Transcriptional Expression of Minichromosome Maintenance 10 as an Independent Negative Predictor of Survival in Hepatocellular Carcinoma

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Abstract

Background: The minichromosome maintenance protein 10 (MCM10) is a replication licensing factor that initiates eukaryotic genome replication by interacting with CDC45-MCM2-7 complex, thereby mediating cellular proliferation. Recent studies have found that MCM10 is involved in tumorigenesis and cancer development. However, research on the role of MCM10 in hepatocellular carcinoma (HCC) is scarce.

Methods: In this study, the transcriptional expression, prognostic efficacy and function of MCM10 in HCC were explored through the public dadabases and bioinformatic tools, including ONCOMINE, Kaplan-Meier plotter, cBioPortal, TIMER and GEPIA. Statistical significance was inferred at a *P*-value < 0.05.

Results: It was found that MCM10 expression was commonly overexpressed in cancer specimens compared to match normal tissues. Up-regulation of MCM10 gene in HCC had a close relationship with patients' survival time. A higher level of MCM10 expression was correlated with shorter overall survival (OS) and progression-free survival (PFS) in HCC patients, especially at early stages (grade 2 or stage 1+2). MCM10 was also demonstrated to be an independent negative predictor of OS and disease-free survival (DFS) in patients with HCC by multivariate Cox regression. Additionally, according to the levels of marker genes for different immune cells in HCC, MCM10 expression was markedly positively correlated with the numbers of tumor-infiltrating B cells, CD8$^+$ T cells, CD4$^+$ T cells, macrophages, neutrophils and dendritic cells, indicating its potential immunogenic role in HCC.

Conclusions: MCM10 could be exploited as a potential independent prognostic indicator as well as therapeutic target for HCC patients.

Background

Hepatocellular carcinoma (HCC) is the most frequent primary hepatic malignancy and globally the second leading cause of cancer-related deaths[1, 2]. In China, patient population accounts for even over half of the total HCC cases, largely because of chronic hepatitis B virus infection[3]. A very large proportion of HCC patients are usually diagnosed at advanced stages, at which point curative treatments are not eligible, contributing to a dismal clinical outcome[4]. Despite improvements in HCC diagnosis and treatments, the prognosis for patients is still poor[5]. Additionally, limitations remain for traditional clinicopathological features such as alpha-fetoprotein (AFP), cancer stage and histological grade to predict prognosis of HCC patients[6]. Accordingly, it has been a research trend to develop new discriminatory markers or therapeutic targets on the molecular level for enabling precision medicine. Most recently, databases on basis of large-scale, genome-wide association studies have led to the development of novel markers for tumor therapies[7].

The minichromosome maintenance (MCM) family is composed of eight conserved members, namely MCM2-7, MCM8, and MCM10[8]. The MCM proteins are of vital importance as DNA helicases in the initiation and elongation of eukaryotic genome replication. Excess MCM proteins are responsible for maintaining genomic integrity by functioning as dormant origins that serve as a backup in DNA duplication[9]. In conformity to their crucial roles in replication, MCM genes are determined as potential prognostic indicators for cancers[10, 11]. Previous studies have found that genetic up-regulation or amplification of several MCM proteins including MCM2, MCM3, MCM6 and MCM7 can promote cell proliferation and HCC tumorigenicity[12−15]. However, research on the role of MCM10 in HCC is scarce. MCM10, along with CDC45-MCM2-7 complex, is a subtype of the MCM family relating to initiating DNA replication and regulating cell proliferation in late G1 or early S phase of cell cycle[16−18]. Accumulating studies have shown that MCM10 expression tended to be higher in a variety of malignancies[19−23]. Over-expression of MCM10 in some cancers had a close relationship with tumor progression and dismal clinical outcomes[19−21]. Furthermore, MCM10 was identified as one of the top-ranking genes which is enriched in cancer-related pathways[24]. Although Liu et al. have reported up-regulation of MCM10 gene in HCC[23], the prognostic implication and molecular mechanisms of MCM10 in HCC remains obscure.

In this study, we concentrated on investigating MCM10 gene expression and its predictive values in HCC patients by using the publicly available databases (including ONCOMINE, Kaplan-Meier plotter, cBioPortal, TIMER, and GEPIA). We for the first time

explored the correlation of MCM10 with the status of different immune infiltrating cells in HCC. Results from our study highlighted the significance of MCM10 as an independent negative predictor of survival in patients with HCC.

