Increased expression of HMMR in renal cell carcinoma is an independent prognostic factor

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Abstract. The present study investigated how abnormal expression of hyaluronan-mediated mobility receptor (HMMR) in renal cell carcinoma (RCC) tissue affects the growth of RCC cells and the association between expression of HMMR and pathological staging and prognosis of patients with RCC. Reverse transcription-quantitative PCR was used to measure the expression of HMMR mRNA in RCC tissue and cell lines. For the prediction of HMMR gene expression, The Cancer Genome Atlas online database was utilized to compare differential gene expression of HMMR in normal renal and RCC tissue. Cell Counting Kit-8 assay was used to examine cell proliferation. The expression of HMMR increased in RCC tissue and renal cancer cell lines. The expression of HMMR was an independent prognostic factor for 5-year and disease-free survival in patients with RCC. The silencing of HMMR expression decreased the expression of Cyclin B1 and inhibited the proliferation of RCC cells. Overexpression of HMMR promoted the expression of Cyclin B1 and cell proliferation. The present study demonstrated that the expression of HMMR was significantly upregulated in RCC and was an independent prognostic factor for RCC. HMMR may be involved in the occurrence of RCC by regulating Cyclin B1.

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Introduction

Renal cell carcinoma (RCC) is a common tumor, accounting for 3-4% of malignant tumors and 90% of all renal malignancies globally (1,2). More than 200,000 new RCC cases and 100,000 RCC-associated deaths occur each year, seriously affecting human health (3). It is reported that the occurrence of RCC is associated with genetic and environmental factors, but the molecular mechanism of its occurrence and development is still unclear (4). The primary treatment for RCC is radical resection, but 30% of patients still have recurrence or distant metastasis following the operation (5). Certain patients already have distant metastasis at their first visit to a doctor and the recurrence and metastasis rates in patients with advanced RCC are high (6). The mechanism of RCC metastasis is still unclear. With the emergence of novel chemotherapeutic and molecular targeted drugs, such as the advent of the tyrosine kinase inhibitor sunitinib (7), progress has been made in the drug treatment of RCC. However, due to the heterogeneity of tumor cells and drug resistance of patients with tumor, there are still problems in the treatment of RCC.

Hyaluronan-mediated mobility receptor (HMMR), also known as CD168 on the cell surface (8), can combine with hyaluronic acid. HMMR is not only expressed on the cell surface, but also distributed in the cytoplasm and nucleus (9). HMMR has the characteristics of an oncogene and can transform cells (10). It is a microtubule-associated protein, which can bind to microtubules, microfilaments and calmodulin, affecting cytoskeleton assembly and cell movement (11). It is also a cell cycle regulator that regulates cell division in the G_2/M phase (12). Moreover, HMMR is a centrosome and mitotic spindle-binding protein that maintains the structural integrity of centrosome and the number and structural integrity of spindle poles (13). All these biological characteristics are associated with the occurrence, development and metastasis of tumors. In clinical studies, HMMR has been shown to be expressed in malignant glioma, as well as breast, bladder and endometrial cancer (14-17). The HMMR gene is highly expressed in mouse embryonic cardiomyocytes and regulates cell cycle as a mitotic regulator (18). HMMR is associated with, and may be a new marker for, cell proliferation (15,19). Although HMMR is highly expressed in a variety of tumor tissue, the mechanism of HMMR in the occurrence and

development of RCC is still unclear. The present study aimed to investigate the mechanism of HMMR in RCC and provide a theoretical basis for identifying novel molecular markers and gene therapy targets of RCC.

Materials and methods

Subjects. A total of 30 patients (age range, 18-95 years; males:females, 1:1) with RCC who received treatment at the Department of Urology Surgery of Xintai People's Hospital (Xintai, China) between January 2015 and October 2019 were included in the study. Inclusion criteria were as follows: Histology or cytology results confirming RCC; adult patients (≥18 years old), of either sex, able to provide consent; suspected or confirmed RCC; written informed consent provided by the patient. Exclusion criteria were a patient age of ≤18 years old and an inability to provide informed consent. Tumor and matched adjacent tissue (distance, ≥5 cm) were collected and stored at -80˚C. Incomplete clinical and pathological data were obtained from the hospital biobank. All procedures performed were approved by the Ethics Committee of Jinan Third People's Hospital. Written informed consent was obtained from all patients or their families.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) was used to cleave 786-O, ACHN, 769-P and Caki-1 (all ATCC) cells and liver tissue, and total RNA was extracted by chloroform and precipitated with isopropanol. The RNA concentration was determined by Nanodrop 2000c ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc.). According to the instructions of PrimeScript RT reagent with gDNA Eraser kit (Takara Biotechnology Co., Ltd.), 1 μg RNA was reverse-transcribed into cDNA. An RT-qPCR reaction system was set up according to the instructions of SYBR Premix Ex Taq kit (Takara Biotechnology Co., Ltd.), and iQ5 (Bio-Rad Laboratories, Inc.) was used to assess the expression of HMMR in RCC tissue and cells.

The forward primer for HMMR was 5'-CTGAGAGTG TCTTGGGAG-3' and the reverse primer was 5'-CAGTGG GTGAGTGACTCTG-3'. GAPDH was used as an internal reference. The forward primer for GAPDH was 5'-CCACTC CTCCACCTTTGACG-3', and the reverse primer was 5'-TGG TGGTCCAGGGGTCTTA-3'. The cycling program was composed of an initial step to activate the enzyme at 95˚C for 3 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 20 sec and 72˚C for 1 sec. The $2^{-\Delta\Delta Cq}$ method (20) was used to calculate the expression of the genes.

