International Urology and Nephrology (2019) 51:1517–1526
https://doi.org/10.1007/s11255-019-02169-3

UROLOGY - ORIGINAL PAPER

MCM5 promotes tumour proliferation and correlates with the progression and prognosis of renal cell carcinoma

Binbin Gong[1] · Ming Ma[1] · Xiaorong Yang[1] · Wenjie Xie[1] · Yanping Luo[1] · Ting Sun[1]

Received: 4 March 2019 / Accepted: 7 May 2019 / Published online: 12 June 2019

Abstract

Background To investigate the role of Minichromosome maintenance protein 5 (MCM5) in the clinical prognosis and biological function of renal cell carcinoma (RCC).

Methods The Oncomine database was analysed to determine the differential expression of MCMs in RCC. A total of 50 RCC tissues were evaluated by immunohistochemistry (IHC), and the association between MCM5 and clinicopathologic features was determined. Kaplan–Meier curves and the log-rank test were applied for survival analysis. MCM5 expression in RCC tissues and cell lines was examined further by Western blotting. To explore the biological function of MCM5 in RCC, RCC cell lines (786-0, 769p) were transfected with shRNA-MCM5 or MCM5. Cell proliferation was assessed using MTT and colony-formation assays. Tumour xenografts were generated in nude mice to confirm the effects of MCM5 on tumour growth.

Results MCM5 was significantly overexpressed in RCC tissues; this outcome was confirmed by the Oncomine database, IHC and Western blotting. IHC and LinkedOmics analysis demonstrated that the MCM5 expression was significantly associated with pathological stage, lymph node status, distant metastasis, and TNM stage ($p < 0.05$) but not with sex, age, position, or tumour size ($p > 0.05$). Furthermore, high MCM5 levels correlated with unfavourable clinical outcomes in RCC ($p < 0.05$). Additionally, MCM5 silencing inhibited RCC cell line proliferation and reduced 786-0 xenograft tumour growth; in contrast, MCM5 upregulation promoted cell proliferation.

Conclusion MCM5 overexpression is associated with malignant status and poor prognosis in RCC. Additionally, MCM5 plays an important role in proliferation and may be a potential prognostic marker and novel therapeutic target for RCC.

Keywords MCM5 · Renal cell carcinoma · Proliferation · Prognosis

Introduction

Renal cell carcinoma (RCC) is one of the ten most common solid tumour cancers, accounting for 2–3% of all malignant diseases in adults globally in 2017, and its incidence and mortality have increased in recent years [1, 2]. It is estimated that 65,340 incident RCC cases and 14,970 RCC deaths occurred in the United States in 2018 [3]. Due to a lack of typical symptoms and early prognostic markers to screen for early RCC, 20–30% of RCC patients present with locally advanced or metastatic disease at the time of diagnosis, and the prognosis of RCC is extremely poor [1, 4, 5]. Advanced RCC has a poor survival rate and may result in metastasis or recurrence, which is predominantly attributed to resistance to both chemotherapy and radiation after the initial radical surgery [6]. Hence, it is urgent to elucidate the molecular mechanisms of RCC to identify novel biomarkers for an early diagnosis and molecular targets for establishing novel therapeutic strategies for RCC.

Minichromosome maintenance protein 5 (MCM5) is located on chromosome 22q13.1, it is a member of the minichromosome maintenance (MCM) protein family. MCM proteins were first recognized in the yeast *Saccharomyces cerevisiae* as mutants defective in the maintenance of minichromosomes, suggesting a role for MCM proteins in plasmid replication and cell cycle progression [7]. MCM proteins are thought to function as regulatory components in the S-phase of the cell cycle, presenting weak helicase activity by binding to chromatin long before the initiation

✉ Yanping Luo
lypyfy@126.com

✉ Ting Sun
sunting6600@163.com

[1] Department of Urology, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi, China

of DNA synthesis [8]. Aside from their utility as cell proliferation markers, MCM proteins have also been considered useful diagnostic and prognostic markers in several human malignancies [9]. Recently, Kaihua Gou et al. performed a meta-analysis to investigate the prognostic value of MCMs in cancers and found that high expression of MCM2, MCM5 and MCM7 might predict a poor prognosis in cancers [10]. Overexpression of MCM5 has been found in several types of cancers, including gastric adenocarcinoma, lung cancer, oral squamous cell carcinoma, malignant skin diseases, cervical cancer and bladder cancer. Moreover, higher MCM5 expression is significantly associated with tumour size, lymph node metastasis, histopathological stage and prognosis of cancer [11-15]. In RCC, there is a significant association between MCM2 and tumour grade, angiogenic phenotype, and overall survival, but not with patient age, sex, tumour size or stage [16]. However, the influence of MCM5 protein expression on RCC progression and prognosis has not been investigated.

In this study, to explore the role of MCM5 in RCC, we performed a series of tests and found that MCM5 is overexpressed in RCC. Furthermore, higher expression of MCM5 was significantly associated with the clinicopathological features of RCC and patient survival. In addition, we further explored the function of MCM5 in RCC in vivo and in vitro.

Materials and methods

Database analysis

The Oncomine microarray database (https://www.oncomine.org) was searched to explore the differential expression of MCM2-7 in paired RCC samples. Several datasets, including GSE6344, GSE14994, and GSE15641, were used to evaluate the mRNA expression of MCM2-7 in RCC. In addition, LinkedOmics (https://www.linked -omics.org/admin.php) was used to explore the relationship between MCM5 expression and patient survival and clinicopathological features of RCC. OncoLnc (https://www.oncolnc.org/) was also used to analyse the association between overall survival (OS) of RCC patients and MCM5.

