

Prognostic value of minichromosome maintenance mRNA expression in early-stage pancreatic ductal adenocarcinoma patients after pancreaticoduodenectomy

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Background: The aim of the current study was to investigate the potential prognostic value of minichromosome maintenance (MCM) genes in patients with early-stage pancreatic ductal adenocarcinoma (PDAC) after pancreaticoduodenectomy by using the RNA-sequencing dataset from The Cancer Genome Atlas (TCGA).

Methods: An RNA-sequencing dataset of 112 early-stage PDAC patients who received a pancreaticoduodenectomy was obtained from TCGA. Survival analysis was used to identify potential prognostic values of MCM genes in PDAC overall survival (OS).

Results: Through mining public databases, we observed that MCM genes (*MCM2*, *MCM3*, *MCM4*, *MCM5*, *MCM6*, and *MCM7*) were upregulated in pancreatic cancer tumor tissue and have a strong positive coexpression with each other. Multivariate survival analysis indicated that a high expression of *MCM4* significantly increased the risk of death in patients with PDAC, and time-dependent receiver operating characteristic analysis showed an area under the curve of 0.655, 0.587, and 0.509 for a 1-, 2-, and 3-year PDAC OS prediction, respectively. Comprehensive survival analysis of *MCM4* using stratified and joint effects survival analysis suggests that *MCM4* may be an independent prognostic indicator for PDAC OS. Gene set enrichment analysis indicated that *MCM4* may participate in multiple biologic processes and pathways, including DNA replication, cell cycle, tumor protein p53, and Notch signaling pathways, thereby affecting prognosis of PDAC patients.

Conclusions: Our study indicates that *MCM2–7* were upregulated in pancreatic cancer tumor tissues, and mRNA expression of *MCM4* may serve as an independent prognostic indicator for PDAC OS prediction after pancreaticoduodenectomy.

Keywords: minichromosome maintenance, mRNA, pancreatic ductal adenocarcinoma, prognosis, pancreaticoduodenectomy.

Introduction

Pancreatic cancer (PC) is not only a highly lethal malignancy but also has a poor prognosis, low early diagnosis rate, and lacks effective treatment strategies.^{1–3} In 2012, it was estimated that there would be approximately 330,700 PC deaths worldwide.⁴ In 2015, the National Central Cancer Registry of China (NCCRC) estimated that there would be approximately 901,000 new cases of PC and 794,000 deaths in China, using data from population-based cancer registries (2009–2011).⁵ The updated nationwide cancer statistics from the NCCRC, which were estimated on population-based cancer registry data collected from all available cancer registries in 2014, suggest an upward

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trend in new cases of PC and deaths in China compared to their previous report (2009–2011).^{5,6}

Due to the clinicopathologic features of PC, treatment and management strategies of PC were focused on developing early diagnostic and prognostic monitoring biomarkers. Previous studies have demonstrated that genetic alterations may have a contribution in PC tumorigenesis and progression.^{7,8} More than 80% of the histologic type of PCs are pancreatic ductal adenocarcinoma (PDAC).^{4,9,10} Numerous studies have reported that minichromosome maintenance (MCM) genes (including *MCM2*, *MCM3*, *MCM4*, *MCM5*, *MCM6*, and *MCM7*) could serve as a diagnostic and prognostic indicator in multiple cancers.^{11–14} However, the potential application value and mechanism of MCM genes in the prognostic prediction of PDAC after pancreaticoduodenectomy remain unclear. Therefore, the purpose of the current study was to investigate the potential prognostic application of MCM2–7 for patients with early-stage PDAC after pancreaticoduodenectomy by using an RNA-sequencing (RNA-Seq) dataset from The Cancer Genome Atlas (TCGA).

Materials and methods

Public database mining of MCM genes

With the development of high-throughput technologies, a large number of genome-wide expression profiling data have been published and freely shared with researchers, especially the whole genome dataset of cancer diseases. In the present study, we used the Genotype-Tissue Expression (GTEx; <https://www.gtexportal.org/home/>, accessed March 20, 2018) website to investigate the mRNA expression distribution of MCM genes in normal organ tissues.^{15–17} The Gene Expression Profiling Interactive Analysis (GEPIA; <http://gepia.cancer-pku.cn/index.html>; accessed March 20, 2018) website was used to investigate the mRNA expression distribution of MCM genes in pancreatic adenocarcinoma (PAAD), which is an online analysis tool based on the RNA-Seq expression data of 9,736 tumors and 8,587 normal samples from TCGA and the GTEx projects.¹⁸ We also used the Metabolic gEne RAPid Visualizer (MERAV; <http://merav.wi.mit.edu/>; accessed March 20, 2018), comprising human gene expression data from normal tissues, primary tumors, and cancer cell lines, to further validate the expression distribution of MCM genes between PC and adjacent normal tissues.¹⁹

Comprehensive survival analysis of MCM genes

The RNA-Seq dataset of PAAD was downloaded from TCGA (<https://portal.gdc.cancer.gov/>; accessed April 20, 2017)²⁰ and the raw data were normalized by *DESeq*.²¹ The

corresponding clinical information was downloaded from the University of California, Santa Cruz Xena (UCSC Xena: <http://xena.ucsc.edu/>, accessed April 20, 2017). To ensure the reliability of prognostic analysis, we have established a strict patient inclusion criteria and exclusion criteria, which have been published in our previous paper and are described as follows: 1) complete survival data available; 2) the histology type was PDAC; 3) pathologic stage I or II; 4) patients underwent pancreaticoduodenectomy.²² PDAC patients with pathologic stage III or IV disease who underwent other types of surgery were excluded.²² To comprehensively investigate the prognostic values of MCM genes in PDAC, multivariate Cox proportional risk regression model analysis was used to screen the genes by adjusting for clinical parameters that were significantly associated with PDAC overall survival (OS) in univariate survival analysis. Subsequently, comprehensive survival analysis of prognostic-MCM genes was performed, including evaluation by the *survivalROC* package in the R platform, stratified survival analysis, nomogram construction, and joint effect survival analysis.

Gene set enrichment analysis

To investigate the biologic processes involved in the clinical outcome of PDAC with different expression levels of prognostic-MCM genes, we carried out a gene set enrichment analysis (GSEA, <http://software.broadinstitute.org/gsea/index.jsp>, accessed December 15, 2017).^{23,24} GSEA was used to deep mine the potential mechanisms of prognostic-MCM genes using the Molecular Signatures Database (MSigDB) c2 (c2.cp.kegg.v6.1.symbols.gmt) and c5 (c5.all.v6.1.symbols.gmt).²⁵ The significantly enriched gene sets in the GSEA were identified with the following criteria: a nominal *P*-value <0.05 and false discovery rate (FDR) <0.25.

Statistical analysis

FDRs in the GSEA were adjusted for multiple testing with the Benjamini–Hochberg procedure to control FDR.^{26–28} Univariate survival analysis of clinical features and MCM genes were compared using the log-rank test; clinicopathologic parameters significantly associated with OS (*P*<0.05) were entered into the multivariate Cox proportional hazards regression model for adjustment, whereas, hazard ratios (HRs) and 95% CIs were used to assess the relative risk in different PDAC patients that were stratified by MCM gene expression. Coexpression relationships between MCM genes were assessed by the Pearson correlation coefficient. All statistical analyses were conducted with SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) and R3.3.0. Statistical significance was set as a *P*-value <0.05.