Cells. Human RCC ACHN and renal proximal convoluted tubule epithelial HK-2 cells were cultured in MEM; human kidney clear cell carcinoma 786-O, Caki-1 and 769-P cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.). Cell lines were authenticated using STR profiling. All media were supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 100 U/ml penicillin/streptomycin, and all cells were incubated at 37˚C and 5% CO_2. When cell density reached 70-90%, cells were digested with trypsin containing EDTA (Gibco), and the medium was replaced every two days. The cells were seeded in 96-well plates. After a density of 70-90% was reached, the cells were transfected with small interfering (si)RNA-HMMR and its negative control (siR-NC) or HMMR and its NC.

Cell Counting Kit (CCK)-8 assay. CCK-8 reagent (Dojindo Laboratories, Inc.) was added to 786-O, ACHN, 769-P and Caki-1 cells, which were cultured for 1 h. Then, absorbance at 450 nm was measured at 0, 24, 48 and 72 h using an enzyme-linked immunosorbent assay (ELISA) reader (Dynatech Laboratories). Each sample was tested in 6 replicate wells.

Cell transfection. siR-NC and siR-HMMR (siR-HMMR-1, siR-HMMR-2, siR-HMMR-3), overexpression-NC and overexpression-HMMR were obtained from Shanghai GenePharma Co., Ltd. and transfected into 786-O and ACHN cells by Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The sequences of HMMR siRNA were as follows: HMMR-1, 5'-CUGAUU UGCAGAACCAACUdTdT-3'; HMMR-2, 5'-GGAGAAUAU UGUUAUAUUAdTdT-3' and HMMR-3, 5'-GGUGUAUAU AGAUAUAUUAdTdT-3'. The siR-NC was non-silencing siRNA (siR-NC-1, 5'-UUAAUAUGCAGUGCCAUAUdTdT-3'; siR-NC-2, 5'-CCAGAAUACAGAUAUAUUAdTdT-3' and siR-NC-3, 5'-GCAGUAUAUUAAUAUAACUdTdT-3') with the same length as siRNA of HMMR. The sequence of overexpression-NC sequence was 5'-GGATCTACACGAATGAGG AGC-3' and the sequence of overexpression-HMMR sequence was 5'-GTACGGTACAGGTCACTTGAT-3'. All 786-O and ACHN cells were seeded in 6-well plates and cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C for ≥24 h before transfection, and rinsed with phosphate-buffered saline (PBS, pH 7.4) before transient transfection. Transfections were performed with 10 nM siR-NC or siR-HMMR mixed with Lipofectamine 2000 at 37˚C for 24 h. The cells were harvested for subsequent experimentation 24 h later.

Transwell assay. Migration and invasion tests on 786-O and ACHN cells were performed in 24-well Transwell chambers (Corning, Inc.) with a polycarbonate membrane. In the Transwell migration assay, $1x10^5$ 786-O cells were seeded in serum-free DMEM in the upper chamber and lower chamber contained DMEM with 10% FBS. Following incubation at 37˚C for ~10 h, the inserts were taken out carefully. The cells were fixed on the lower side of the insert membrane with 5% glutaraldehyde for 10 min, followed by staining with 1% crystal violet in 2% ethanol for an addition 20 min (all at 25˚C). The inserts were washed in PBS for several seconds to remove excess dye, then observed under a light microscope (Nikon, 100x magnification). Cells from five randomly selected fields were counted. The procedure of Transwell invasion assay were the same as aforementioned, except the upper chambers were coated with 20 μg extracellular Matrix gel (Sigma-Aldrich; Merck KGaA). The Matrigel temperature was 37˚C and the precoating time was 10 h.

Flow cytometry. Cell cycle was analyzed by flow cytometry. 786-O cells were collected, treated with trypsin, washed with PBS and then fixed with cold ethanol at room temperature for 3 min. Then, propidium iodide (Sigma-Aldrich; Merck KGaA) was used to stain the cells at room temperature for