Clinical specimens

A total of 50 matched pairs of primary RCC tissues (*T*) and adjacent normal tissues (*N*) were collected from patients who underwent radical or partial nephrectomy without any neoadjuvant treatment before surgery between January 2017 and July 2018 at the First Affiliated Hospital of Nanchang University. All patients were diagnosed as having clear cell RCC by pathologists. All tumours were staged according to the 2010 American Joint Committee on Cancer TNM classification and graded according to the Fuhrman grading system by two senior pathologists. Detailed information, including age, sex, tumour size, TNM stage, and Fuhrman grade, were recorded. For Western blot assays, we collected 16 pairs of snap-frozen normal renal tissues and tumour RCC tissues. For immunohistochemistry (IHC) analysis, we evaluated 50 paraffin-embedded RCC tissues and their corresponding adjacent normal renal tissues. All participating patients signed informed consent forms. The study was approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University.

Cell line culture and lentiviral transfection

The human RCC cell lines 786-0 and 769p were purchased from the Cell Bank, Chinese Academy of Science (Shanghai, China). According to the manufacturer's protocol, all cell lines were cultured in RPMI-1640 with 10% foetal bovine serum (FBS), 100 mg/ml streptomycin and 100 U/ml penicillin and maintained in a humidified incubator containing 5% CO_2 at 37 °C. The MCM5 ectopic expression and knockdown lentiviruses and their negative control (NC) were purchased from Cyagen (Guangzhou, China). Transfection was conducted according to the manufacturer's instructions, and stable cell lines (786-0, 769p) with overexpressed or silenced MCM5 were selected for 1 week with 1.5 μg/ml puromycin. After selection, MCM5 expression was examined by real-time polymerase chain reaction (RT-PCR) and Western blot to confirm effective overexpressing and silencing.

Immunohistochemistry

MCM5 expression was assessed by IHC. First, 5-μm paraffin-embedded tissue sections were dewaxed in xylene and dehydrated in a series of descending ethanol concentrations. Then, the sections were treated with 3% hydrogen peroxide for 15 min at room temperature, placed in a high-pressure cooker for antigen retrieval and incubated with 10% normal goat serum for 15 min at room temperature to block nonspecific binding. Sections were incubated with MCM5 antibody (1:250 dilution, ab75975, Abcam, UK) overnight at 4 °C and then incubated with an anti-rabbit secondary antibody at 37 °C for 30 min, followed by treatment with diaminobenzidine tetrahydrochloride (DAB) and counterstaining with haematoxylin. All slides were evaluated independently by two pathologists. The staining intensity was defined as follows: 0 (negative); 1 (weak); 2 (moderate); and 3 (strong). The staining fraction was scored as follows: 0 (0% stained); 1 (1–25% stained); 2 (26–50% stained); 3 (51–75% stained); and 4 (>75% stained). The immunoreactive score (IRS) was calculated by multiplying the intensity scores and staining fraction score.

RT-PCR

Following lentiviral transfection and establishment of stable cell lines, total RNA was extracted from cells by 1.0 ml TRIzol. The total RNA was reverse transcribed to cDNA by PrimeScript™ RT Master Mix (Takara, Japan). RT-PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System with TB Green™ Premix Ex Taq™ II (Takara, Japan) to detect MCM5 mRNA. GAPDH was amplified in parallel as an internal control. MCM5 expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The following primers were used: MCM5 forward, 5′-AGCATTCGTAGC CTGAAGTCG-3′ and reverse, 5′-CGGCACT- GGATAG AGATGCG-3′; and GAPDH forward, 5′-TCACTCACATTT GCC-TCCCTC-3′ and reverse, 5′-CTGAAGGGCAAGGCC ATGTA-3′.

Western blot analysis

Total protein was extracted from RCC tissue samples and cells using radioimmunoprecipitation assay (RIPA) buffer, and supernatants were collected for Western blot assays. Protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (CW0014S, CWBIO, China). Total protein samples were subjected to 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% bovine serum albumin (BSA), the membranes were incubated with primary antibodies (MCM5: 1:2500 dilution, ab75975, Abcam, UK; GAPDH: 1:3000 dilution, HC301-01, TransGen Biotech, China) at 4 °C overnight. After extensive washing, the membrane was incubated with the secondary antibody at room temperature for 1 h and was then exposed to an enhanced chemiluminescence reagent (Millipore, USA).

MTT proliferation assay

Cell proliferation was evaluated using MTT assays. Cells were seeded in 96-well plates at 5×10^3 cells per well with four time points, days 1, 2, 3 and 4 after transfection. Then, 50 µl of 1 × MTT was added to each well and incubated for 4 h at 37 °C, followed by the addition of 150 µl DMSO to dissolve the formazan crystals. Finally, the absorbance values were measured at a 550 nm wavelength with a spectrophotometric plate reader.

Colony-formation assay

The transfected cells were seeded on a 6-well plate at a density of 1×10^3 cells/well. After 2 weeks, cell colonies that formed on the plates were fixed with 4% paraformaldehyde for 15 min at room temperature and visualized by staining with Giemsa stain. The colonies (> 50 cells) were counted under a light microscope at 200 × magnification.

Node mouse xenograft assays

Four- to six-week-old male nude mice were purchased from Hunan Slake Jingda Laboratory Animal Company (Hunan, China). A total of 5×10^6 786-0 cells stably transfected with shRNA-MCM5 and shRNA-NC were injected subcutaneously into the right flanks of the mice. Tumour growth was examined every 7 days, and tumour volume was calculated using the following equation: Volume = (length × width^2)/2 (mm^3). Studies on animals were conducted with approval from the ethics committee of the First Affiliated Hospital of Nanchang University.

Statistical analysis

All experiments were performed in triplicate. SPSS 19.0 software was used to perform the statistical analysis. Numerical data are expressed as the mean ± standard deviations, and Student's *t* test was used to assess differences in MCM5 expression between the two groups. In addition, the correlation between MCM5 expression and clinicopathological parameters of patients with RCC was evaluated using the Chi square test. Survival curves for patients were plotted using the Kaplan–Meier method, with log-rank tests for statistical significance. All p values < 0.05 were considered statistically significant.

Results

Expression of MCM genes in RCC tissues and cell lines.