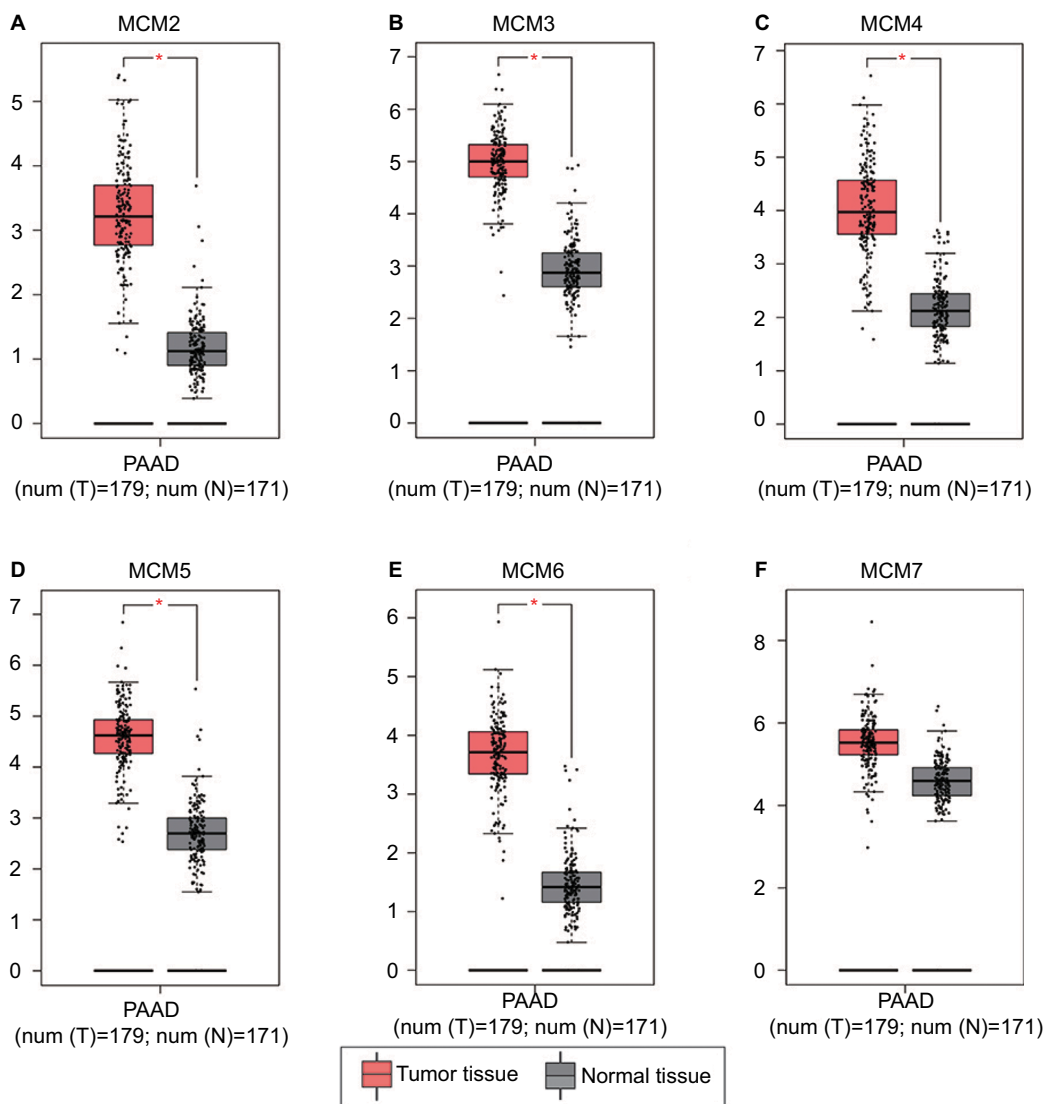


Figure 2 Expression distribution of MCM2–7 between PAAD tumor and normal tissues.

Notes: Expression distribution box plot of *MCM2* (A); *MCM3* (B); *MCM4* (C); *MCM5* (D); *MCM6* (E); *MCM7* (F). Expressions of MCM genes were upregulated in PDAC tumor tissues. * $P < 0.05$.

Abbreviations: MCM, minichromosome maintenance; PAAD, pancreatic adenocarcinoma.

the expression of MCM2–7 genes was not significantly differentially distributed between stage I and stage II tumor tissues (Figure 5A), whereas expressions of *MCM2*, *MCM4*, and *MCM7* were significantly differentially distributed between histologic grade G1/G2 and G3/G4 tumor tissues (Figure 5B). Furthermore, we also explored the ability of *MCM2*, *MCM4*, and *MCM7* (Figure 5C–E) to distinguish histologic grade G1/G2 and G3/G4 tumor tissues using the area under the curve (AUC) of a receiver operating characteristic (ROC) curve. ROC analysis indicated that *MCM2* ($P = 0.005$, AUC = 0.670, 95% CI = 0.558–0.782; Figure 5C) and *MCM7* ($P = 0.011$,

AUC = 0.654, 95% CI = 0.534–0.775; Figure 5E) may have potential in distinguishing histologic grade G1/G2 and G3/G4 tumor tissues in PDAC, and a combination of *MCM2* and *MCM6* could improve diagnostic efficiency ($P = 0.003$, AUC = 0.681, 95% CI = 0.566–0.795; Figure 5F).

Survival analysis of MCM2–7 in PDAC OS

Baseline information of these 112 PDAC patients is shown in Table 1, and clinical parameters of histologic grade, radical resection, radiation therapy, and targeted molecular therapy were significantly associated with PDAC OS, which

