferation of Hep3B and Huh-7 cells.* The present study used si-CDC20 to silence CDC20 expression. Hep3B and Huh-7 cells were used, as the expression levels of CDC20 were significantly different; the expression levels of CDC20 in Huh-7 were relatively high, whereas those in Hep3B cells were relatively low. Post-transfection with si-CDC20, the expression levels of CDC20 were significantly decreased in Hep3B and Huh-7 cells ( $P<0.05$ ; Fig. 3A and B). Furthermore, cellular immunofluorescence confirmed the reduction in the expression levels of



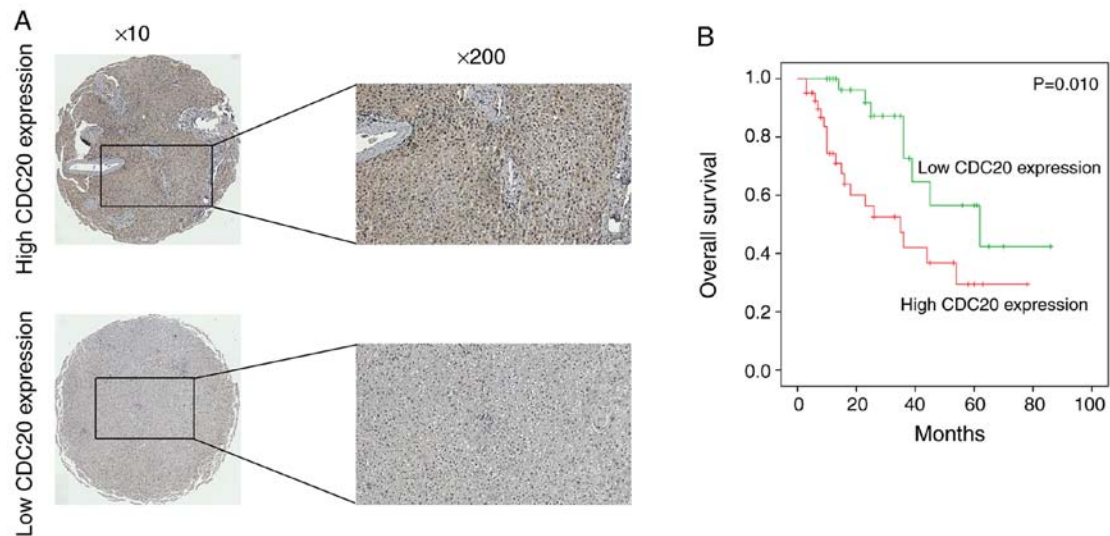


Figure 2. CDC20 expression and its relationship with prognosis in HCC. (A) Immunohistochemical staining analysis of CDC20 expression in HCC. (B) Kaplan-Meier analysis of overall survival of patients with HCC and high or low CDC20 expression (analyzed using the 71 clinical HCC samples collected). CDC20, cell division cycle 20; HCC, hepatocellular carcinoma.