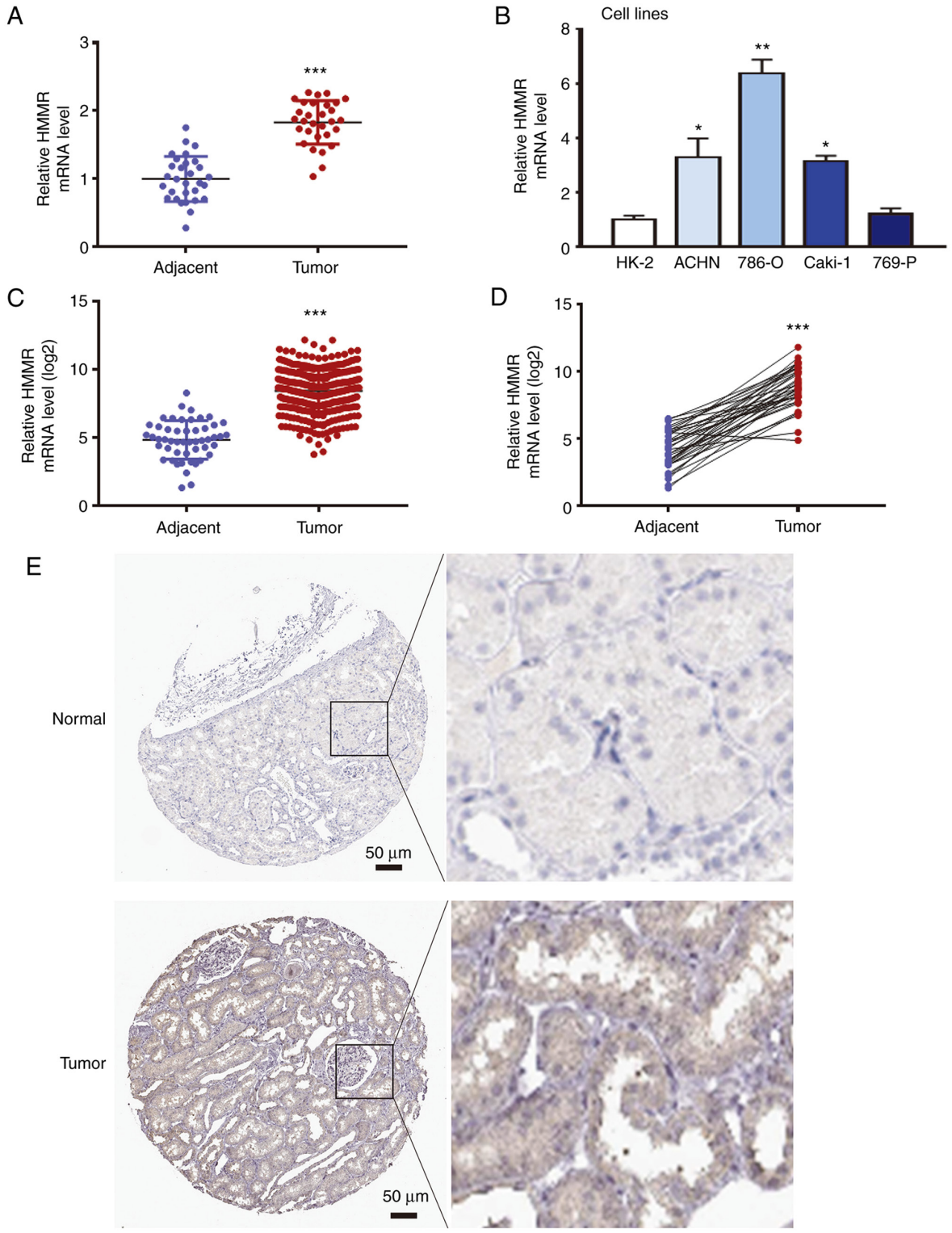

Figure 1. Expression of HMMR in RCC tissue and cell lines. (A) Relative expression of HMMR mRNA in RCC and adjacent tissue. Statistical significance was analyzed by paired two-tailed Student's t-test. ***P<0.001 vs. adjacent. (B) Relative expression of HMMR in RCC cell lines. Statistical significance was analyzed by one-way ANOVA. *P<0.05, **P<0.01 vs. HK-2 cells. (C) Relative expression of HMMR mRNA in RCC and adjacent tissue from TCGA database. Statistical significance was analyzed using paired two-tailed Student's t-test. ***P<0.001 vs. adjacent. (D) Relative expression of HMMR mRNA in RCC and corresponding adjacent tissue in TCGA database. ***P<0.001 vs. adjacent. (E) Immunohistochemical staining of HMMR in RCC and normal tissue obtained from The Human Protein Atlas database (proteinatlas.org). HMMR, hyaluronan-mediated mobility receptor; RCC, renal cell carcinoma; TCGA, The Cancer Genome Atlas.

15 min, and cell proportions of each phase were detected by flow cytometry (CytoFLEX; Beckman-Coulter, Inc.). Lastly, flow cytometry data were analyzed by WinList 7.0 (Verity Software House).

Bioinformatics. For the prediction of HMMR gene expression, The Cancer Genome Atlas (TCGA) online database (https://portal.gdc.cancer.gov/) was utilized to compare differential expression of the HMMR gene in normal renal and

Table I. Clinical and pathological data of patients with renal cell carcinoma.

Case	Sex	Age, years	Tumor stage
1	Male	85	III
2	Male	52	III
3	Female	87	I
4	Female	56	II
5	Male	49	IV
6	Male	72	III
7	Male	31	III
8	Female	45	II
9	Male	64	II
10	Female	39	III
11	Male	55	I
12	Female	48	II
13	Male	66	III
14	Male	60	IV
15	Female	33	II
16	Female	73	III
17	Female	53	I
18	Female	41	IV
19	Female	54	II
20	Male	37	III
21	Male	51	III
22	Male	95	III
23	Female	51	IV
24	Male	29	II
25	Male	55	II
26	Male	89	III
27	Male	85	I
28	Female	82	IV
29	Female	77	II
30	Female	34	III