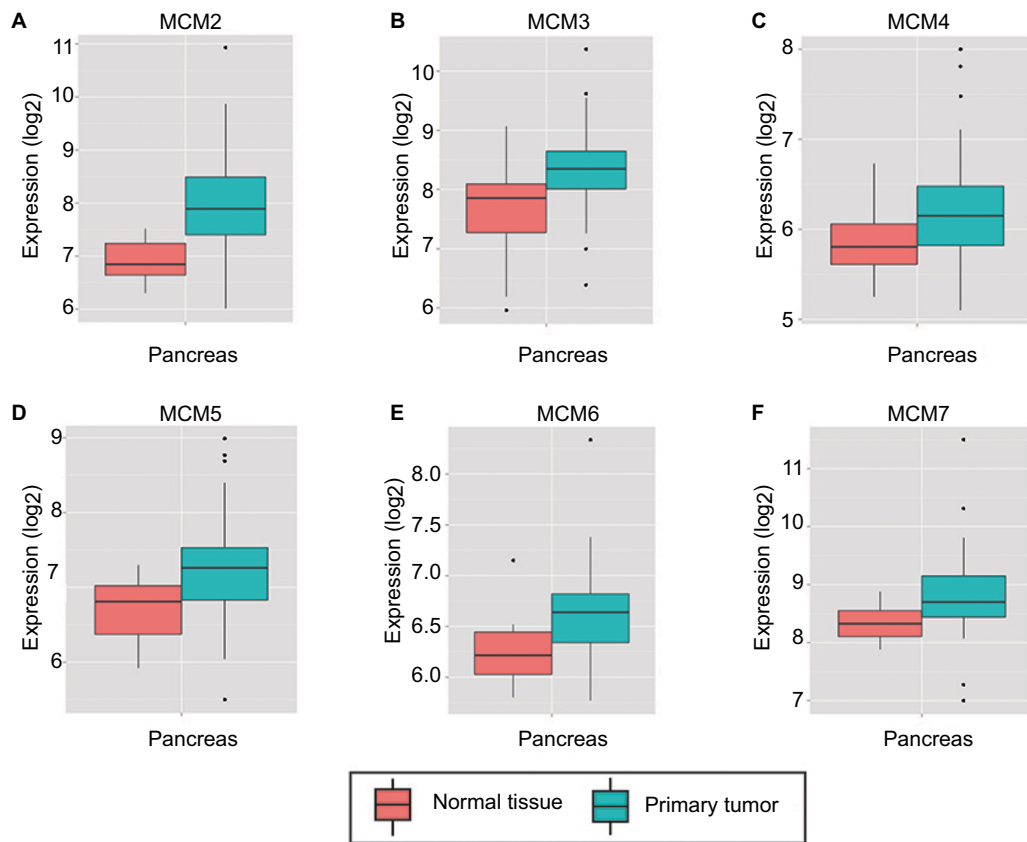


Figure 3 Expression distribution of MCM2–7 between PC tumor and normal tissues that was carried out by MERAV. Expressions of MCM genes were upregulated in PC tumor tissues.
Notes: Expression distribution box plot of *MCM2* (A); *MCM3* (B); *MCM4* (C); *MCM5* (D); *MCM6* (E); *MCM7* (F).
Abbreviations: MCM, minichromosome maintenance; MERAV, Metabolic gEne RApid Visualizer; PC, pancreatic cancer.

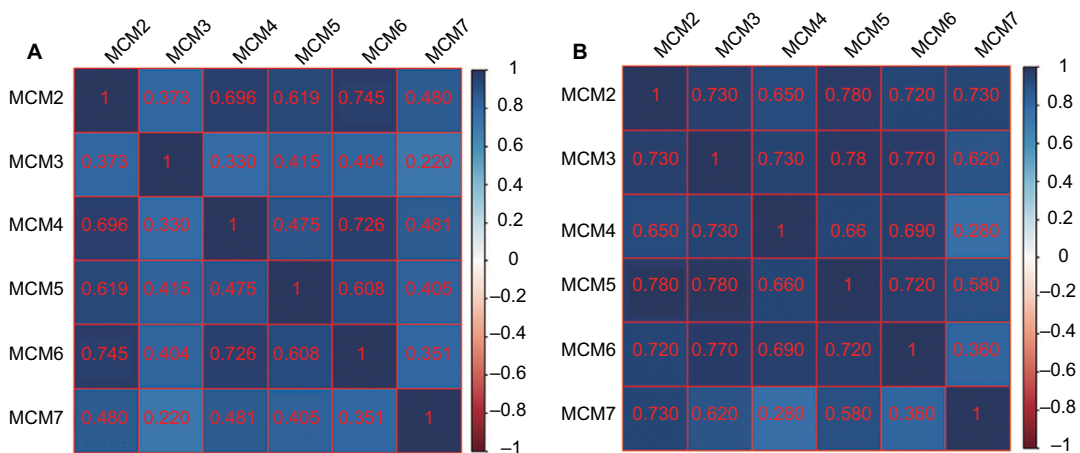


Figure 4 Coexpression matrix of MCM genes in PDAC tumor tissues and human normal pancreas tissues, and demonstrated that MCM2–7 were strongly positively correlation and coexpressed with each other in both PDAC tumor and normal pancreas tissues.
Notes: (A) Coexpression matrix of MCM genes in PDAC tumor tissues; (B) coexpression matrix of MCM genes in human normal pancreas tissues.
Abbreviations: MCM, minichromosome maintenance; PDAC, pancreatic ductal adenocarcinoma.

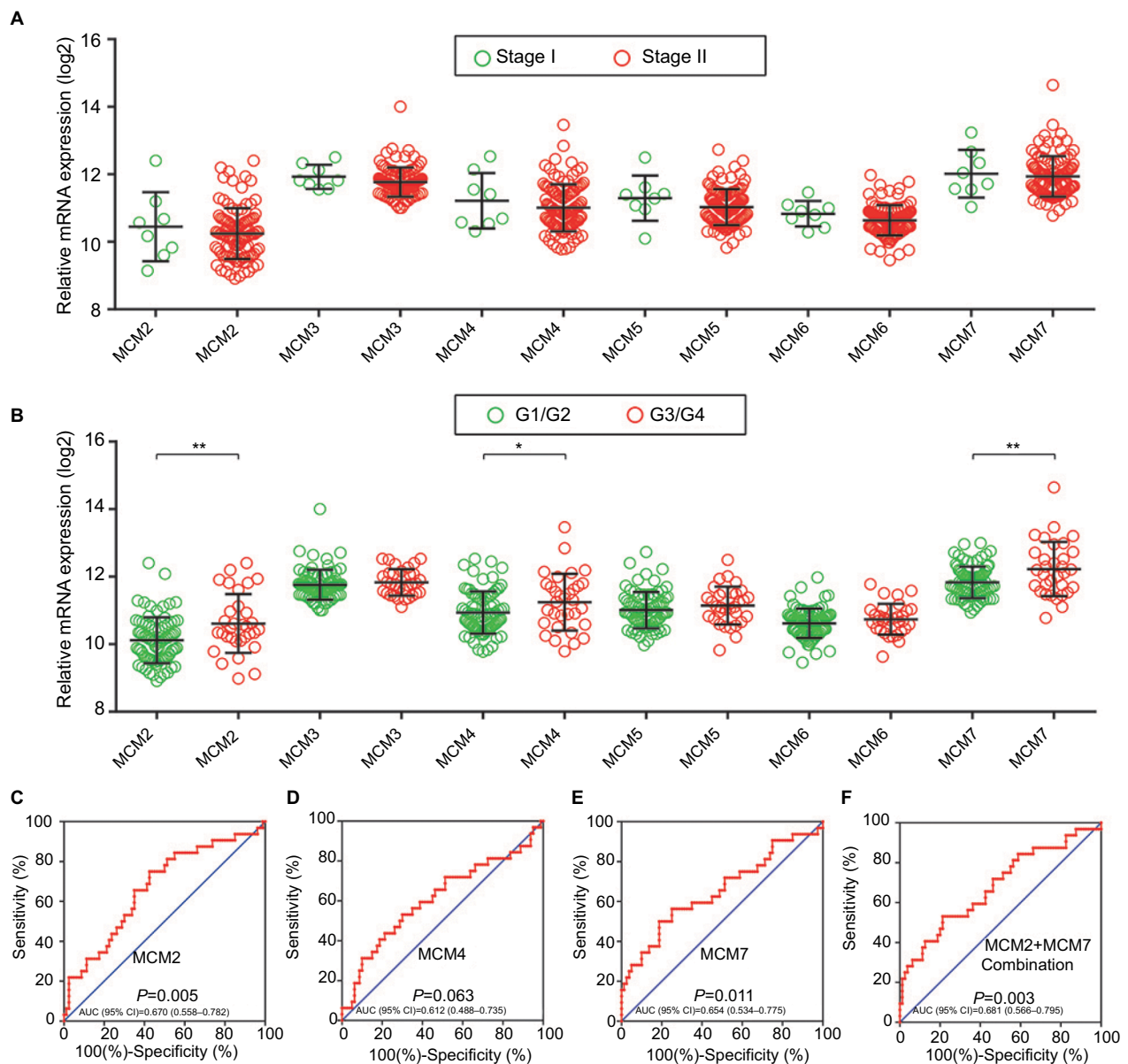


Figure 5 Gene expression distribution of MCM genes in different PDAC tumor stages and histologic grades, and ROC curves of MCM genes to distinguish different PDAC histologic grades.