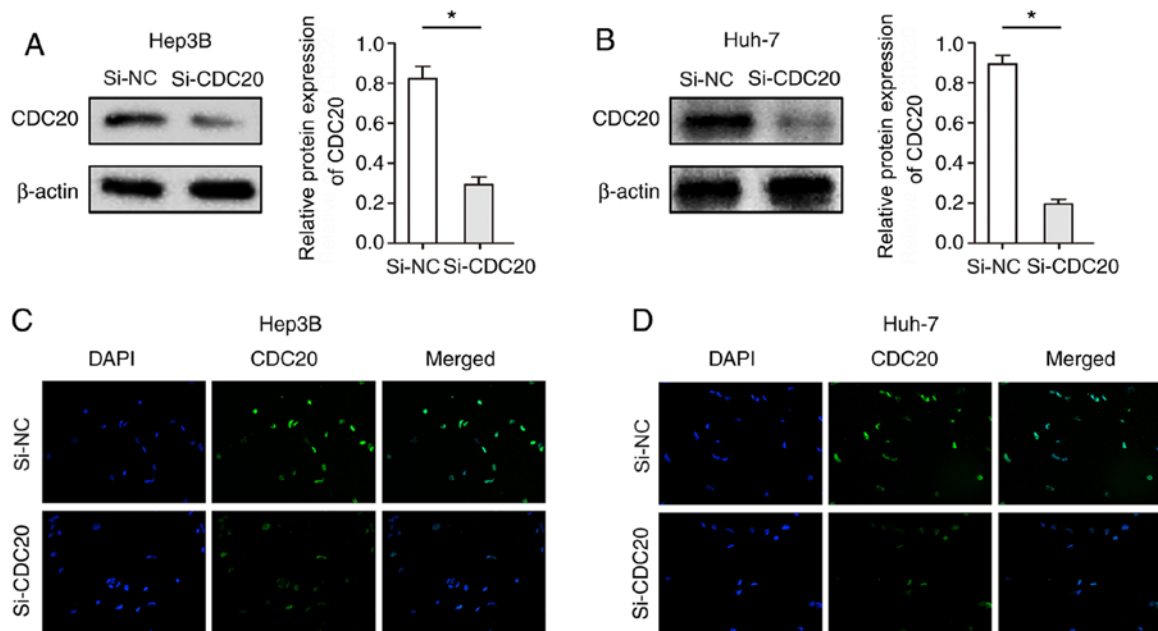


Figure 3. Knockdown of CDC20 in hepatocellular carcinoma cells. Protein expression levels of CDC20 in (A) Hep3B and (B) Huh-7 cells, as determined by western blotting. Protein expression of CDC20 in (C) Hep3B and (D) Huh-7 cells, as determined by immunofluorescence staining (magnification, x200). \* $P < 0.05$ . CDC20, cell division cycle 20; NC, negative control; Si, small interfering RNA.

CDC20 in Hep3B and Huh-7 cells transfected with si-CDC20 (Fig. 3C and D). The results of the CCK-8 assay revealed that knockdown of CDC20 significantly inhibited the proliferation of Hep3B and Huh-7 cells ( $P < 0.05$ ; Fig. 4A and B). In addition, the number of Hep3B and Huh-7 EdU-positive cells was significantly decreased in the si-CDC20 group compared with that in the si-NC group ( $P < 0.01$ ; Fig. 4C and D).

**Knockdown of CDC20 inhibits the migration and invasion of Hep3B and Huh-7 cells.** The present study examined the effect of CDC20 on the migration and invasion of Hep3B and Huh-7 cells through Transwell assays. The results revealed that knockdown of CDC20 inhibited Hep3B and Huh-7 cell

migration and invasion compared with those in the si-NC group ( $P < 0.05$ ; Fig. 5A and B).

**Knockdown of CDC20 inhibits the expression of EMT-related proteins.** The present study demonstrated that the proliferation, migration and invasion of HCC cells was reduced when expression of CDC20 was decreased. Therefore, whether the changes were related to alterations in EMT- and proliferation-associated proteins was assessed. The results of western blotting revealed that CDC20 silencing decreased N-cadherin, vimentin and Ki-67 expression levels, and increased E-cadherin expression levels compared with those in the si-NC group ( $P < 0.05$ ; Fig. 6A and B).