Methods And Materials

ONCOMINE database

The transcriptional expression of MCM10 in different cancer types was analyzed using the ONCOMINE database (https://www.oncomine.org), which is an integrated publicly accessible online cancer microarray database for DNA or RNA sequence analysis[25]. A students' t-test was performed to generate the P-value for different MCM10 expression between cancer specimens and paracarcinoma tissues. The cut-off of P-value and fold change were determined as follows: P-value: 0.05, fold change: 1.5, gene rank: 10%, data type: mRNA.

Kaplan-Meier plotter

Kaplan-Meier plotter (https://kmplot.com/analysis/) is a public database that contains expression data of 54,000 genes and survival information based on 18,674 samples of 21 diverse human tumors including 371 liver, 1440 gastric, 6234 breast, 2190 ovarian, and 3452 lung cancer samples[26]. In our study, the prognostic value of MCM10 was evaluated by overall survival (OS), relapse-free survival (RFS), progression-free survival (PFS), distant metastasis-free survival (DMFS), disease-specific survival (DSS), post-progression survival (PPS), and first progression (FP) in HCC, breast, ovarian, gastric, lung, esophageal, renal papillary cell carcinoma, pancreatic, pheochromocytoma and paraganglioma, sarcoma, thymoma, thyroid and endometrial cancers. The probe set used for MCM10, which was identified by Affymetrix ID, was 223570_at. All patients were split into low and high expression groups based on the median mRNA level of MCM10 and corroborated by K-M survival curves. Accordingly, the hazard ratios (HRs), 95% confident intervals (CIs) and log-rank P-values were shown. Statistical significance was inferred at a P-value < 0.05.

TCGA data and cBioPortal

The Cancer Genome Atlas (TCGA) provides a huge amount of sequencing and pathological data spanning more than 30 types of cancer diseases[27]. In this study, clinicopathological parameters and mRNA sequencing of MCM10 in TCGA liver cancer data were downloaded from the cBioPortal website (https://www.cbioportal.org)[28]. After excluding patients with missing follow-up data, 364 HCC cases subjected to MCM10 expression were included in the final analysis and their clinicopathological characteristics were summarized in Supplementary Table 2. The effects of clinicopathological characteristics and mRNA expression of MCM10 on survival of HCC patients were analyzed by Cox proportional hazards model. The variables which exhibited a correlation with $P \leq 0.1$ in univariate Cox analysis were further examined in multivariate regression. Statistical analysis was conducted using SPSS software version 20.0 (Chicago, IL, USA). $P < 0.05$ was identified as statistically significant.

Immune infiltrates analysis in TIMER database

TIMER (https://cistrome.shinyapps.io/timer/) is an open-access web portal designed for systematical analysis of tumor-infiltrating immune cells across 32 kinds of human cancer diseases[29]. In TIMER, the abundance of immune infiltrates was determined according to the statistical analysis of gene expression profiles. In this study, we used TIMER to explore MCM10 expression in diverse cancers and the correlations of MCM10 gene expression with the abundance of different immune infiltrating cells including B cells, CD4$^+$ T cells, CD8$^+$ T cells, macrophages, neutrophils and dendritic cells (DCs). Meanwhile, the associations of MCM10 expression with specific marker genes of various immune cells were also analyzed. The related marker genes applied to the analysis of immune infiltrating cells including DCs, neutrophils, monocytes, tumor-associated macrophages (TAMs), natural killer (NK) cells, B cells, T cells, cytotoxic T lymphocytes (CTLs), T-helper 1 (Th1) cells, Th2 cells, follicular helper T (Tfh) cells, Th17 cells, regulatory T cells (Tregs), and exhausted T cells were according to the information from previous studies[30,31].

GEPIA dataset

GEPIA (http://gepia.cancer-pku.cn/) is a web-based server for analyzing the RNA sequencing expression using 9736 tumors from the TCGA and GTEx projects, which offers access to the analysis of the effect of MCM10 expression on survival in 33 cancer types[32]. And the correlations of MCM10 gene expression with related marker genes of immune cells in TIMER database were further confirmed in GEPIA using Spearman's correlation analysis. Statistical significance was inferred at a P-value < 0.05.