Notes: (A) Gene expression distribution of MCM genes in different PDAC tumor stages; (B) gene expression distribution of MCM genes in different PDAC histologic grades; ROC curves of MCM2 (C), MCM4 (D), MCM7 (E) and MCM2 + MCM7 (F) combination to distinguish different PDAC histological grades. * $P<0.05$, ** $P<0.01$.

Abbreviations: AUC, area under the curve; MCM, minichromosome maintenance; PDAC, pancreatic ductal adenocarcinoma; ROC, receiver operating characteristic.

needed to be adjusted into a multivariate Cox proportional hazards regression model. Survival analysis of the MCM2–7 genes (Figure 6A–F) demonstrated that *MCM4* was significantly associated with PDAC OS, and high expression of *MCM4* was significantly increased with the risk of death in PDAC (adjusted $P=0.003$, adjusted HR=2.409, 95% CI=1.351–4.294, Table 2, Figure 6C) and promoted a poor OS (high *MCM4* vs low *MCM4*; 458 days vs 634 days, Table 2, Figures 6C and 7A). We also performed a time-dependent ROC analysis, which was carried out using *survivalROC* in

the R platform, to evaluate the predictive accuracy of *MCM4* expression in PDAC OS, and demonstrated that *MCM4* expression performed well in predicting PDAC OS. The AUC of the time-dependent ROC curve was 0.655, 0.587, and 0.509 for 1-, 2-, and 3-year survival (Figure 7B), respectively.

Comprehensive analysis of *MCM4* in PDAC OS

To perform a comprehensive investigation of the role of *MCM4* in the prognosis of PDAC, we used a stratified analysis and

Table 1 Correlation between OS and clinicopathologic features of PDAC patients^a

Variables	Events/total (n=112)	MST (days)	HR (95% CI)	Log-rank P-value
Age (years)				0.066
≤60	20/38	593	1	
>60	49/74	485	1.636 (0.962–2.780)	
Gender				0.523
Female	36/53	511	1	
Male	33/59	592	0.855 (0.529–1.382)	
Alcohol history ^b				0.349
No	25/43	592	1	
Yes	38/61	511	1.276 (0.765–2.128)	
Pathologic stage				0.943
Stage I	4/8	236	1	
Stage II	65/104	518	1.038 (0.375–2.872)	
Pathological T				0.466
T1/T2	7/14	498	1	
T3	62/98	518	1.340 (0.608–2.949)	
Pathological N				0.091
N0	9/21	634	1	
N1	60/91	511	1.818 (0.899–3.678)	
Pathological M				0.319
M0	31/55	593	1	
Mx	38/57	485	1.278 (0.787–2.075)	
Histologic grade				0.010
G1/G2	45/80	596	1	
G3/G4	24/32	470	1.919 (1.156–3.185)	
Radical resection ^c				0.009
R1/Rx	29/44	381	1	
R0	39/66	603	0.514 (0.310–0.852)	
Radiation therapy ^d				0.029
No	48/70	473	1	
Yes	15/30	691	0.527 (0.293–0.947)	
Targeted molecular therapy ^e				<0.001
No	24/29	224	1	
Yes	41/73	634	0.168 (0.095–0.296)	

Notes: ^aThe data in this table also have been shown in our previous publication²². ^bAlcohol history information is unavailable in eight patients; ^cRadical resection information is unavailable in two patients. ^dRadiation therapy information is unavailable in 12 patients. ^eTargeted molecular therapy information is unavailable in ten patients.

Abbreviations: HR, hazard ratio; MST, median survival time; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma.

joint effect survival analysis to assess the prognostic value of *MCM4* in PDAC, and developed a nomogram, which included clinical parameters and the *MCM4* gene, to evaluate individualized prognostic risk scores. Stratified analysis suggests that high expression of *MCM4* was associated with a significantly increased risk of death in PDAC patients except in patients with pathologic stage I, who received radiation therapy and targeted molecular therapy; young patients (≤60 years); female patients, without or with alcohol history; and patients without radical resection (Figure 8A). The nomogram indicated that expression of *MCM4* also had a certain contribution to the prognosis of PDAC (Figure 8B). Joint effect survival analysis suggests that a combination of *MCM4* and those clinical parameters, which were significantly correlated to PDAC OS, showed a

better performance in PDAC OS, compared with single clinical parameters (Figure 9A–D and Table 3).

GSEA investigation for *MCM4* in PDAC OS

To further explore the potential mechanism of *MCM4* in PDAC OS, we also developed a single-gene GSEA to investigate the potential biologic processes and pathways between different *MCM4* expression levels. Enrichment of c5 suggests that high expression of *MCM4* may be involved in DNA repair, DNA replication, cell cycle, and cell and nuclear division biologic processes (Figure 10A–F, Table S1), whereas enrichment of c2 indicates that high expression of *MCM4* may participate in the cell cycle, DNA replication,