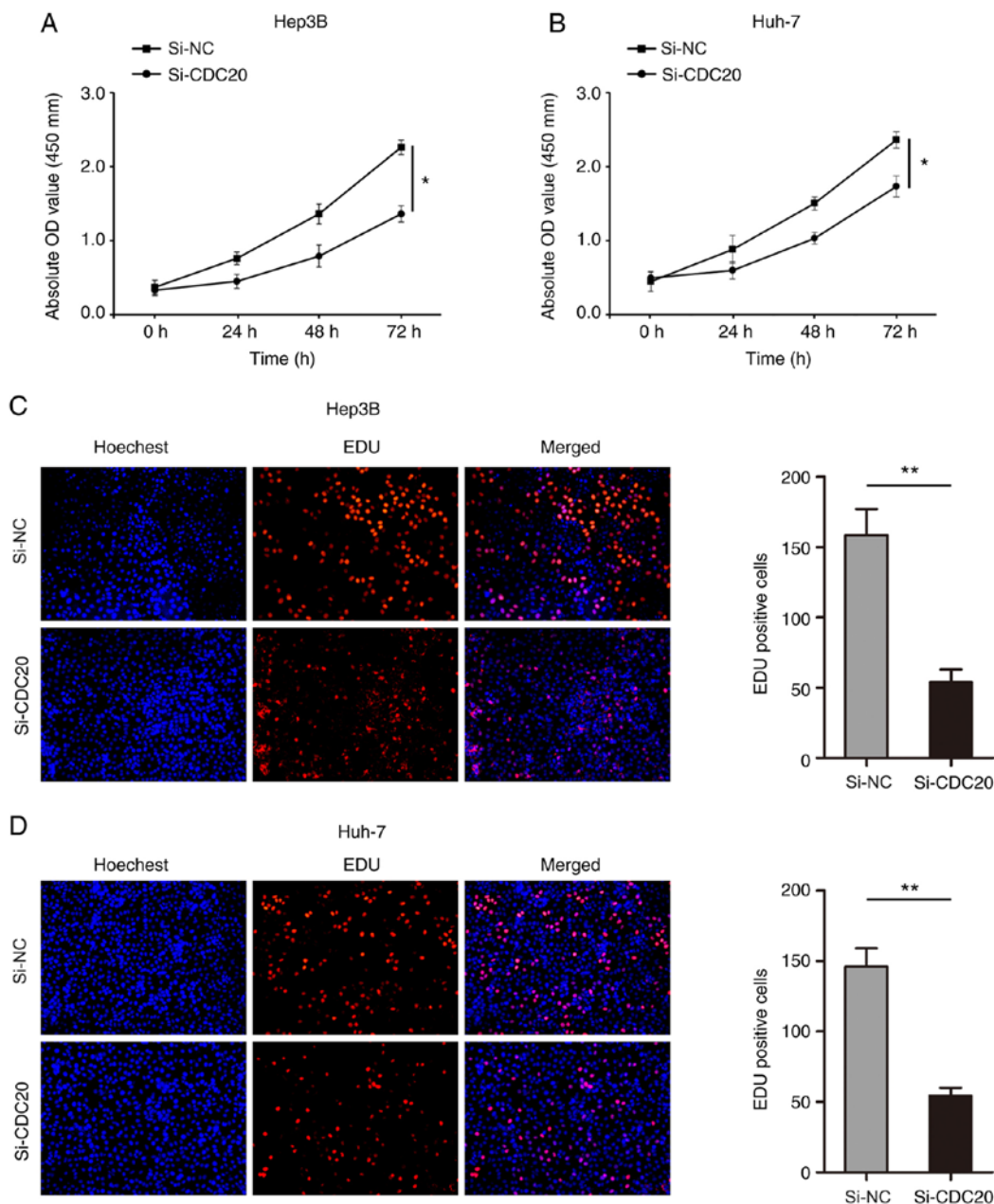


Figure 4. Effect of CDC20 on the proliferation of hepatocellular carcinoma cells. Cell Counting Kit-8 assays were carried out to examine the effects of silencing CDC20 on the proliferative ability of (A) Hep3B and (B) Huh-7 cells. Cell proliferation was detected by EdU staining in (C) Hep3B and (D) Huh-7 cells after knockdown of CDC20 (magnification, x200). \* $P < 0.05$ , \*\* $P < 0.01$ . CDC20, cell division cycle 20; EdU, 5-ethynyl-2'-deoxyuridine; NC, negative control; Si, small interfering RNA.

## Discussion

The present study confirmed that CDC20 was highly expressed in HCC, and its high expression was significantly associated with the poor prognosis of patients with HCC. In addition, it was revealed that CDC20 could regulate the malignant biological behavior of HCC through EMT, which lays the foundation for further research into the specific mechanism underlying the effects of CDC20 on HCC.