The mRNA expression levels of MCMs in cancer and normal tissues were investigated using the Oncomine database. With the following thresholds, p value < 0.05 and fold change > 2, MCMs were determined to be overexpressed in RCC tissues. The comparisons, including datasets GSE6344, GSE14994 and GSE15641, revealed that there were no significant changes in MCM3 or MCM4 mRNA expression between cancerous and normal tissues (Fig. 1). In the GSE14994 dataset, MCM2 was significantly higher in the RCC specimens than in the normal specimens (Fig. 1). In addition, the expression levels of MCM5, MCM6 and MCM7 were higher in RCC tissues according to the analysis of GSE6344, GSE14994 and GSE15641 (Fig. 1). Furthermore, MCM5 had the highest mRNA expression in RCC tissues (GSE6344: fold change, 9.142, $p = 3.57E{-}12$; GSE14994: fold change, 3.838, $p = 1.18E{-}13$, GSE1564: fold change, 2.224,

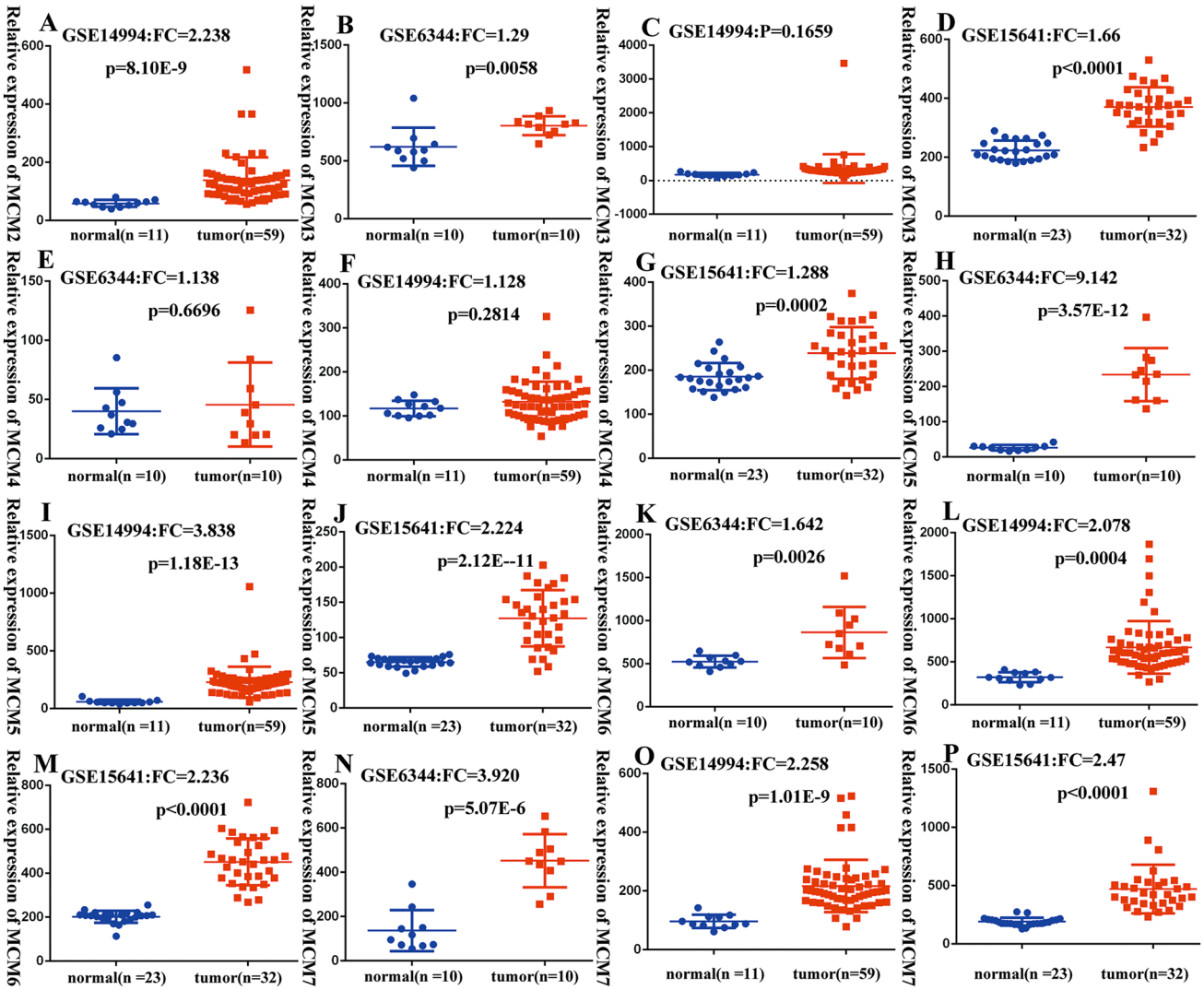

Fig. 1 Differential expression of MCMs (MCM2-7) in normal and RCC specimens from the Oncomine database: **a** MCM2; **b**–**d** MCM3; **e**–**g** MCM4; **h**–**j** MCM5; **k**–**m** MCM6; and **n**–**p** MCM7

$p = 2.12E{-}11$) compared with the expression of MCM2, MCM6 and MCM7 (Fig. 1). Then, we explored MCM5 expression in RCC cell lines using the Cancer Cell Line Encyclopedia (CCLE) database and found that MCM5 was expressed at higher levels in 786-0, 769P, Caki-2 and A498 cells than in HK2 cells (Fig. 2c).

To verify MCM5 expression in RCC tissues, IHC was performed to detect MCM5 protein levels in 50 samples of RCC tissues and normal tissues, and as expected, MCM5 expression was significantly increased in tumour tissues compared with that in paired normal tissues (Fig. 3a, b, $p = 0.0001$). Representative immunostaining images of negative, weak, moderate, and strong MCM5 expression are shown in Fig. 3c. Furthermore, proteins were extracted from 16 RCC specimens or RCC cell lines and examined by Western blot, which showed that MCM5 expression was significantly upregulated in tumour tissues (Fig. 2a, b) and cell lines (Fig. 2d, e).