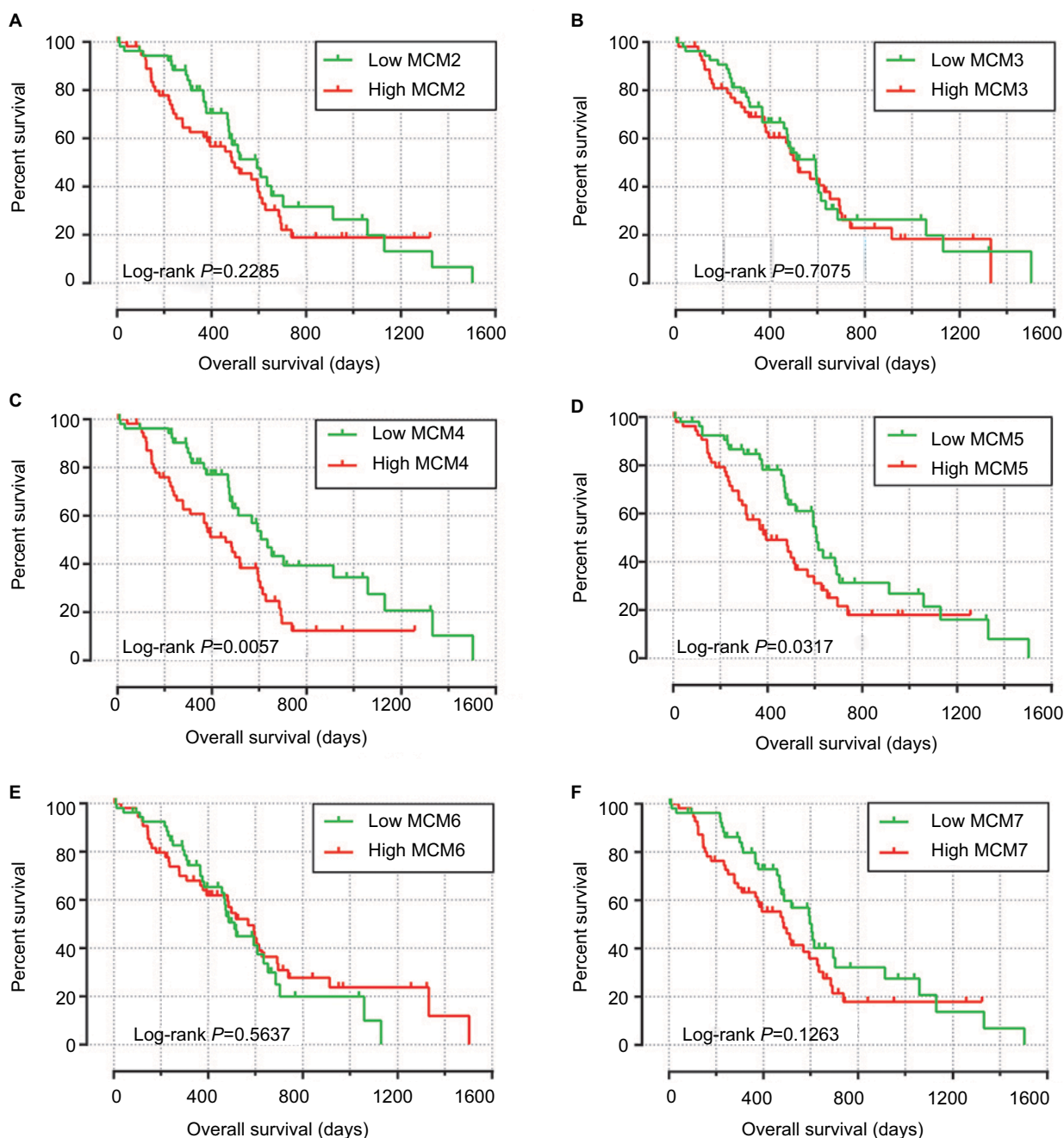


Figure 6 Kaplan–Meier survival curves for MCM genes in pancreatic ductal adenocarcinoma of The Cancer Genome Atlas cohort.

Notes: Overall survival stratified by *MCM2* (A), *MCM3* (B), *MCM4* (C), *MCM5* (D), *MCM6* (E), and *MCM7* (F).

Abbreviation: MCM, minichromosome maintenance.

tumor protein p53 (TP53), PC, and Notch signaling pathways, as well as pathways in cancer (Figure 10G–L; Table S2).

Discussion

The replication of DNA is a fundamental step in the cell cycle, and the MCM protein family in this process has been investigated extensively in numerous studies over the past decade.^{31–35} The MCM2–7 complex provides essential replicative helicase function in late mitosis and early G1 in

the cell cycle.^{35–37} The functions of MCM2–7 are involved in the basic biologic processes of cell cycle maintenance in cells; therefore, dysregulation of the MCM protein family may cause cancer or developmental defects.^{38–41} MCM genes may be potential diagnostic and prognostic biomarkers for cancers, and promising targets for anticancer drug development and targeted therapy.⁴²

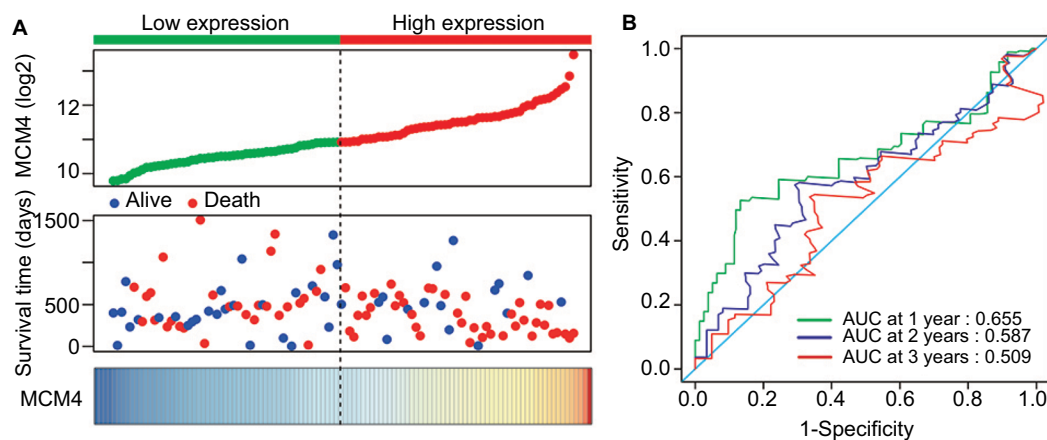
Extensive studies have shown that the dysregulation of MCM2–7 can be observed in various cancers and may have

Table 2 Prognostic values of MCM genes expression in PDAC OS of TCGA cohort

Gene expression	Events/total (n=112)	MST (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
MCM2						
Low	31/56	592				
High	38/56	498	1.345 (0.828–2.185)	0.230	1.614 (0.929–2.805)	0.089
MCM3						
Low	33/56	592				
High	36/56	517	1.096 (0.679–1.769)	0.708	0.773 (0.453–1.320)	0.346
MCM4						
Low	28/56	634				
High	41/56	458	1.990 (1.210–3.272)	0.007	2.409 (1.351–4.294)	0.003
MCM5						
Low	32/56	607				
High	37/56	393	1.692 (1.041–2.749)	0.034	1.185 (0.693–2.028)	0.535
MCM6						
Low	33/56	511				
High	36/56	568	0.867 (0.534–1.408)	0.564	1.104 (0.645–1.889)	0.718
MCM7						
Low	31/56	603				
High	38/56	485	1.456 (0.897–2.364)	0.129	1.183 (0.660–2.119)	0.572

Note: ^aAdjusted for histologic grade, radiation therapy, radical resection, and targeted molecular therapy.

Abbreviations: MCM, minichromosome maintenance; MST, median survival time; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; TCGA, The Cancer Genome Atlas.

**Figure 7** Prognostic value evaluation of MCM4 in patients with PDAC.

Notes: (A) From top to bottom are the expression values of *MCM4*, patients' survival status distribution, and the expression heat map of *MCM4* in the low- and high-expression groups. (B) Receiver operating characteristic curve for predicting overall survival in PDAC patients by the *MCM4*.