Results

Expression level of MCM10 in patients with HCC and other cancers

A comparative gene expression analysis investigating the mRNA expression of MCM10 was performed on 20 kinds of cancers and match normal samples using the ONCOMINE database (Fig. 1A and Supplementary Table 1). Noticeably, MCM10 exhibited a strikingly up-regulated expression pattern in most types of cancers, including liver, bladder, breast, cervical, colorectal, esophageal, gastric, head and neck, kidney, lung, lymphoma, melanoma, ovarian, pancreatic, as well as sarcoma cancer tissues. But down-regulation of MCM10 was observed in leukemia and brain cancer.

Next, using the TIMER web portal, the mRNA expression level of MCM10 between diverse types of cancer diseases and the corresponding normal tissues was further detected in TCGA database. As shown in Fig. 1B, in comparison with match nontumoral tissues, MCM10 gene was remarkably over-expressed in liver hepatocellular carcinoma (LIHC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck cancer (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) tissues, with no down-regulated expression pattern in the TIMER database. In short, these results found that the mRNA expression of MCM10 was commonly up-regulated in cancer tissues, implying a potential role of MCM10 in tumor formation and progression.

Prognostic value of MCM10 expression in different types of cancers

The analysis of the prognostic efficacy of MCM10 expression in different types of human tumors was conducted by the Kaplan-Meier Plotter database. As shown in Fig. 2, MCM10 mRNA expression was of prognostic significance in most of the cancer types. Overexpressed mRNA level of MCM10 was markedly related to poorer prognosis in HCC (OS: HR = 1.78, P = 0.0011; PFS: HR = 1.91, P = 1.4e-05; RFS: HR = 1.87, P = 0.00018; DSS: HR = 2.29, P = 0.00028; Fig. 2A-D), breast cancer (OS: HR = 1.67, P = 0.0013; RFS: HR = 1.46, P = 1.5e-06; DMFS: HR = 1.55, P = 0.0085; Fig. 2E, F, H), lung cancer (OS: HR = 1.8, P = 2.8e-12; FP: HR = 1.66, P = 0.00026; Fig. 2L, N), ovarian cancer (OS: HR = 1.29, P = 0.014; PFS: HR = 1.26, P = 0.016; Fig. 2O-P), kidney renal papillary cell carcinoma (OS: HR = 2.46, P = 0.0038; RFS: HR = 4.68, P = 0.00032; Fig. 2T-U), pancreatic ductal adenocarcinoma (OS: HR = 1.59, P = 0.026; RFS: HR = 3.05, P = 0.01; Fig. 2V-W), pheochromocytoma and paraganglioma (OS: P = 0.024; Fig. 2X), sarcoma (OS: HR = 1.69, P = 0.01; RFS: HR = 1.84, P = 0.014; Fig. 2Z-AA), thyroid carcinoma (RFS: HR = 4.73, P = 0.00057; Fig. 2AD), and uterine corpus endometrial carcinoma (OS: HR = 1.61, P = 0.025; Fig. 2AE). Conversely, higher mRNA expression of MCM10 was remarkably associated with favorable prognosis in esophageal squamous cell carcinoma (OS: HR = 0.44, P = 0.049; Fig. 2R) and thymoma (OS: HR = 0.08, P = 0.0024; Fig. 2AB). However, no significant correlation was found between MCM10 expression and PPS in breast cancer (Fig. 2G), OS, PPS and FP in gastric cancer (Fig. 2I-K), PPS in lung cancer (Fig. 2M), PPS in ovarian cancer (Fig. 2Q), RFS in esophageal squamous cell carcinoma (Fig. 2S), RFS in pheochromocytoma and paraganglioma (Fig. 2Y), OS in thyroid carcinoma (Fig. 2AC), and RFS in uterine corpus endometrial carcinoma (Fig. 2AF).

Further, we also used the GEPIA database to determine the prognostic effects of MCM10 expression on cancer patients. A higher level of MCM10 expression predicted shorter OS in KICH, LUAD, mesothelioma (MESO), pheochromocytoma and paraganglioma (PCPG) and skin cutaneous melanoma (SKCM), shorter DFS in PRAD, THCA and uveal melanoma (UVM), shorter OS and DFS in HCC, adrenocortical carcinoma (ACC), KIRP, brain lower grade glioma (LGG), pancreatic adenocarcinoma (PAAD) and sarcoma (SARC). Consistently, higher mRNA expression of MCM10 was markedly related to favorable OS of thymoma (THYM) patients

(Supplementary Fig. 1). Overall, these results indicated that MCM10 expression may exert predictive function of the prognosis in several cancers even though their associations vary according to the cancer type.