Association between MCM5 expression and clinicopathological features of RCC patients

Due to the high expression of MCM5 in RCC, we supposed that MCM5 may play an important role in the pathogenesis of RCC. To explore the correlation between MCM5 and the clinicopathological parameters of RCC patients, data from LinkedOmics were downloaded, and the correlations were analysed by Chi square test. The results showed that MCM5 expression significantly correlated with multiple variables, including N classification ($p = 0.0065$), M classification ($p = 0.002$), T classification ($p \leq 0.05$) and pathologic stage ($p \leq 0.05$) (Fig. 3d). Moreover, to determine

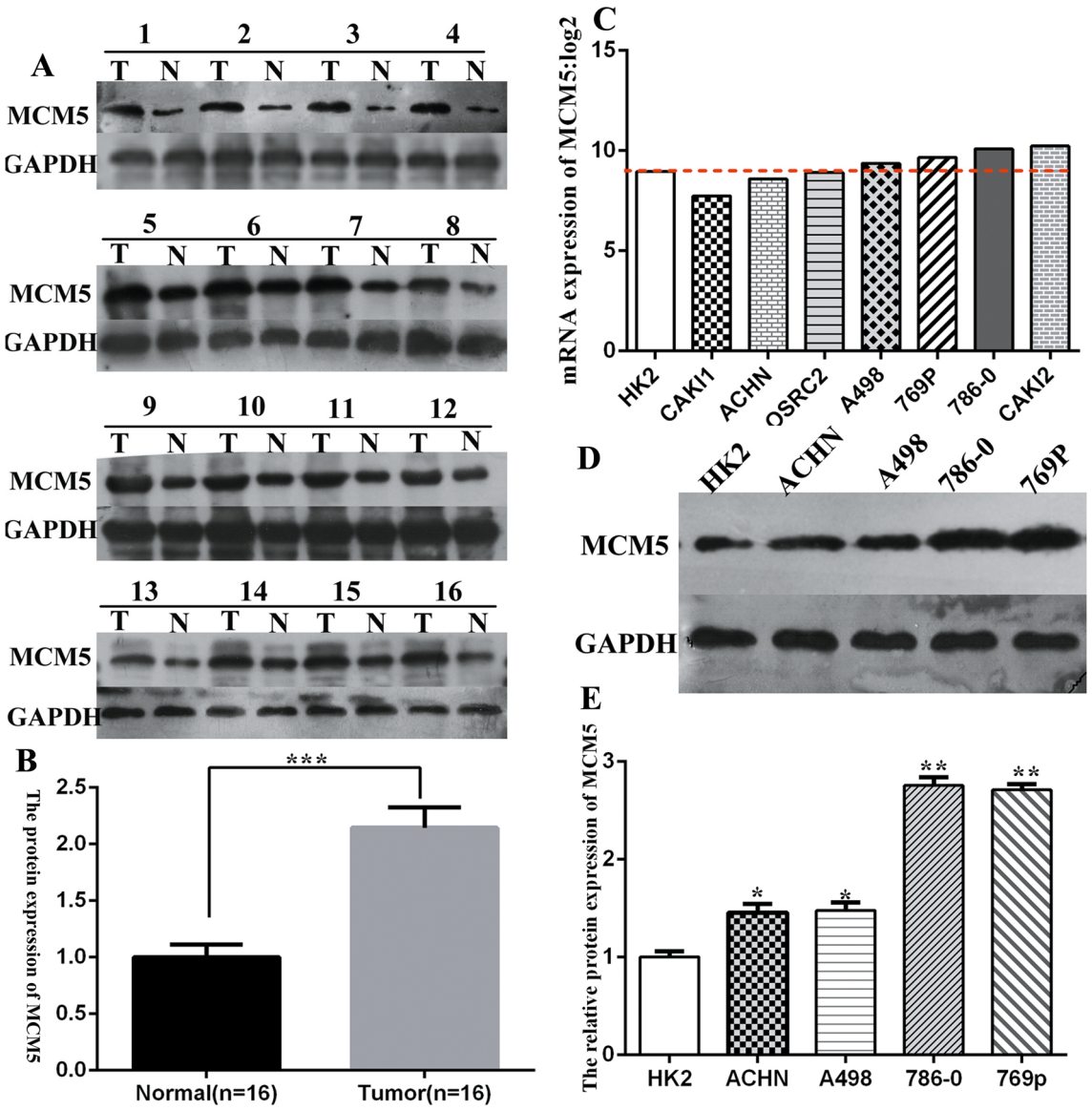

Fig. 2 MCM5 expression in RCC tissues and RCC cell lines: **a**, **b** MCM5 protein expression in RCC tissues. **c** MCM5 mRNA expression in RCC cell lines from CCLE (https://portals.broadinstitute.org/ccle). **d**, **e** MCM5 protein expression in RCC cell lines

the association of clinicopathological characteristics with MCM5 expression, IHC analysis was performed in 50 primary RCC samples and paired adjacent normal tissues; we found that MCM5 expression significantly correlated with tumour stage ($p = 0.01$) and T stage ($p = 0.022$). However, no association was found between MCM5 gene expression level and sex, age, position, tumour size or Fuhrman grade ($p > 0.05$) (Table 1). The above results indicated that high MCM5 expression positively correlates with adverse clinicopathological features, especially related to progression and metastasis, which further suggests that MCM5 may be a promising potential prognostic factor of RCC.