CDC20 is a regulator of the APC, which can accelerate mitotic exit and interact with the spindle assembly checkpoint (SAC) (20). The SAC protein aggregates at the centromere in the prometaphase stage of mitosis and induces conformational changes of Mad2, which helps it bind to CDC20 and BubR1 to

form the mitotic checkpoint complex, an APC inhibitor (20). The high expression of CDC20 in tumor tissues has been reported to promote the malignant progression of tumors (21). Conversely, inhibition of CDC20 activity may regulate cell division cycle and accelerate cell apoptosis (21). A previous study demonstrated that decreasing the expression of CDC20 inhibited the migration of pancreatic cancer cells and metastatic breast cancer cells, and triterpene mixture extracted from the mushroom *Poria cocos*, purified triterpene dehydropropionic acid and polyvalerate C could significantly inhibit the expression of CDC20 to regulate the malignant biological behavior of tumor cells (22). In estrogen receptor-positive breast cancer, CDC20 mRNA was reported to be highly expressed, and high CDC20 expression was closely related to tumor size and poor tumor

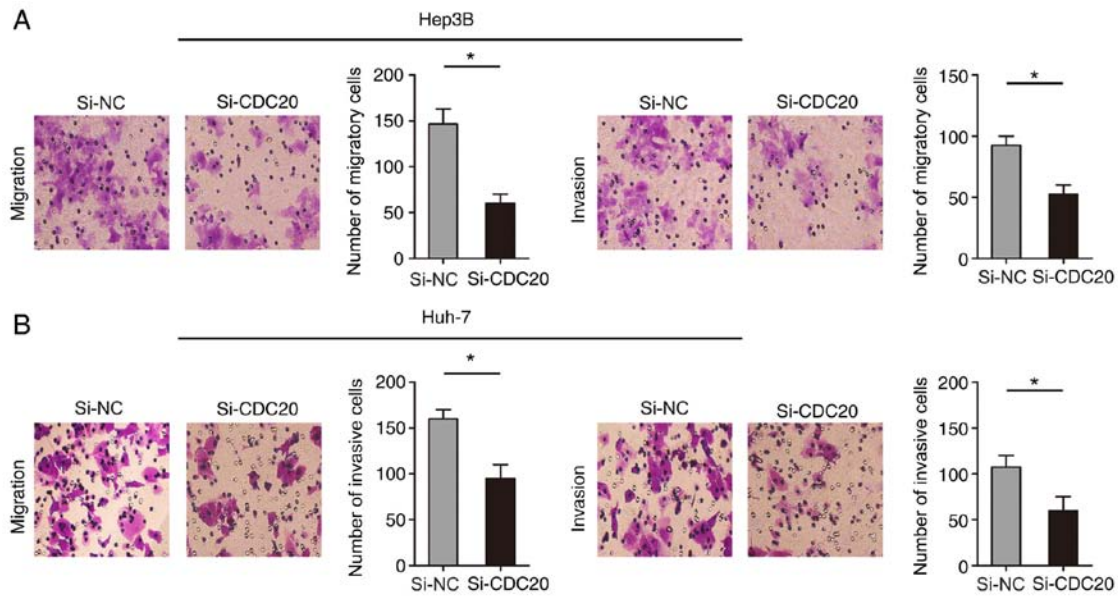


Figure 5. Effect of CDC20 on the migration and invasion of hepatocellular carcinoma cells. Migration and invasion assays were used to examine the effects of silencing CDC20 on (A) Hep3B and (B) Huh-7 cell migration and invasion (magnification, x100). \* $P<0.05$ . CDC20, cell division cycle 20; NC, negative control; Si, small interfering RNA.