Association of MCM10 expression with clinicopathological parameters of HCC patients

Using the Kaplan-Meier plotter, the association of MCM10 expression with different clinical factors of HCC patients was investigated (Table 1). The results found that overexpressed mRNA level of MCM10 was associated with both unfavorable OS and PFS in males (OS: HR = 1.95, P = 0.0034; PFS: HR = 1.9, P = 0.00042), Asians (OS: HR = 4.15, P = 1.2e-05; PFS: HR = 2.6, P = 7.6e-05), non-alcoholics (OS: HR = 1.71, P = 0.023; PFS: HR = 1.97, P = 0.00091), and patients without hepatitis virus (OS: HR = 2.43, P = 0.00015; PFS: HR = 3.92, P = 1.4e-09). Moreover, HCC patients in grade 2 (OS: HR = 1.93, P = 0.013; PFS: HR = 2.58, P = 1.8e-05) or stage 1 + 2 (OS: HR = 1.65, P = 0.043; PFS: HR = 1.65, P = 0.0092) with higher MCM10 expression had distinctively shorter OS and PFS. Nevertheless, no remarkable correlation was observed between MCM10 expression and OS and PFS in patients with hepatitis virus, patients in stage 2, patients in T2 stage, and patients with vascular invasion. In short, these results indicated that the prognostic value of MCM10 expression was correlated with clinicopathological characteristics of HCC patients.

Table 1
Correlation of MCM10 mRNA expression and prognosis in hepatocellular carcinoma with different
clinicopathological factors by Kaplan-Meier plotter.

Clinicopathological factors	OS			PFS		
	N	HR	P-value	N	HR	P-value
Gender						
Male	246	1.95 (1.24−3.06)	**0.0034**	246	1.9 (1.32−2.74)	**0.00042**
Female	118	1.51 (0.86−2.64)	0.15	120	1.73 (1.03−2.9)	**0.035**
Race						
White	181	1.2 (0.76−1.9)	0.42	183	1.97 (1.32−2.93)	**0.00069**
Asian	155	4.15 (2.08−8.25)	**1.2e−05**	155	2.6 (1.59−4.24)	**7.6e−05**
Alcohol consumption						
Yes	115	1.25 (0.66−2.36)	0.49	115	2.44 (1.42−4.21)	**0.00091**
None	202	1.71 (1.07−2.74)	**0.023**	204	1.97 (1.31−2.97)	**0.00091**
Hepatitis virus						
Yes	150	1.18 (0.62−2.25)	0.61	152	1.21 (0.76−1.91)	0.42
None	167	2.43 (1.51−3.89)	**0.00015**	167	3.92 (2.44−6.29)	**1.4e−09**
Stage						
1	170	1.3 (0.71−2.39)	0.4	170	1.72 (1.04−2.85)	**0.031**
2	83	1.95 (0.87−4.35)	0.098	84	1.49 (0.83−2.69)	0.18
1 + 2	253	1.65 (1.01−2.68)	**0.043**	254	1.65 (1.13−2.41)	**0.0092**
3	83	2.18 (1.19−4.02)	**0.01**	83	1.2 (0.7−2.06)	0.51
4	5	-	-	5	-	-
3 + 4	87	2.22 (1.23−4)	**0.0068**	88	1.18 (0.7−2)	0.53
Grade						
1	55	3.56 (1.27−9.95)	**0.011**	55	1.67 (0.75−3.7)	0.2
2	174	1.93 (1.14−3.25)	**0.013**	175	2.58 (1.65−4.04)	**1.8e−05**
3	118	2.5 (1.32−4.73)	**0.0037**	119	1.63 (0.99−2.68)	0.055
4	12	-	-	12	-	-
AJCC_T						
1	180	1.45 (0.81−2.59)	0.21	180	1.66 (1.02−2.7)	**0.038**
2	90	2 (0.94−4.25)	0.065	92	1.55 (0.9−2.69)	0.11
3	78	2.08 (1.12−3.87)	**0.018**	78	1.19 (0.68−2.1)	0.54
4	13	-	-	13	-	-
Vascular invasion						
None	203	1.32 (0.79−2.2)	0.29	204	1.59 (1.02−2.48)	**0.041**

Clinicopathological factors	OS			PFS		
	N	HR	P-value	N	HR	P-value
Micro	90	1.43 (0.67−3.06)	0.36	91	1.51 (0.85−2.67)	0.15
Macro	16	-	-	16	-	-
Bold values indicate *P* < 0.05.						
OS: overall survival; PFS: progression-free survival; N: number; HR: hazard ratio.						