Abbreviations: MCM, minichromosome maintenance; PDAC, pancreatic ductal adenocarcinoma.

a diagnostic value. Work by Saydam et al demonstrated that expression of MCM2–7 mRNAs was markedly increased in meningiomas tumor tissue.⁴³ A prospective cohort study from India suggests that MCM (MCM2 and MCM5) immunocytochemistry may have considerable advantages for first-line cervical screening in developing countries.⁴⁴ Immunohistochemical detection of MCM2 in the urine of bladder cancer (BC) patients and in the stools of colorectal cancer (CRC) patients could serve as a novel method for the diagnosis of these cancers.^{45,46} A study by Wang et al observed that mRNA expression of MCM2 in colon adenocarcinoma (COAD) was

significantly increased in adenomas, as well as upregulation in adenomas with high-grade dysplasia, or in older patients, and demonstrates an application value in the early diagnosis of COAD.⁴⁷ Immunohistochemical detection of MCM5 in human secretions also has a diagnostic value for cancers of the corresponding organs, such as urine sediments for prostate cancer,¹³ gastric aspirates for esophageal cancer,¹⁴ and bile aspirates or biliary brush cytology for pancreaticobiliary malignancy.^{48,49} Moreover, the levels of MCM6 mRNA and protein in the plasma are also markedly increased, and significantly associated with tumor stage progression and lymph

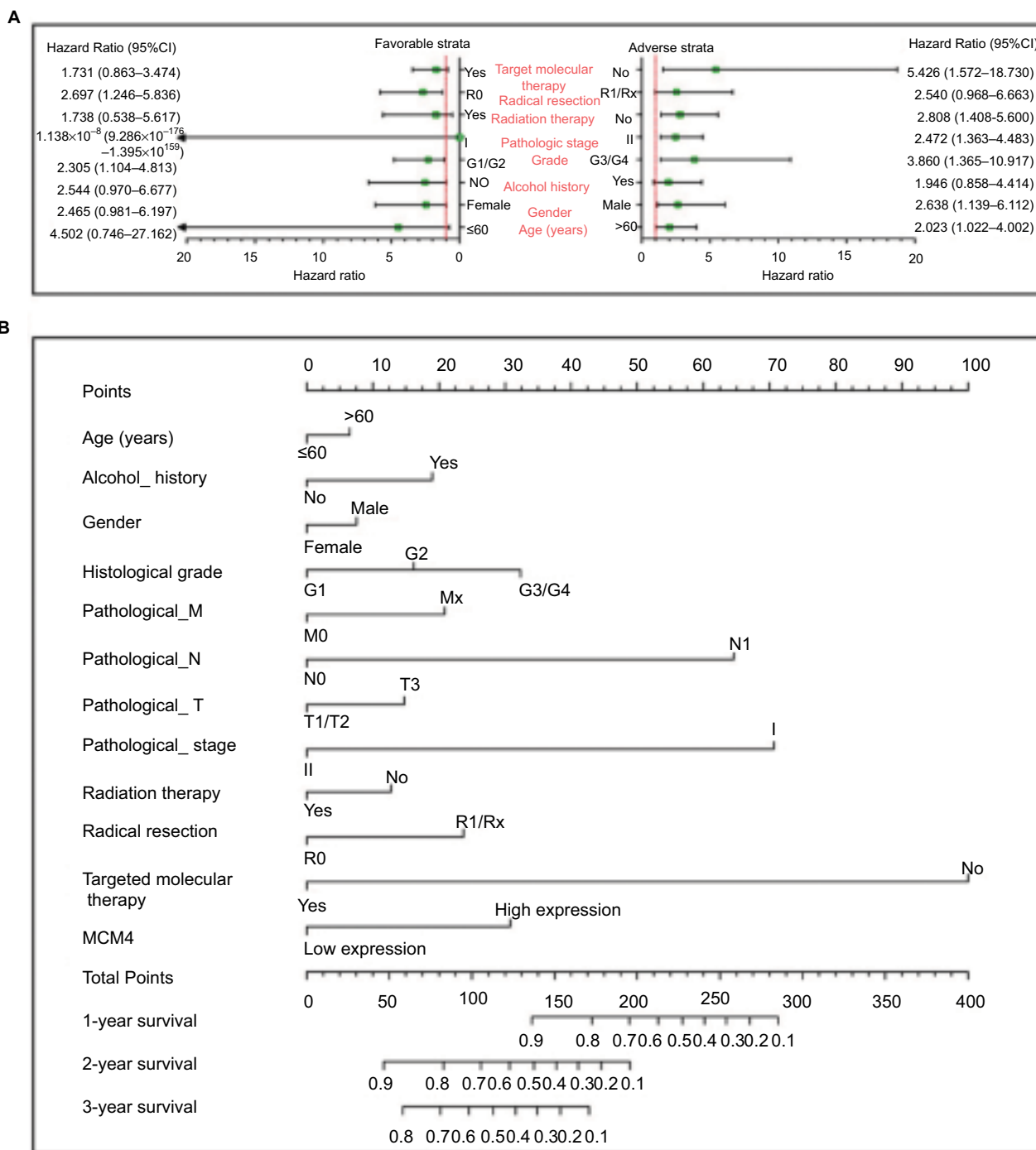


Figure 8 The relationship between *MCM4* and clinical information. **Notes:** (A) Stratified analysis of association between *MCM4* and overall survival in pancreatic ductal adenocarcinoma. (B) Nomogram for predicting the 1-, 2-, and 3-year event (death) with *MCM4* and clinical information. **Abbreviation:** MCM, minichromosome maintenance.

node metastasis in hepatocellular carcinoma (HCC).⁵⁰ Similar to previous studies, by using the GEPIA online analysis tool, we observed that *MCM2–7* were upregulated in the majority of human tumor tissues, as well as in PC tumor tissue. However, due to the limitation that we cannot obtain normal

pancreas tissue expression data from GEPIA, we could not assess the diagnostic values of *MCM2–7* in PC.

In addition, extensive investigations indicate that *MCM* genes may serve as proliferation markers in various cancers. Immunohistochemical analysis of *MCM3* might be useful