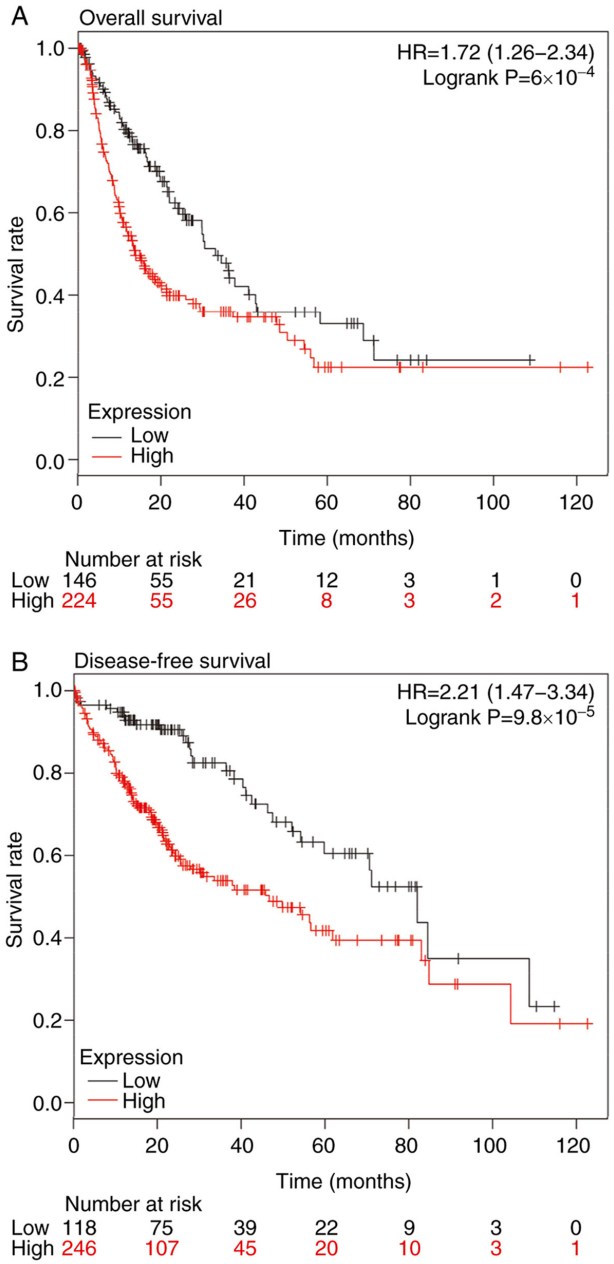

Figure 2. Association between HMMR expression and survival rates of patients with RCC based on The Cancer Genome Atlas database. Association between HMMR expression and (A) overall and (B) disease-free survival rate of patients with RCC. HMMR, hyaluronan-mediated mobility receptor; RCC, renal cell carcinoma.

RCC tissue. For survival analysis, TCGA (cancergenome.nih.gov/) database was used to study the association between gene expression of HMMR and survival time and recurrence rate of RCC. For Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, the Metascape (metascape.org) database was used to find the GO classification items and associated pathways of enriched HMMR and associated proteins, and to search for their possible functions. The STRING database was applied to construct the protein-protein interaction network. For proteomics data analysis, The Human Protein Atlas database (proteinatlas.org) was used to obtain data on the expression of HMMR protein in normal renal and cancer tissue.

Statistical analysis. Data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc.). All data are expressed as the mean ± SEM from three independent experiments. Statistical significance was analyzed using a paired two-tailed Student's t-tests for two groups and one-way ANOVA followed by Tukey's post hoc test for multiple groups. χ^2 test was performed to test differences in prognosis between groups. Survival curves were analyzed by Kaplan-Meier and log-rank analysis. Cox model was used to analyze the association between characteristic data (including age, sex, grade and stage) and patient survival. HMMR expression was ranked from low to high. The lowest and highest 25% were defined as low- and high-expression group, respectively. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of HMMR is increased in RCC tissue and renal cancer cell lines. Table I shows the clinical and pathological

Table II. Cox regression model analysis of overall survival in renal cell carcinoma.

	Univariate analysis			Multivariate analysis		
Characteristic	HR	95% CI	P-value	HR	95% CI	P-value
Age, years (<60 vs. ≥60)	1.031	0.981-1.051	0.588	1.030	0.988-1.049	0.421
Sex (male vs. female)	0.681	0.587-1.249	0.441	0.769	0.575-1.343	0.876
Grade (G1 + G2 vs. G3 + G4)	1.215	0.784-1.588	0.467	1.263	0.639-1.611	0.791
Stage (I-II vs. III-IV)	1.664	1.426-2.369	$2.67x10^{-5}$	1.317	0.572-3.527	0.622
T1 + T2 vs. T3 + T4	1.573	1.297-2.588	$3.58x10^{-5}$	1.239	0.474-3.249	0.775
M1 vs. M0	4.634	2.246-6.473	0.037[a]	1.436	0.394-5.312	0.157
N0 + N1 vs. N2 + N3	3.371	0.599-6.482	0.511	1.966	0.571-8.175	0.387
HMMR (low vs. high)	1.235	1.187-1.244	$6.63x10^{-5}$[b]	1.216	1.041-1.273	$2.46x10^{-5}$[b]

[a]P<0.05; [b]P<0.0001.