Independent Prognostic Value Of Mcm10 Expression In Hcc Patients

In the current study we performed further analysis to assess the independent prognostic significance of MCM10 expression for OS and DFS in HCC patients. The clinicopathological parameters (Supplementary Table 2) and MCM10 mRNA sequencing of 364 patients in TCGA liver cancer data were downloaded from the cBioPortal. The variables which exhibited an association with $P \leq 0.1$ in univariate analysis were further analyzed in multivariate Cox regression. Our results showed that in univariate analysis for OS, vascular invasion (HR = 1.384, 95% CI: 1.001−1.914, *P* = 0.050), a more advanced pathologic stage (HR = 1.660, 95% CI: 1.355−2.035, *P* < 0.001) as well as a higher level of MCM10 expression (HR = 2.152, 95% CI: 1.506−3.075, *P* < 0.001) had an inverse correlation with OS of HCC patients (Table 2). Subsequently, multivariate Cox regression demonstrated that high MCM10 expression (HR = 1.705, 95% CI: 1.111−2.616, *P* = 0.015) was independently related to unfavorable OS of HCC patients. Similarly, in univariate analysis for DFS, HCC patients with vascular invasion (HR = 1.687, 95% CI: 1.288−2.210, *P* < 0.001), high pathologic stage (HR = 1.731, 95% CI: 1.450−2.066, *P* < 0.001) as well as high MCM10 expression (HR = 2.266, 95% CI: 1.666−3.081, *P* < 0.001) had remarkably shorter DFS (Table 2). And multivariate analysis found that a higher level of MCM10 mRNA expression (HR = 2.042, 95% CI: 1.423−2.929, *P* < 0.001) was independently associated with shorter DFS of HCC patients. Taken together, the above results implied the independent prognostic value of MCM10 expression in HCC patients.

Variables	OS				DFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age (years)	1.012 (0.999–1.026)	0.076	1.018 (1.001–1.035)	0.038	0.998 (0.986–1.010)	0.735		
Gender	1.236 (0.866–1.766)	0.243			1.126 (0.817–1.553)	0.469		
Weight (kg)	0.993 (0.983–1.003)	0.175			0.999 (0.991–1.006)	0.705		
PLT (10^9/L)	1.000 (1.000–1.000)	0.735			1.000 (1.000–1.000)	0.759		
Creatinine (mg/dl)	1.002 (0.986–1.018)	0.794			1.002 (0.986–1.017)	0.830		
Albumin (g/L)	0.987 (0.945–1.032)	0.576			0.999 (0.995–1.003)	0.668		
TB (µmol/L)	0.975 (0.845–1.124)	0.723			1.047 (0.960–1.141)	0.299		
PT (s)	1.015 (0.978–1.055)	0.432			1.002 (0.970–1.034)	0.919		
AFP (ng/ml)	1.000 (1.000–1.000)	0.432			1.000 (1.000–1.000)	0.282		
Child-Pugh stage	1.523 (0.836–2.775)	0.170			1.250 (0.729–2.143)	0.418		
Adjacent hepatic tissue inflammation	1.158 (0.797–1.683)	0.441			1.170 (0.869–1.575)	0.302		
Liver fibrosis ishak score category	0.930 (0.800–1.080)	0.342			1.049 (0.937–1.174)	0.408		
Vascular invasion	1.384 (1.001–1.914)	**0.050**	1.070 (0.748–1.529)	0.712	1.687 (1.288–2.210)	**0.000**	1.269 (0.937–1.718)	0.123
Histologic grade	1.123 (0.888–1.422)	0.333			1.103 (0.904–1.347)	0.334		
Pathologic stage	1.660 (1.355–2.035)	**0.000**	1.475 (1.140–1.907)	**0.003**	1.731 (1.450–2.066)	**0.000**	1.558 (1.241–1.956)	**0.000**

Variables	OS				DFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
MCM10	2.152 (1.506– 3.075)	**0.000**	1.705 (1.111– 2.616)	**0.015**	2.266 (1.666– 3.081)	**0.000**	2.042 (1.423– 2.929)	**0.000**
Bold values indicate *P* < 0.05.								
OS: overall survival; DFS: disease-free survival; HR: hazard ratio.								