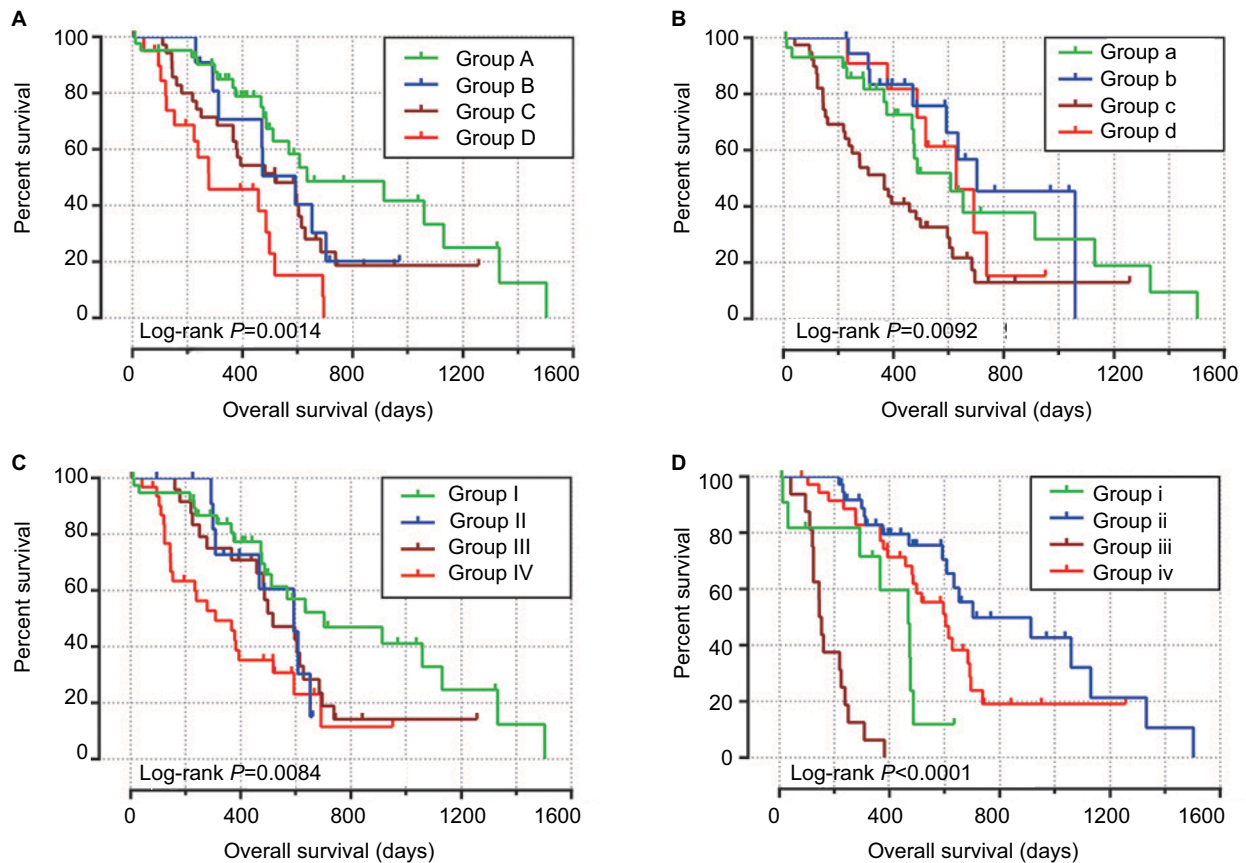


Figure 9 Joint effects analysis of overall survival stratified by *MCM4* and pancreatic ductal adenocarcinoma clinical parameters.

Notes: Joint effects analysis stratified by *MCM4* and following clinical parameters: Histologic grade (A), radiation therapy (B), radical resection (C), and targeted molecular therapy (D).

Abbreviation: MCM, minichromosome maintenance.

as a proliferation marker in oral squamous cell carcinoma (OSCC),⁵¹ papillary thyroid carcinoma,⁵² and salivary gland tumors.⁵³ The potential application of MCM5 in identifying cell proliferation can be found in Merkel cell carcinoma⁵⁴ and malignant skin diseases.⁵⁵ Similar studies of MCM7 also have been reported in gastric cancer (GC),⁵⁶ esophageal lesions,⁵⁷ reactive mesothelial cells, and malignant cells.^{58,59} Because of the close relationship between MCM gene and cell proliferation, MCM genes are also found to be associated with tumor progression. Overexpression of MCM7 was significantly associated with prostate cancer progression, relapse, local invasion, and a worse tumor grade,⁶⁰ as well as in OSCC development and metastasis.⁶¹ Work by Das et al observed that MCM4, MCM5, MCM6, and MCM10 were significantly overexpressed in cervical cancer tumor tissues, and upregulated in advanced tumor stages, which indicates that these genes were significantly associated with cervical cancer carcinogenesis and progression.⁶² Moreover, there was a significantly positive association between the expression of MCM2 and histopathologic grade, and the expression was

markedly upregulated in poorly differentiated HCC tissues, which may serve as a biomarker for HCC progression.⁶³

The prognostic values of MCM2–7 have also been identified in multiple types of cancer.^{64–66} Due to the upregulation of MCM2–7 in multiple types of cancer, we speculate that they might play an oncogene role in cancer prognosis, and high expression of MCM genes may promote a poor survival. The following series of evidence from a review of the literature will support our inference. Work by Kang et al also demonstrated that knockdown of MCM7 in GC cells may suppress its oncogenic function.⁶⁵ Immunohistochemical detection of MCM2 can be used as a biomarker for clinical outcome prediction, and high expression of MCM2 was associated with a significantly increased risk of death in patients with muscle-invasive urothelial cancer,⁶⁶ nonbenign epithelial ovarian tumors,⁶⁷ non-small-cell lung cancer (NSCLC),^{12,68} and GC,^{69–71} or recurrence in patients with BC⁷² and GC.⁷¹ Similar results could also be observed with MCM5, MCM6, and MCM7. High expression of MCM5 was associated with a significantly increased risk of death in patients with NSCLC⁷³

Table 3 Joint effects survival analysis of clinical factors and the MCM4 expression with OS in PDAC patients

Group	MCM4	Variables	Events/total (n=112)	MST (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
Histologic grade								
A	Low expression	G1 + G2	20/44	634	1		1	
B	Low expression	G3 + G4	8/12	592	1.652 (0.711–3.837)	0.243	1.782 (0.713–4.457)	0.217
C	High expression	G1 + G2	25/36	518	1.830 (0.991–3.380)	0.054	2.146 (1.051–4.383)	0.036
D	High expression	G3 + G4	16/20	278	3.788 (1.893–7.579)	<0.001	5.185 (2.373–11.329)	<0.001
Radiation therapy ^b								
a	Low expression	No	17/31	607	1		1	
b	Low expression	Yes	8/19	702	0.686 (0.289–1.626)	0.392	1.403 (0.513–3.836)	0.510
c	High expression	No	31/39	366	2.047 (1.097–3.819)	0.024	2.977 (1.509–5.871)	0.002
d	High expression	Yes	7/11	627	1.010 (0.408–2.497)	0.983	0.657 (1.880–5.378)	0.239
Radical resection ^c								
I	Low expression	R0	20/41	702	1		1	
II	Low expression	R1/Rx	7/13	592	1.732 (0.708–4.232)	0.229	1.457 (0.555–3.826)	0.445
III	High expression	R0	19/25	517	1.775 (0.924–3.408)	0.085	2.413 (1.162–5.014)	0.018
IV	High expression	R1/Rx	22/31	308	2.975 (1.560–5.673)	0.001	3.498 (1.724–7.101)	0.01
Targeted molecular therapy ^d								
i	Low expression	No	8/13	467	1		1	
ii	Low expression	Yes	18/37	702	0.239 (0.098–0.583)	0.002	0.204 (0.076–0.547)	0.002
iii	High expression	No	16/16	145	5.036 (2.007–12.638)	0.001	4.303 (1.625–11.399)	0.003
iv	High expression	Yes	23/36	603	0.411 (0.180–0.940)	0.035	0.357 (0.148–0.859)	0.021

Notes: ^aAdjusted for histologic grade, radiation therapy, radical resection, and targeted molecular therapy. ^bRadiation therapy information is unavailable in 12 patients; ^cradical resection information is unavailable in two patients. ^dTargeted molecular therapy information is unavailable in 10 patients.