Prognostic value of MCM5 expression in patients with RCC

We evaluated the relationship between MCM5 mRNA expression and OS in RCC patients through using Kaplan–Meier analysis with the log-rank test based on LinkedOmics database. We found that MCM5 expression was markedly associated with OS: patients with high MCM5 expression had a shorter OS than those with low MCM5 expression [Fig. 3e, hazard ratio (HR) 1.46, 95% confidence interval (CI) 1.08–1.96, $p = 0.0128$]. Moreover, an analysis using OncoLnc resulted in similar findings,

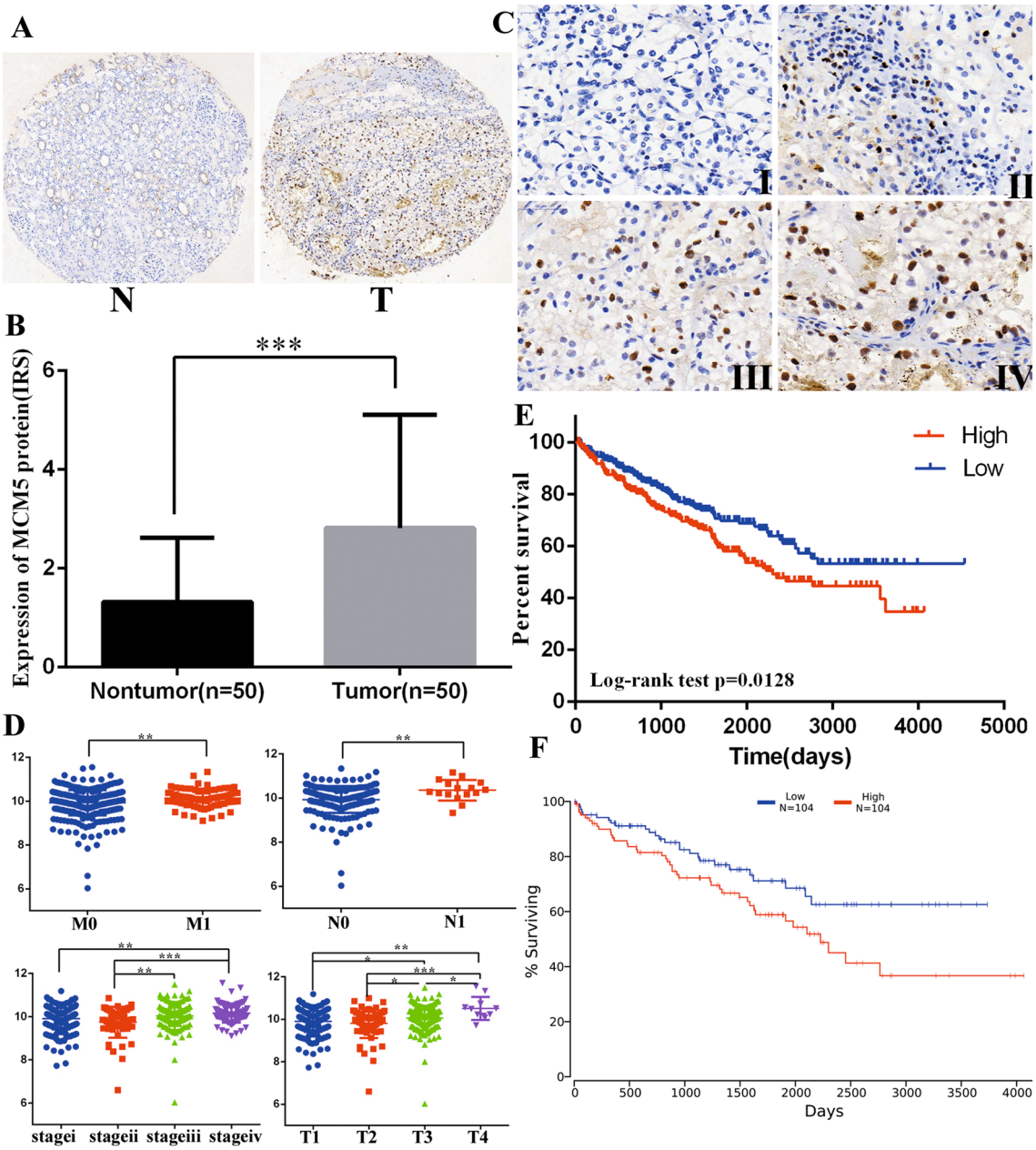

Fig. 3 Association between MCM5 expression and clinicopathological features of RCC patients: **a** Representative IHC staining images of MCM5 expression in non-tumour tissues and tumour tissues. **b** The results of MCM5 staining were evaluated by staining scores. **c** The representative immunostaining of MCM5 in RCC specimens as follows: I, negative; II, weak; III, moderate; and IV, strong. **d** MCM5 overexpression was significantly associated with metastatic status, lymph node status, pathological stage and TNM stage. **e**, **f** RCC patients with a high level of MCM5 showed worse OS than those with a low level of MCM5 (E: LinkedOmics, F: OncoLnc)

which demonstrated that high MCM5 expression may be an adverse prognostic marker in RCC (Fig. 3f; $p = 0.0368$).

The biological function of MCM5 in vivo and in vitro

To study the biological function of MCM5 in RCC, we downregulated or upregulated MCM5 expression by lentiviral transfection, and the different expression levels were confirmed by RT-PCR and Western blot assays (Fig. 4a, b). An MTT assay was performed to evaluate the impact of MCM5 on the proliferation ability of RCC cells. In the 786-0 and 769p cell lines, the growth curves determined by MTT assays showed that MCM5 downregulation inhibited cell proliferation (Fig. 4c). Similarly, MCM5 overexpression markedly enhanced the proliferation of 786-0 and 769p cells (Fig. 4d). The plate colony-formation assay was used

Table 1 Association between MCM5 expression level and clinical characteristics of renal cancer patients

Variable	Total	No. of patients (%)		*p* value[b]
		High[a]	Low[a]	
Gender				
Male	37	19	18	0.747
Female	13	6	7	
Age (years)				
≤55	25	10	16	0.089
>55	25	15	9	
Position				
Left	23	11	12	0.777
Right	27	14	13	
Tumour size (cm)				
≤4.0	30	13	17	0.248
>4.0	20	12	8	
Tumour stage				
I+II	43	18	25	0.01
III+IV	7	7	0	
Fuhrman grade				
I+II	31	15	16	0.771
III+IV	19	10	9	
T stage				
T1+T2	44	19	25	0.022
T3+T4	6	6	0	

[a]Cutoff point: mean

[b]Calculated using Chi square test

to examine the colony-formation ability of the cells. The size and quantity of the formed colonies were reduced when MCM5 expression was downregulated (Fig. 4e); in contrast, the number of colonies increased when MCM5 was upregulated (Fig. 4f). Furthermore, 786-0 cells transfected with shRNA-NC or shRNA-MCM5 were inoculated into nude mice, and the volumes of the 786-0 tumours were measured every 7 days. The tumour volumes in the shRNA-MCM5 group were significantly lower than those in the shRNA-NC group; therefore, the downregulation of MCM5 inhibited the tumour growth in vivo (Fig. 4g, h).