Correlation of MCM10 expression with immune infiltration levels in HCC

Previous studies have unveiled that tumor-infiltrating lymphocytes may have function of prediction of sentinel lymph node status and survival time of patients in cancers[33, 34]. Accordingly, we tried to explore the correlation of MCM10 gene expression with the abundance of immune infiltrates in HCC and other cancer types based on the TIMER and GEPIA, due to a majority of the homologous TCGA in theses two databases. Our results found that the expression level of MCM10 was markedly associated with tumor purity in 20 cancer types, and immune infiltrating levels of B cells in 16 cancer types, CD8$^+$ T cells in 21 cancer types, CD4$^+$ T cells in 16 cancer types, macrophages in 17 cancer types, neutrophils in 22 cancer types, and DCs in 19 cancer types, respectively (Fig. 3, Supplementary Fig. 2). In HCC, high mRNA expression of MCM10 was remarkably related to poor prognosis and high immune infiltration. The mRNA expression level of MCM10 had a positive relationship with the immune infiltrating levels of B cells (r = 0.473, *P* = 1.31e-20), CD8$^+$ T cells (r = 0.343, *P* = 7.33e-11), CD4$^+$ T cells (r = 0.282, *P* = 1.06e-07), macrophages (r = 0.444, *P* = 6.71e-18), neutrophils (r = 0.39, *P* = 5.91e-14), and DCs (r = 0.475, *P* = 1.40e-20), respectively (Fig. 3). Interestingly, in THYM, high MCM10 expression was related to favorable prognosis but positively associated with immune infiltrating levels of B cells (r = 0.837, *P* = 3.86e-31), CD8$^+$ T cells (r = 0.598, *P* = 2.22e-12), CD4$^+$ T cells (r = 0.591, *P* = 8.88e-12), macrophages (r = 0.586, *P* = 7.22e-12), as well as DCs (r = 0.741, *P* = 4.30e-21), respectively (Supplementary Fig. 2AI). Overall, these findings indicated that MCM10 gene expression could have an immunogenic impact on the tumor microenvironment despite variations between MCM10 expression, infiltrating levels of immune cells and prognosis in diverse types of cancers.

Correlation analysis between MCM10 expression and marker genes of various immune cells

Further analysis of the associations between MCM10 gene expression and specific marker genes of various immune cells including DCs, neutrophils, monocytes, TAMs, NK cells, B cells, T cells in HCC and CHOL was performed by the TIMER and GEPIA. Meanwhile, different subsets of T cells, namely CTLs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs, and exhausted T cells, were also analyzed. Previous studies found that tumor purity in clinical samples could have an effect on the analysis of immune infiltration by genomic methods[35], thus we conducted the analysis after adjusting the correlation for purity. As shown in Table 3, MCM10 gene expression in HCC had remarkable relationship with related marker genes of DCs, neutrophils, monocytes, TAMs, NK cells, B cells, and different subsets of T cells. However, in CHOL, such correlation was not significant.

Table 3
Correlation analysis between MCM10 and gene markers of immune cells in TIMER and GEPIA.