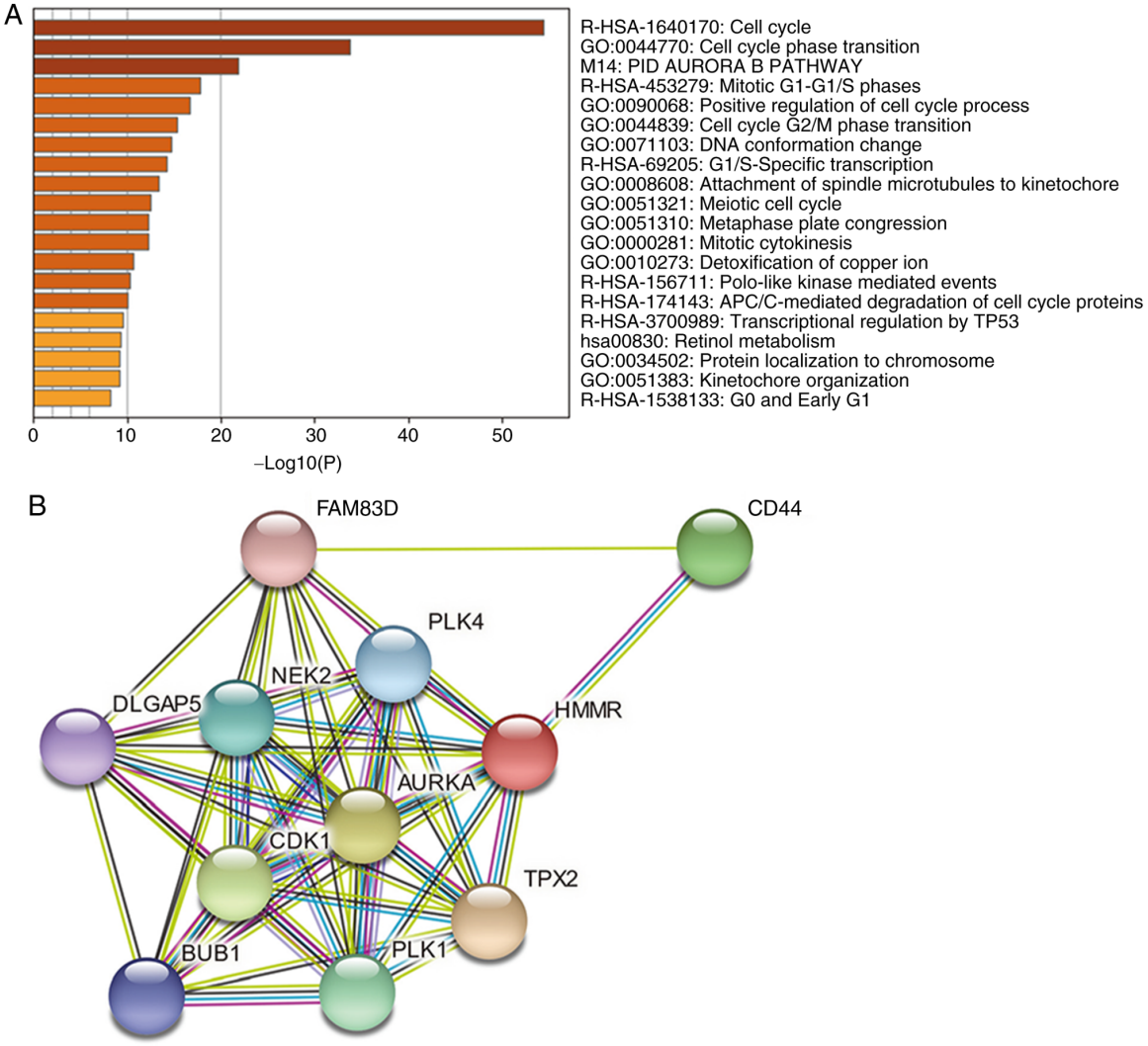

Figure 3. Analysis of HMMR gene enrichment and protein-protein interaction. (A) Heatmap showing the top 20 functional pathways enriched in patients with high expression of HMMR. (B) Proteins that interact with HMMR. HMMR, hyaluronan-mediated mobility receptor.

data of patients with RCC included in the study (Table I). To examine the expression of HMMR in tissue and cells, RT-qPCR was performed. The data showed that HMMR mRNA level in RCC tissue was significantly higher than that in adjacent tissue (Fig. 1A). Moreover, HMMR mRNA levels in renal cancer ACHN, 786-O and Caki-1 cells were

Table III. Top 20 function pathways enriched in patients with high expression of HMMR.

GO	Category	Description	Count	%	Log10(P)	Log10(q)
R-HSA-1640170	Reactome Gene Sets	Cell cycle	87	22.54	-54.46	-50.14
GO:0044770	GO Biological Processes	Cell cycle phase transition	66	17.10	-33.82	-30.58
M14	Canonical Pathways	PID AURORA B PATHWAY	18	4.66	-21.85	-18.98
R-HSA-453279	Reactome Gene Sets	Mitotic G_1-G_1/S phases	25	6.48	-17.82	-15.07
GO:0090068	GO Biological Processes	Positive regulation of cell cycle process	32	8.29	-16.71	-14.02
GO:0044839	GO Biological Processes	Cell cycle G_2/M phase transition	29	7.51	-15.36	-12.78
GO:0071103	GO Biological Processes	DNA conformation change	31	8.03	-14.67	-12.13
R-HSA-69205	Reactome Gene Sets	G_1/S-specific transcription	12	3.11	-14.25	-11.75
GO:0008608	GO Biological Processes	Attachment of spindle microtubules to kinetochore	12	3.11	-13.4	-10.96
GO:0051321	GO Biological Processes	Meiotic cell cycle	25	6.48	-12.49	-10.1
GO:0051310	GO Biological Processes	Metaphase plate congression	14	3.63	-12.3	-9.93
GO:0000281	GO Biological Processes	Mitotic cytokinesis	15	3.89	-12.27	-9.9
GO:0010273	GO Biological Processes	Detoxification of copper ion	8	2.07	-10.64	-8.36
R-HSA-156711	Reactome Gene Sets	Polo-like kinase mediated events	8	2.07	-10.34	-8.07
R-HSA-174143	Reactome Gene Sets	APC/C-mediated degradation of cell cycle proteins	14	3.63	-10.05	-7.81
R-HSA-3700989	Reactome Gene Sets	Transcriptional regulation by TP53	26	6.74	-9.59	-7.38
hsa00830	KEGG Pathway	Retinol metabolism	12	3.11	-9.36	-7.15
GO:0034502	GO Biological Processes	Protein localization to chromosome	13	3.37	-9.22	-7.03
GO:0051383	GO Biological Processes	Kinetochore organization	8	2.07	-9.17	-6.98
R-HSA-1538133	Reactome Gene Sets	G_0 and Early G_1	8	2.07	-8.17	-6.05