Discussion

DNA replication is one of the most important steps in cell division, and replication origins are assembled and activated once per cell cycle to initiate DNA replication [17]. MCM proteins play a critical role in DNA replication and cell cycle progression, and MCM2-7 proteins interact with one another, forming a heterohexamer complex that is markedly associated with the origins of DNA replication [10, 18]. In addition, the meta-analysis performed by Kaihua Gou suggested that the elevated expression levels of MCM2, MCM5 and MCM7 might serve as predictive biomarkers for poor prognosis in cancers [10]. In this study, we found that the expression levels of MCM2, MCM5, MCM6, MCM7, but not those of MCM3 and MCM4, were upregulated in RCC. Furthermore, MCM5 had the highest mRNA expression in RCC tissues compared with the expression of MCM2, MCM6 and MCM7. Zhong et al. noted that MCM2, MCM5, MCM6 and MCM7 were overexpressed and may be important prognostic markers for patients with RCC via bioinformatics analysis [19]. In RCC, both Ki-67 and MCM-2 are markers of proliferation and are closely linked to grade and can thus act as surrogate markers for grade in an objective and reproducible manner [20]. In addition, SETD2 loss of function leads to aberrant MCM7 and promotes renal cancer branched evolution [21]. RCC is a public health challenge as it is difficult to detect in early stages, and it has a high mortality rate owing to its high metastatic potential. Hence, there is an urgent need to investigate the underlying molecular mechanisms of RCC pathogenesis and identify a novel diagnostic and treatment targets to improve the prognosis of RCC. Although MCM5 has been reported to participate in the genesis of many cancers, the role of MCM5 in RCC remains unclear. In the present study, we first demonstrated that MCM5 was overexpressed in RCC tissues. Furthermore, MCM5 overexpression was positively associated with clinicopathological parameters. These findings suggested that MCM5, which deserves further intensive study, may play an important role in RCC progression.

Recently, MCM5 was found to be overexpressed in thyroid cancer, ovarian cancer, colorectal cancer, breast cancer, oesophageal cancer, gastric cancer, pancreaticobiliary cancer, cervical cancer, bladder cancer and prostate cancer [10, 14]. Similarly, MCM5 is significantly overexpressed in RCC. Furthermore, we used IHC and bioinformatics analysis of LinkedOmics and found that MCM5 expression was closely related to lymph node metastasis, distant metastasis and clinical stage (TNM), but not age, sex, position or tumour size in RCC patients. Similarly, MCM5 expression closely correlated with cervical adenocarcinoma patients' clinical stage, lymph node metastasis, distant metastasis and histological grade [14]. In oral squamous cell carcinoma (OSCC), MCM5 levels were significantly higher than in normal oral mucosa; this elevated expression was observed in mild, moderate and severe oral epithelial dysplasia and OSCC ($p<0.001$). In addition, there was a significant correlation between higher MCM5 expression and larger tumour size ($p=0.032$), positive lymph node metastasis ($p=0.003$), more advanced clinical stage ($p=0.002$), higher histological grade ($p=0.002$), deeper invasion depth ($p=0.0001$), and perineural invasion ($p=0.0047$) [11]. Giaginis et al. reported that MCM-5 expression in gastric adenocarcinoma was significantly associated with tumour size ($p=0.0295$),