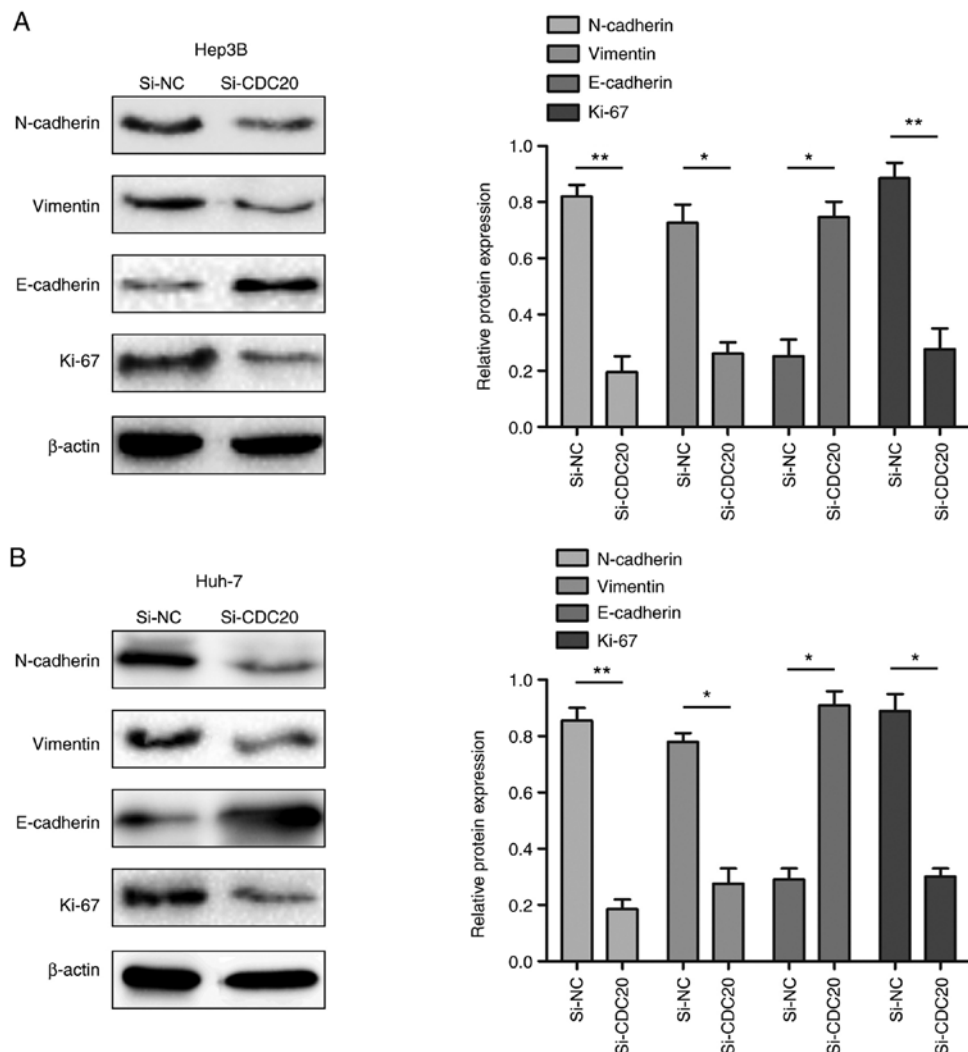


Figure 6. Effect of CDC20 on epithelial-mesenchymal transition in hepatocellular carcinoma cells. Protein expression levels of N-cadherin, vimentin, E-cadherin and Ki-67 after transfection of (A) Hep3B and (B) Huh-7 cells with si-CDC20. \* $P<0.05$ , \*\* $P<0.01$ . CDC20, cell division cycle 20; NC, negative control; Si, small interfering RNA.



grade (23). High mRNA expression levels of CDC20 were also significantly associated with poor prognosis. Notably, the high expression of CDC20 has been reported to be closely associated with the adverse effects of endocrine therapy in patients receiving hormone therapy; CDC20 is therefore considered an independent predictor of adverse clinical outcomes after endocrine therapy (23). A previous study also demonstrated that CDC20 expression was high and the expression of BIM was decreased in glioma cell lines resistant to temozolomide (24). Furthermore, inhibition of CDC20 expression could inhibit the EMT characteristics of drug-resistant cell lines and regulate the malignant growth of glioma drug-resistant cell lines (24). CDC20 has also been revealed to be highly expressed in primary cutaneous squamous cell carcinoma and may promote the malignant biological behavior of tumor cells through the Wnt/ $\beta$ -catenin signaling pathway (25). In addition, CDC20 is a known key downstream gene of the MDM2-p53 signaling pathway in diffuse large B-cell lymphoma, and has been shown to regulate tumor cell proliferation, apoptosis and cell cycle changes (26). A previous study reported that CDC20 was very lowly expressed in normal liver tissues, and to the best of our knowledge, there are no reports of CDC20 expression in other liver diseases (1). Notably, the results of the present study demonstrated that CDC20 was highly expressed in HCC, and its high expression was significantly associated with the poor prognosis of patients with HCC. Our future studies aim to collect information on the expression of CDC20 in HCC from a public database, and further analyze the relationship between the expression of CDC20 and the poor prognosis of HCC.