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

significantly higher than in HK-2 cells (Fig. 1B). A total of 530 patients with RCC were screened through TCGA database and the expression of HMMR was detected in RCC tissue. The data showed that the expression of HMMR mRNA in RCC was significantly increased compared with normal tissue (Fig. 1C). In addition, the expression of HMMR mRNA in RCC was significantly higher than that in corresponding adjacent tissue (Fig. 1D). The Human Protein Atlas (proteinatlas.org/) showed that HMMR protein expression levels in patients with RCC were higher than in normal renal tissue (Fig. 1E). These results suggested that the expression of HMMR is increased in RCC tissue and renal cancer cell lines.

Expression of HMMR is an independent prognostic factor for 5-year and disease-free survival in patients with RCC. To investigate the association between HMMR and the prognosis of patients with RCC, survival analysis of 530 patients with RCC was performed using the expression of HMMR and 5-year overall and disease-free survival rate. The data showed that the 5-year overall and disease-free survival rate of patients with RCC with high expression of HMMR were both decreased (Fig. 2A and B). To analyze whether HMMR was an independent factor affecting the prognosis of patients with RCC, all samples were divided into low- and high-expression groups according to the mean HMMR expression in RCC tissue. All the samples were divided into two age groups (using 60 years as the cut-off point) and χ^2 test was performed. Univariate analysis showed that there was no significant difference in 5-year survival rate between different genders and ages, and the prognosis of patients with RCC with high expression of HMMR was worse than that of patients with low expression of HMMR. Multivariate regression analysis showed that high expression of HMMR was an independent prognostic factor for RCC (Table II). These results suggested that expression of HMMR is an independent prognostic factor for 5-year overall and disease-free survival in patients with RCC.

Analysis of HMMR gene enrichment and protein interaction. GO enrichment analysis was performed on the differentially genes between the high- and low-HMMR expression groups. The data showed that differentially expressed genes were primarily enriched in biological behaviors such as 'cell cycle', 'cell cycle G_2/M phase transition' and 'DNA conformation change' (Fig. 3A; Table III). Kyoto Encyclopedia of Genes and Genomes pathway analysis of HMMR-associated differential genes showed that the high HMMR expression group involved 'PID AURORA B PATHWAY', 'mitotic G_1-G_1/S phases', 'transcriptional regulation by TP53' and 'retinol metabolism' ($P<0.05$ vs. low HMMR expression group; Fig. 3A; Table III). Prediction of HMMR-interacting proteins using STRING database showed that 10 proteins, including NIMA-related kinase 2, DLG-associated protein 5, CDK1, BUB1 mitotic checkpoint serine/threonine kinase, polo-like kinase (PLK)1, TPX2 microtubule nucleation factor, aurora kinase A, PLK4,