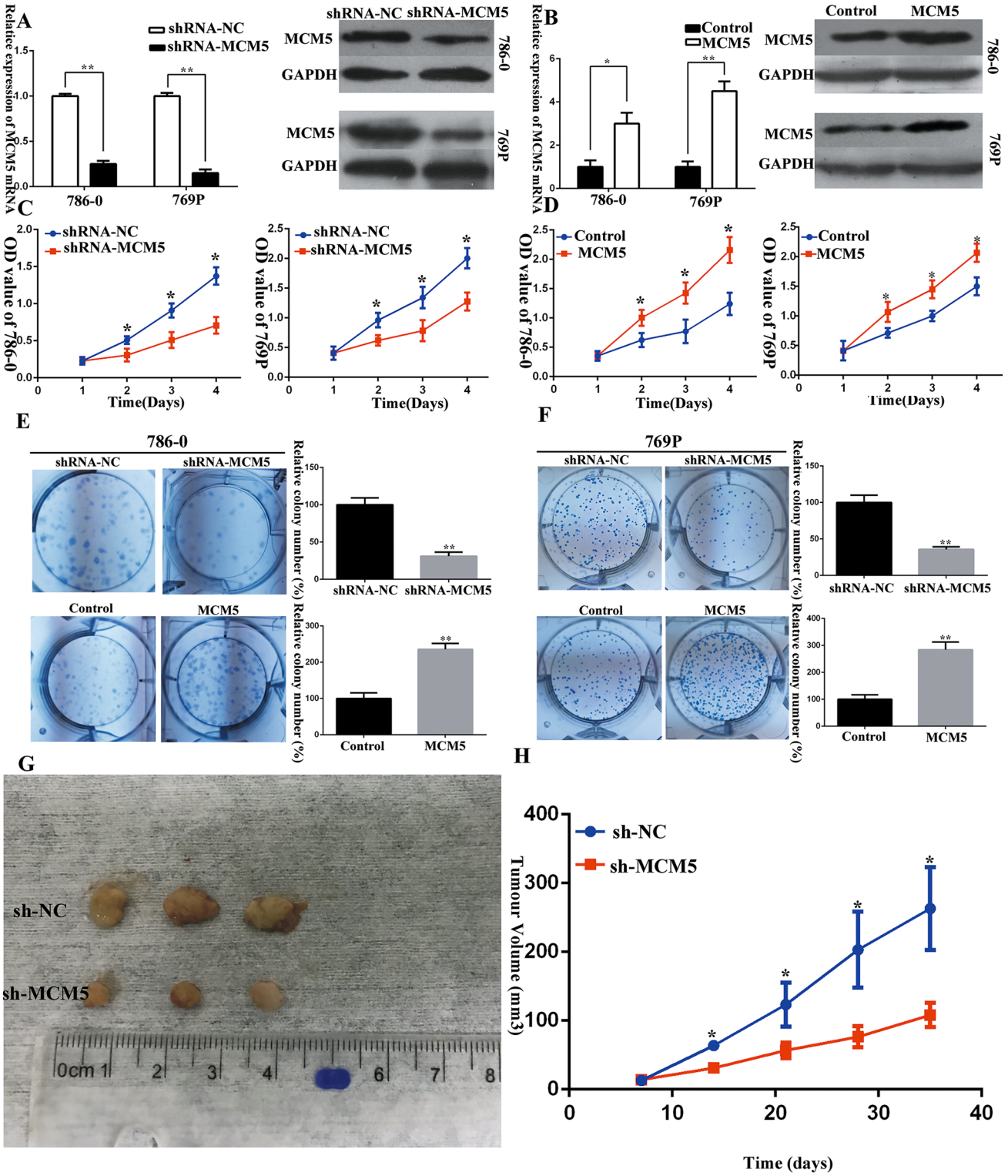

Fig. 4 The biological function of MCM5 in vivo and in vitro: **a** MCM5 was successfully knocked down in 786-0 and 769p cells, which was confirmed by Western blot and RT-PCR. **b** MCM5 overexpression in 786-0 and 769p cells was confirmed by Western blot and RT-PCR. **c** MCM5 knockdown in 786-0 and 769p cells suppressed cell proliferation. **d** MCM5 overexpression promoted the proliferation of 786-0 and 769p cells. **e**, **f** MCM5 overexpression could enhance the colony formation of 786-0 and 769p cells. Downregulation of MCM5 expression inhibited 786-0 and 769p cell colony formation. **g** Representative images of nude mouse xenograft tumours. **h** Statistical analysis of xenograft tumour sizes revealed that tumour growth was markedly inhibited by MCM5 silencing

presence of lymph node metastases ($p = 0.0216$) and tumour histopathological stage ($p = 0.0098$) [13]. Moreover, in lung cancer, patients with distant metastasis expressed higher levels of MCM5 in their tumours ($p = 0.008$) [12], and similar results were found in cervical carcinoma and colon cancer [8, 22]. Furthermore, MCM5 plays a critical prognostic role in several cancers, such as colon cancer [23], breast cancer [24], bladder cancer [25], pancreatic ductal adenocarcinoma [26], oral squamous cell carcinoma [11], lung cancer [12] and cervical cancer [14]. In the present study, we found that RCC patients with high MCM5 expression had a worse prognosis than those with low MCM5 expression, and high MCM5 expression was an independent unfavourable prognostic factor. However, elevated MCM5 expression significantly correlated with favourable outcomes, and MCM5 gene expression negatively correlated with gastric adenocarcinoma pathologic stage progression [27]. Meanwhile, MCM5 serves as a potential diagnostic marker in several types of human cancer. In oesophageal cancer, the MCM5 levels in gastric aspirates had high specificity (85%, CI 66–96%) and sensitivity (85%, CI 62–97%) between patients with and without oesophageal cancer [28]. Brems-Eskildsen et al. demonstrated that MCM5 expression in urine had high sensitivity and specificity in the diagnosis of bladder cancer and was markedly associated with subsequent tumour recurrence [25]. Similarly, MCM5 detection appears to be a simple, accurate and noninvasive method for identifying patients with prostate cancer and pancreaticobiliary malignancy [29, 30]. Therefore, MCM5 may be a potential diagnostic and prognostic biomarker for renal cancer.

MCM5 may play an oncogenic role in several cancers and is closely related to the diagnosis, progression and prognosis of tumours based on the above analysis. MCM5 is a key protein to maintain proper assembly of MCM2-7, which are components of the complex controlling replication origins, and plays an important role in tumour proliferation [31, 32]. Mukesh et al. demonstrated that the expression levels of MCM5 and its transcriptional regulator E2F1 are negatively regulated by p53 and play a role in negating the growth arrest function of p53 [33]. Furthermore, MCM2 and MCM5 expression significantly correlated with proliferative capacity (Ki67) and p53 cell cycle regulator expression in colon cancer and ovarian adenocarcinomas [8, 34]. In our study, we found that MCM5 overexpression promoted the proliferation of RCC cell lines, and MCM5 downregulation inhibited RCC cell proliferation. Moreover, the volume of tumours in nude mice decreased with MCM5 knockdown. In addition, MCM5 silencing reduced thyroid carcinoma cell proliferation and may be involved in the inhibition of proliferation induced by BET inhibitors (BETi) [35]. Moreover, Sox10 regulates skin melanocyte proliferation by activating the DNA replication licencing factor MCM5 [32]. Hsu et al. reported that MCM5 was modulated by oestrogen receptor β to promote bladder cancer development in vitro and in vivo [36]. The above results consistently suggest that MCM5 promotes renal cancer proliferation and progression and may be a promising potential therapeutic target.

Conclusion

In conclusion, MCM5 expression was higher in RCC, and its overexpression was associated with malignant status and poor prognosis in RCC. Additionally, MCM5 plays an important role in proliferation and may be a potential prognostic marker and novel therapeutic target for RCC.

Funding This study is supported by National Natural Science Foundation of China (NSFC) [81460137].

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study has been approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University.

Informed consent Informed consent was provided by all patients who provided clinical specimens.