One of the important biological characteristics of HCC is its high levels of metastasis, which can lead to poor prognosis of patients (27). At present, although the mechanism of tumor metastasis is not fully understood, existing studies have revealed that tumor metastasis is a complex process involving multiple factors and stages, which depends on the interaction between tumor cells and internal environmental factors, such as promoting tumor cell growth, invasion, migration and angiogenesis (28). A large number of studies have demonstrated that EMT may have an important role in tumor metastasis. EMT is a process during which epithelial cells lose polarity, tight junctions and adhesion junctions under the action of certain factors, and thus gain infiltrative and migratory abilities, and acquire interstitial cell morphology and characteristics (29-31). The loss of an epithelial phenotype and the acquisition of stromal characteristics are the main features of EMT. EMT includes the following: i) Decreased expression of cell adhesion molecules, which leads to the loss of epithelial cell intercellular adhesion, and ii) the keratin cytoskeleton is transformed into a vimentin cytoskeleton, and cells transform into spindle cells. In addition, EMT sometimes affects cell function and morphology (30,31). This phenotypic transformation enables tumor cells to eliminate intercellular adhesion and thus become more aggressive.

The EMT process has been reported to be associated with changes in the expression levels of slug, snail and twist, which can regulate the malignant proliferation of HCC (32). Previous studies have reported that inhibition of CDC20 expression in cisplatin-resistant osteosarcoma cells significantly reversed the EMT phenotype and altered the expression of EMT biomarkers (24,33). In estrogen receptor-positive luminal A breast cancer cells, EMT changes have been shown to serve an

important regulatory role in breast cancer migration, invasion and metastasis (34). In the present study, CDC20 silencing decreased the expression levels of N-cadherin, vimentin and Ki-67, and increased E-cadherin expression suggesting that CDC20 expression may promote the metastasis of HCC through EMT. However, how this mechanism is achieved and the further regulatory mechanism require in-depth research. The present study demonstrated that CDC20 could regulate the malignant biological behavior of HCC through EMT, and these findings may provide a scientific and objective basis for further exploring the role of CDC20 in HCC from the perspective of EMT. Although changes in the expression level of CDC20 may cause changes in EMT, it is still unclear which specific molecule it acts on, and its specific regulatory mechanism is unclear. In addition, our future studies aim to analyze the mechanism of CDC20 in other liver diseases.

Research on CDC20 in HCC has mainly been conducted through bioinformatics analysis, and studies exploring the specific mechanism by which CDC20 affects HCC via cell function analysis are rare. The present study explored the role of CDC20 in HCC from the perspective of cell function and aimed to determine the specific molecular mechanism of CDC20. In addition, the relationship between CDC20 and the characteristics of patients with HCC was assessed. The present research is innovative and comprehensively analyzed the role of CDC20 in HCC. In conclusion, the present results indicated that the expression of CDC20 was significantly increased in HCC cells, and CDC20 overexpression was associated with poor HCC prognosis. Furthermore, CDC20 may promote proliferation, migration and invasion of HCC cells and could affect the biological function of HCC cells through EMT.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

GY, GW, YX and JL conceived and designed the experiments. GY, GW, YX and JS conducted experiments. GY, WL, TT and JL performed data analysis and wrote the paper. GY, GW, YX and JL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All procedures involving human participants were approved by the Ethics Committee of Affiliated Hospital of North Sichuan Medical College, and complied with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The participants signed an extensive informed consent form after being informed about the benefits and risks associated with the present study.

## Patients consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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