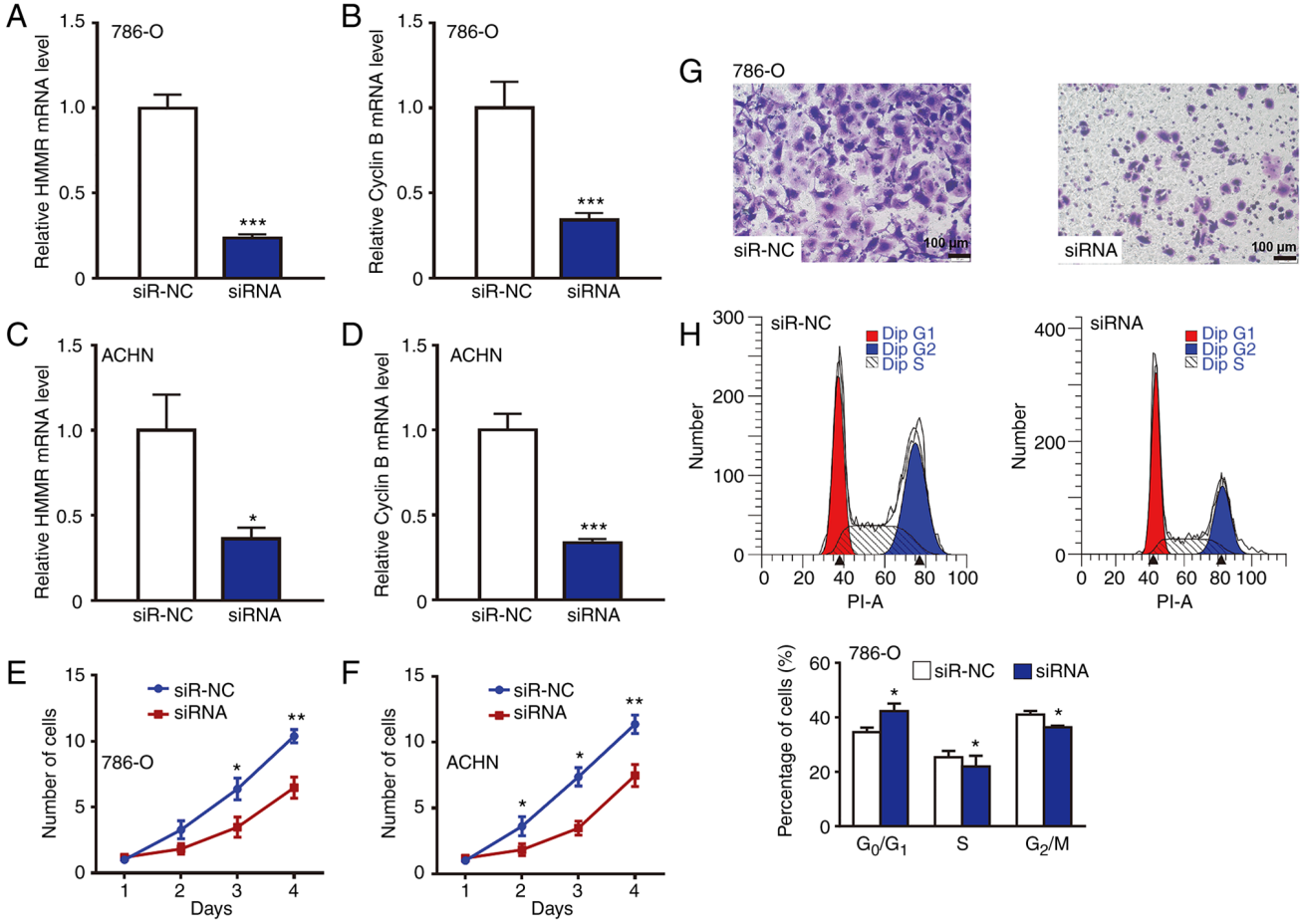

Figure 4. Proliferation of renal cell carcinoma cells with inhibited expression of HMMR. Relative expression of (A) HMMR and (B) Cyclin B mRNA in 786-O cells with siRNA knockdown. Relative expression of (C) HMMR and (D) Cyclin B mRNA in ACHN cells with siRNA knockdown. Proliferation of (E) 786-O and (F) ACHN cells with HMMR knockdown. Statistical significance was analyzed using a paired two-tailed Student's t-test. *P<0.05, **P<0.01, ***P<0.001 vs. siR-NC. (G) Migration ability of 786-O cells with HMMR knockdown, assessed by Transwell assay. (H) Flow cytometric plots and cell cycle distribution of 786-O cells with HMMR knockdown. *P<0.05, **P<0.01, ***P<0.001 vs. siR-NC. HMMR, hyaluronan-mediated mobility receptor; si, small interfering; NC, negative control.

family with sequence similarity 83 member D and CD44, interact with HMMR (Fig. 3B). These genes were shown to be upregulated in RCC.

HMMR promotes proliferation of RCC cells. To examine the effect of HMMR on RCC cell proliferation, 786-O and ACHN cells were first transfected by siRNA of HMMR and Cyclin B1 to inhibit their expression. RT-qPCR showed that HMMR and Cyclin B1 mRNA levels in transfected 786-O or ACHN cells were significantly lower than those in the siR-NC group (Fig. 4A-D). CCK-8 assay showed that the proliferation of 786-O and ACHN cells with HMMR knockdown was decreased compared with that in siR-NC group (Fig. 4E and F). Transwell assay showed that 786-O cells with HMMR knockdown exhibited decreased migration and invasion ability (Fig. 4G). Flow cytometry showed that 786-O cells with HMMR knockdown exhibited an increased percentage of cells in G_0/G_1 phase (Fig. 4H). Moreover, HMMR was overexpressed in 769-P and Caki-1 cells via lentiviral infection. RT-qPCR showed that expression of HMMR and Cyclin B1 mRNA in the overexpression group was significantly higher than that in control group (Fig. 5A-D). CCK-8 assay showed that proliferation of 769-P and Caki-1 cells with overexpression of HMMR was enhanced compared with that in control group (Fig. 5E and F). These results indicated that HMMR promoted the proliferation of RCC cells.

Discussion

To the best of our knowledge, the present study was the first to observe high expression of HMMR in RCC tissue and then verify this result in RCC cell lines. Analysis of data from TCGA database showed that HMMR expression was associated with sex, tumor grade and prognosis of patients with RCC. HMMR is highly expressed in mouse embryonic cardiomyocytes, and high expression of HMMR is associated with proliferation of hepatocytes (21,22). However, when cardiomyocytes are exposed to oxygen, expression of HMMR is decreased significantly (21,22). HMMR is overexpressed in numerous types of tumor, including breast, bladder and endometrial cancer (14-17), and knockdown of HMMR inhibits the growth of tumor cells (23,24). However, the specific molecular regulatory mechanism is still unclear.

As a protooncogene, HMMR promotes cell cycle and proliferation through G_2/M phase (25). HMMR may be a novel marker for cell proliferation. To confirm the association