Abbreviations: HR, hazard ratio; MST, median survival time; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma.

and cervical cancer,⁷⁴ and patients with high expression of MCM6 also have poor OS and increased risk of death in NSCLC,⁷⁵ low-grade chondrosarcoma,⁷⁶ mantle cell lymphoma,⁷⁷ and endometrioid endometrial adenocarcinoma.⁷⁸ The application value of MCM7 in the prognosis of cancer has been widely investigated. Previous studies have substantiated that MCM7 expression can serve as an independent prognostic factor for human CRC,⁷⁹ HCC,⁸⁰ lung cancer,^{81,82} Hodgkin's lymphoma,⁸³ OSCC,⁸⁴ and esophageal squamous cell carcinoma,⁸⁵ and promotes a poor prognosis. In addition, MCM7 also plays a crucial role in monitoring recurrence

or progression-free survival, and evidence from previous studies suggests that MCM7 can be used as a biomarker for recurrence of CRC,⁸⁶ meningiomas,⁸⁷ and GC,⁶⁵ as well as progression-free survival of non-muscle-invasive BC,⁸⁸ pituitary adenoma,⁸⁹ and ovarian cancer.⁹⁰ A previous study by Peng et al reported that MCM genes were significantly overexpressed in PC tumor tissue and correlated to PC progression and prognosis, which is based on PC patients from TCGA.⁹¹ However, the study by Peng et al did not consider the influence of different operation methods and histologic subtypes for PC prognosis. The advantage of this current

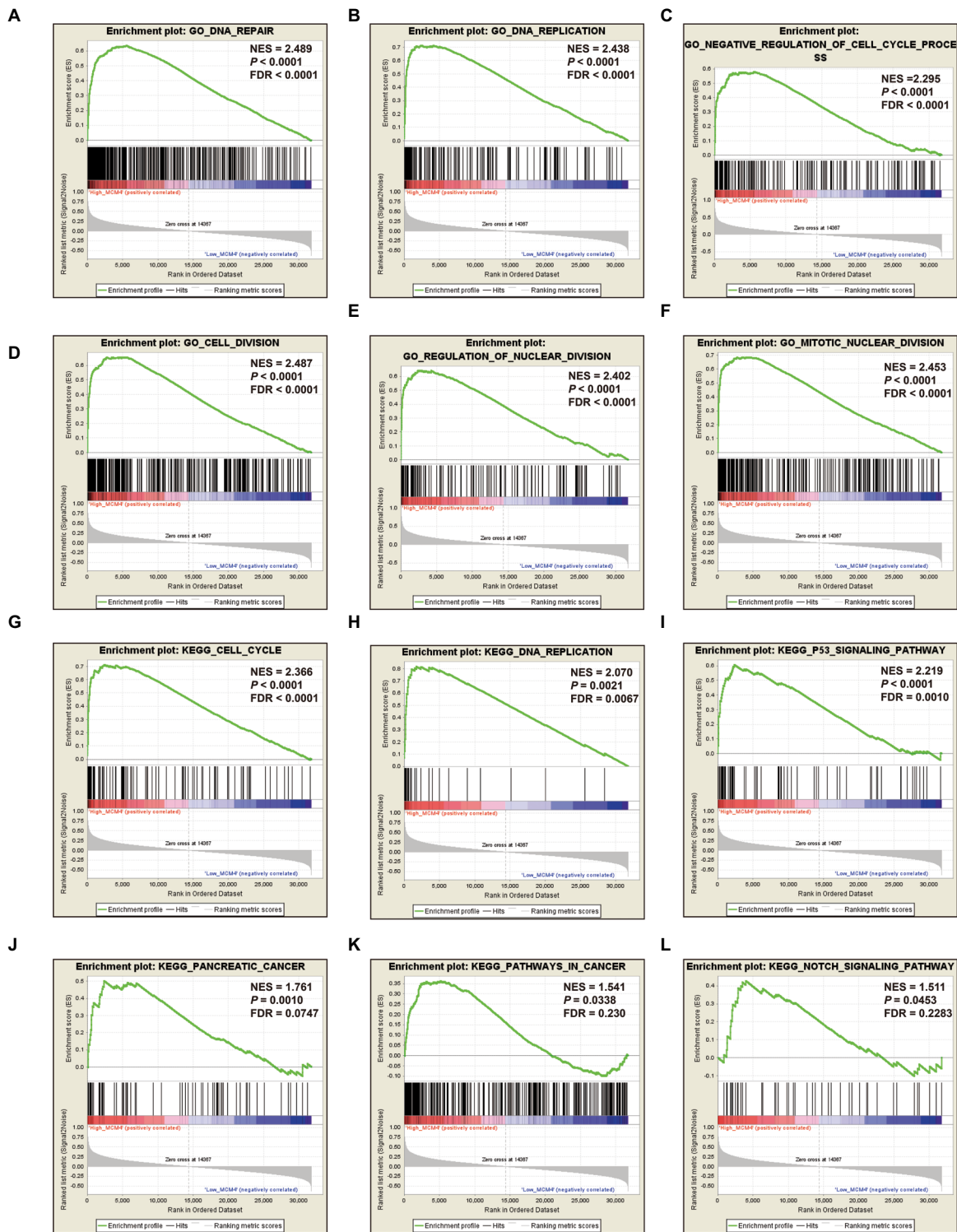


Figure 10 GSEA results of *MCM4* in PDAC patients.

Notes: (A–F) GSEA results of c2 reference gene sets for high *MCM4* expression groups; (G–L) GSEA results of c5 reference gene sets for high *MCM4* expression groups.

Abbreviations: ES, enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; MCM, minichromosome maintenance; NES, normalized enrichment score; PDAC, pancreatic ductal adenocarcinoma.

study was to take into full consideration the effect of operation method and histologic subtypes in PC prognosis, and only included patients with PDAC histologic subtype and underwent pancreaticoduodenectomy; therefore, the results

obtained from our study may be more reliable. In addition, we also developed a comprehensive survival analysis for *MCM4* and constructed a nomogram base on the clinical parameters and *MCM4* mRNA expression levels, indicating that *MCM4*

may be an independent prognostic indicator for PDAC OS prediction after pancreaticoduodenectomy.

The exploration of a potential mechanism by GSEA revealed that *MCM4* may take part in the biologic processes and pathways of DNA replication, cell cycle, TP53, and Notch signaling, which may affect PDAC prognosis. By reviewing the literature, we also found that a number of previous studies supported our results and substantiated that *MCM4* played a crucial role in the cell cycle,^{92,93} DNA replication,^{94,95} DNA repair,⁹⁶ and TP53 signaling pathway⁹⁷; these biologic processes and pathways have already demonstrated a role in cancer prognosis.

As all the data in the present study come from public databases, there are several limitations that need to be recognized. First, the clinical information from TCGA database was not comprehensive, and the results of survival analysis still need to be verified. Second, due to strict inclusion and exclusion criteria, the sample size in our study was relatively small; therefore, an additional large verification cohort to validate our results is necessary. Third, due to a relatively small sample size, and because most of the patients died within 3 years, we cannot assess the survival prediction accurately for more than 3 years. Fourth, because adjacent normal tissues of PDAC were rare, we cannot perform an ROC analysis to assess the diagnostic value of MCM2–7 in PDAC.

Despite these limitations, in the present study we have identified the prognostic application of *MCM4* mRNA expression in patients with PDAC, and also investigated the potential mechanism of different *MCM4* expression levels in PDAC prognosis through a GSEA approach. Once these results are confirmed, *MCM4* may have potential in the clinical application of prognostic monitoring, cancer management, and targeted therapy of PDAC.

Conclusions

Through a comprehensive analysis of MCM genes, we observed that MCM2–7 were upregulated in PC tumor tissues, and mRNA expression of *MCM4* may serve as an independent prognostic indicator for PDAC prognosis prediction. The potential mechanism of *MCM4* in PDAC OS, which was investigated by GSEA, indicated that *MCM4* may play a role in PDAC prognosis through participating in the biologic processes and pathways of DNA replication, cell cycle, TP53, and Notch signaling. However, these results still need further verification and investigation.

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Disclosure

The authors report no conflicts of interest in this work.

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