References

1. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67:7–30
2. Bhatt JR, Finelli A (2014) Landmarks in the diagnosis and treatment of renal cell carcinoma. Nat Rev Urol 11:517–525
3. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
4. Jonasch E, Gao J, Rathmell WK (2014) Renal cell carcinoma. BMJ 349:g4797
5. Chen D, Zhu M, Su H et al (2018) LINC00961 restrains cancer progression via modulating epithelial-mesenchymal transition in renal cell carcinoma. J Cell Physiol 234(5):7257–7265
6. Li P, Wong YN, Armstrong K et al (2016) Survival among patients with advanced renal cell carcinoma in the pretargeted versus targeted therapy eras. Cancer Med 5:169–181
7. Tye BK (1999) MCM proteins in DNA replication. Annu Rev Biochem 68:649–686
8. Giaginis C, Georgiadou M, Dimakopoulou K et al (2009) Clinical significance of MCM-2 and MCM-5 expression in colon cancer: association with clinicopathological parameters and tumor proliferative capacity. Dig Dis Sci 54:282–291
9. Giaginis C, Vgenopoulou S, Vielh P et al (2010) MCM proteins as diagnostic and prognostic tumor markers in the clinical setting. Histol Histopathol 25:351–370
10. Gou K, Liu J, Feng X et al (2018) Expression of minichromosome maintenance proteins (MCM) and cancer prognosis: a meta-analysis. J Cancer 9:1518–1526
11. Yu SY, Wang YP, Chang JY et al (2014) Increased expression of MCM5 is significantly associated with aggressive progression

and poor prognosis of oral squamous cell carcinoma. J Oral Pathol Med 43:344–349
12. Liu YZ, Wang BS, Jiang YY et al (2017) MCMs expression in lung cancer: implication of prognostic significance. J Cancer 8:3641–3647
13. Giaginis C, Giagini A, Tsourouflis G et al (2011) MCM-2 and MCM-5 expression in gastric adenocarcinoma: clinical significance and comparison with Ki-67 proliferative marker. Dig Dis Sci 56:777–785
14. Wang D, Li Q, Li Y et al (2018) The role of MCM5 expression in cervical cancer: Correlation with progression and prognosis. Biomed Pharmacother 98:165–172
15. Kelly JD, Dudderidge TJ, Wollenschlaeger A et al (2012) Bladder cancer diagnosis and identification of clinically significant disease by combined urinary detection of Mcm5 and nuclear matrix protein 22. PLoS ONE 7:e40305
16. Rodins K, Cheale M, Coleman N et al (2002) Minichromosome maintenance protein 2 expression in normal kidney and renal cell carcinomas: relationship to tumor dormancy and potential clinical utility. Clin Cancer Res 8:1075–1081
17. Donaldson AD, Blow JJ (1999) The regulation of replication origin activation. Curr Opin Genet Dev 9:62–68
18. Bell SP, Dutta A (2002) DNA replication in eukaryotic cells. Annu Rev Biochem 71:333–374
19. Zhong H, Chen B, Neves H et al (2017) Expression of minichromosome maintenance genes in renal cell carcinoma. Cancer Manag Res 9:637–647
20. Mehdi MZ, Nagi AH, Naseem N (2016) MCM - 2 and Ki - 67 as proliferation markers in renal cell carcinoma: a quantitative and semi-quantitative analysis. Int Braz J Urol 42:1121–1128
21. Kanu N, Gronroos E, Martinez P et al (2015) SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair. Oncogene 34:5699–5708
22. Murphy N, Ring M, Heffron CC et al (2005) Quantitation of CDC6 and MCM5 mRNA in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix. Mod Pathol 18:844–849
23. Burger M (2009) MCM2 and MCM5 as prognostic markers in colon cancer: a worthwhile approach. Dig Dis Sci 54:197–198
24. Eissa S, Matboli M, Shehata HH et al (2015) MicroRNA-10b and minichromosome maintenance complex component 5 gene as prognostic biomarkers in breast cancer. Tumour Biol 36:4487–4494
25. Brems-Eskildsen AS, Zieger K, Toldbod H et al (2010) Prediction and diagnosis of bladder cancer recurrence based on urinary content of hTERT, SENP1, PPP1CA, and MCM5 transcripts. BMC Cancer 10:646
26. Liao X, Han C, Wang X et al (2018) Prognostic value of minichromosome maintenance mRNA expression in early-stage pancreatic ductal adenocarcinoma patients after pancreaticoduodenectomy. Cancer Manag Res 10:3255–3271
27. Wang D, Zhu H, Guo M (2018) Expression and prognostic value of cell-cycle-associated genes in gastric adenocarcinoma. BMC Gastroenterol 18:81
28. Williams GH, Swinn R, Prevost AT et al (2004) Diagnosis of oesophageal cancer by detection of minichromosome maintenance 5 protein in gastric aspirates. Br J Cancer 91:714–719
29. Keane MG, Huggett MT, Chapman MH et al (2017) Diagnosis of pancreaticobiliary malignancy by detection of minichromosome maintenance protein 5 in biliary brush cytology. Br J Cancer 116:349–355
30. Dudderidge TJ, Kelly JD, Wollenschlaeger A et al (2010) Diagnosis of prostate cancer by detection of minichromosome maintenance 5 protein in urine sediments. Br J Cancer 103:701–707
31. Remus D, Beuron F, Tolun G et al (2009) Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing. Cell 139:719–730
32. Su Z, Zheng X, Zhang X et al (2017) Sox10 regulates skin melanocyte proliferation by activating the DNA replication licensing factor MCM5. J Dermatol Sci 85:216–225
33. Agarwal MK, Ruhul Amin AR, Agarwal ML (2007) DNA replication licensing factor minichromosome maintenance deficient 5 rescues p53-mediated growth arrest. Cancer Res 67:116–121
34. Gakiopoulou H, Korkolopoulou P, Levidou G et al (2007) Minichromosome maintenance proteins 2 and 5 in non-benign epithelial ovarian tumours: relationship with cell cycle regulators and prognostic implications. Br J Cancer 97:1124–1134
35. Mio C, Lavarone E, Conzatti K et al (2016) MCM5 as a target of BET inhibitors in thyroid cancer cells. Endocr Relat Cancer 23:335–347
36. Hsu I, Chuang KL, Slavin S et al (2014) Suppression of ERbeta signaling via ERbeta knockout or antagonist protects against bladder cancer development. Carcinogenesis 35:651–661