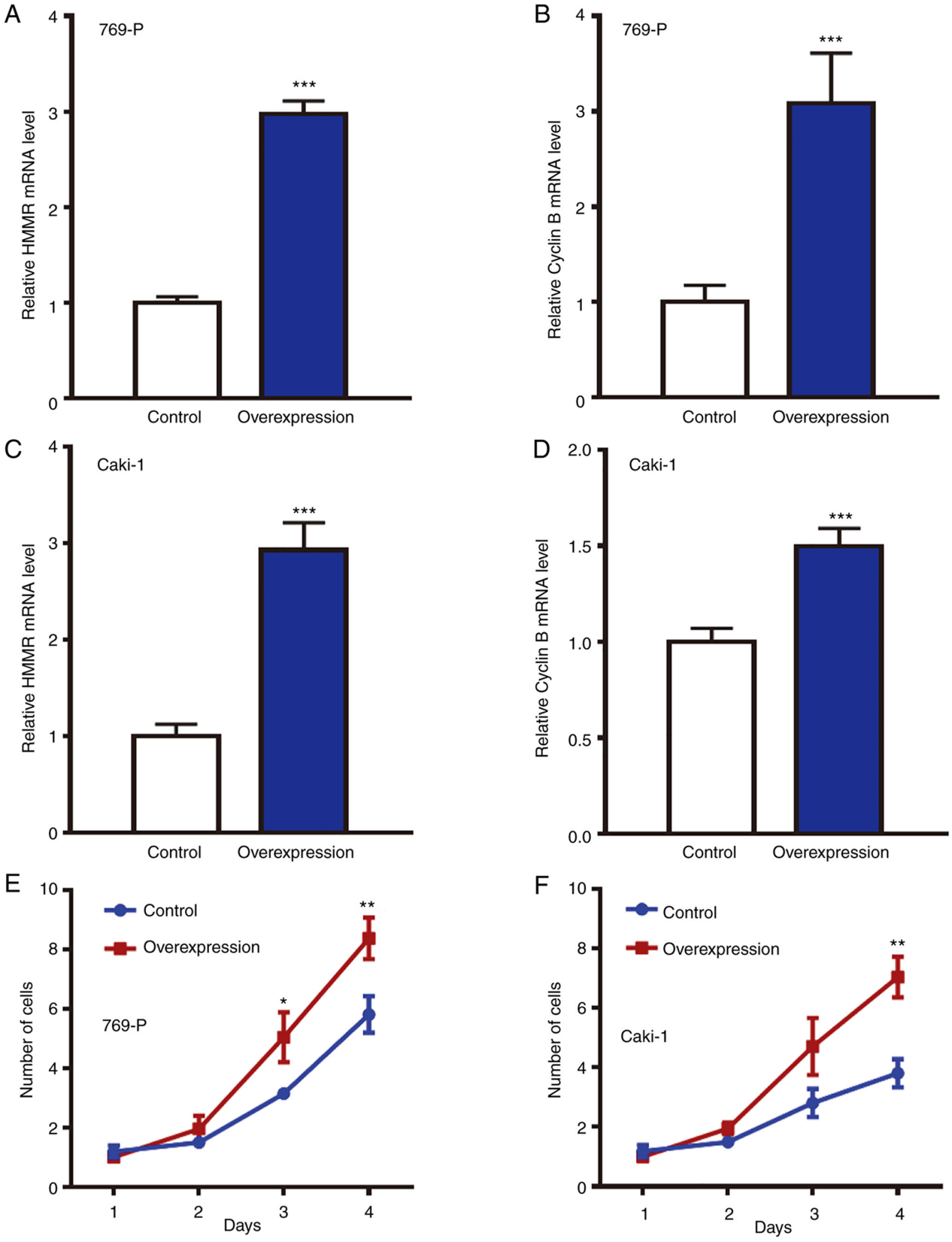

Figure 5. Proliferation of renal cell carcinoma cells overexpressing HMMR. Relative expression of (A) HMMR and (B) Cyclin B mRNA in overexpressing 769-P cells. Relative expression of (C) HMMR and (D) Cyclin B mRNA in overexpressing Caki-1 cells. Proliferation of (E) 769-P and (F) Caki-1 cells with overexpression of HMMR. Statistical significance was analyzed using a paired two-tailed Student's t-test. *P<0.05, **P<0.01, ***P<0.001 vs. control. HMMR, hyaluronan-mediated mobility receptor.

between HMMR and RCC cell proliferation, RCC cell lines with high or low HMMR expression were selected as research models. Following HMMR knockdown, the proliferation of 786-O and ACHN cells decreased; following HMMR

overexpression, the proliferation of 769-P and Caki-1 cells was enhanced. Cyclin B1 is a key marker for G_2/M phase, and high expression of Cyclin B1 accelerates cell cycle and promotes cell proliferation. Following overexpression of HMMR in 769-P and Caki-1 cells, Cyclin B1 level was increased. In 786-O and ACHN cell lines, by knocking out HMMR, Cyclin B1 level was lowered. These results suggested that HMMR affected cell cycle and proliferation of RCC by regulating the expression of Cyclin B1. However, the molecular mechanism of action of HMMR is unclear. P53 inhibits HMMR expression via hyaluronic acid-mediated signaling and metabolic pathways (26,27). In prostate cancer, estrogen receptor regulates HMMR expression (28,29). A study of estrogen-dependent tumor cell lines demonstrated that HMMR is a downstream molecule in the signaling pathway by which estrogen promotes tumor formation (28). Therefore, HMMR expression in RCC cells may be associated with hormone regulation (23). HMMR exerts a variety of functions in cells, including the phosphorylation of PTK2/FAK1 (29), but its specific molecular mechanism and associated signaling pathways are unclear. Further work is needed to determine the molecular mechanism of HMMR.

The present study had several limitations. First, only Caki-1 cell line was available in the laboratory. Future experiments should investigate the role of HMMR in Caki-2 cells. The lack of rescue experiments is another limitation of the present study and will be performed in future. Third, human liver samples were obtained primarily from the hospital biobank. Therefore, only data for sex, age and classification was available.

In conclusion, the present study demonstrated that HMMR was significantly upregulated in RCC tissue and an independent prognostic factor for RCC. HMMR may be involved in the occurrence of RCC by regulating Cyclin B1.

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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

LL, DW and HZ contributed to the design of the study. LL performed the experiments. LL and DW analyzed the data. LL and HZ interpreted results and prepared the manuscript. All authors have read and approved the final version of the manuscript. LL, DW and HZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All procedures were approved by the Ethics Committee of Jinan Third People's Hospital (approval no. 20200201081). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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