Description	Gene marker	TIMER				GEPIA			
		HCC		CHOL		HCC		CHOL	
		Cor	P	Cor	P	R	P	R	P
DC	CD11c (ITGAX)	0.481	2.43e-21	−0.092	0.599	0.36	1.3e-12	0.12	0.48
	NRP1	0.271	3.32e-07	0.233	0.178	0.24	2.2e-06	0.32	0.055
	CD1C	0.195	2.73e-04	−0.272	0.113	0.11	0.032	−0.19	0.27
	HLA-DPB1	0.295	2.22e-08	−0.292	0.089	0.2	0.00011	−0.13	0.44
	HLA-DQB1	0.255	1.64e-06	0.061	0.727	0.097	0.062	0.11	0.52
Neutrophils	CCR7	0.202	1.61e-04	−0.06	0.731	0.093	0.075	0.12	0.48
	CD11b (ITGAM)	0.422	2.69e-16	0.16	0.359	0.36	1.4e-12	0.3	0.075
	CD59	0.072	0.184	−0.072	0.683	0.045	0.39	0.077	0.66
	CD66b (CEACAM8)	0.136	1.17e-02	0.168	0.334	0.13	0.011	0.17	0.33
Monocyte	CD115 (CSF1R)	0.311	3.56e-09	0.017	0.922	0.21	5.7e-05	0.14	0.42
	CD86	0.475	8.27e-21	−0.138	0.428	0.34	3.5e-11	0.069	0.69
TAM	CCL2	0.175	1.10e-03	0.146	0.402	0.085	0.1	0.19	0.26
	IL10	0.375	5.68e-13	0.156	0.372	0.2	7.8e-05	0.36	0.033
	CD32 (FCGR2A)	0.48	2.70e-21	0.043	0.807	0.43	4.9e-18	0.17	0.31
	CD68	0.335	1.62e-10	0.016	0.927	0.26	6.6e-07	0.14	0.42
	CD163	0.228	1.95e-05	0.036	0.837	0.078	0.14	0.051	0.77
	VSIG4	0.221	3.35e-05	0.002	0.990	0.13	0.011	0.11	0.53
NK cell	CD56 (NCAM1)	0.266	5.35e-07	−0.151	0.386	0.21	4.5e-05	−0.064	0.71
	CD16 (FCGR3A)	0.428	8.12e-17	0.176	0.313	0.35	3.5e-12	0.29	0.085
	KIR2DL1	−0.038	0.486	−0.295	0.086	2.3e-05	1	−0.11	0.51
	KIR2DL3	0.202	1.61e-04	−0.06	0.732	0.16	0.0022	−0.0083	0.96

Description	Gene marker	TIMER				GEPIA			
		HCC		CHOL		HCC		CHOL	
		Cor	P	Cor	P	R	P	R	P
	KIR2DL4	0.234	1.09e-05	−0.228	0.188	0.26	4.3e−07	−0.2	0.25
	KIR3DL1	0.004	0.947	−0.108	0.536	−0.034	0.52	−0.087	0.62
	KIR3DL2	0.125	2.06e-02	−0.169	0.332	0.13	0.012	−0.048	0.78
	KIR3DL3	0.022	0.690	−0.097	0.580	0.13	0.014	0.037	0.83
B cell	CD19	0.32	1.21e-09	−0.016	0.929	0.26	4e−07	0.14	0.41
	CD20 (MS4A1)	0.177	9.66e-04	−0.044	0.803	0.07	0.18	0.038	0.83
	CD79A	0.269	3.79e-07	−0.022	0.902	0.17	0.0014	−0.038	0.83
T cell (general)	CD3D	0.381	2.15e-13	−0.221	0.201	0.24	2.7e−06	−0.007	0.97
	CD3E	0.327	4.57e-10	−0.096	0.585	0.18	0.00039	0.092	0.59
CTL	CD8A	0.31	4.02e-09	−0.061	0.728	0.21	4.9e−05	0.045	0.79
	CD8B	0.294	2.50e-08	−0.095	0.586	0.2	0.00013	−0.042	0.81
	EOMES	0.266	5.23e-07	−0.039	0.825	0.16	0.0015	0.093	0.59
Th1	STAT4	0.308	5.04e-09	−0.004	0.982	0.26	3.1e−07	0.1	0.56
	TBX21	0.168	1.76e-03	−0.099	0.572	0.081	0.12	0.099	0.57
	STAT1	0.435	2.38e-17	0.464	5.04e-03	0.4	6.3e−16	0.53	0.00089
	CXCR3	0.385	1.28e-13	−0.099	0.571	0.26	2.5e−07	0.083	0.63
Th2	GATA3	0.338	1.16e-10	−0.233	0.178	0.24	3.5e−06	−0.088	0.61
	CCR4	0.309	4.42e-09	0.069	0.695	0.25	6.9e−07	0.076	0.66
	CXCR4	0.438	1.28e-17	0.009	0.960	0.32	1.7e−10	0.14	0.41
Tfh	IL21	0.201	1.70e-04	−0.098	0.577	0.18	0.00046	−0.078	0.65
	BCL6	0.17	1.57e-03	0.11	0.529	0.19	0.00024	0.21	0.23
Th17	IL17A	0.077	0.152	−0.074	0.671	0.061	0.25	0.054	0.76

Description	Gene marker	TIMER				GEPIA			
		HCC		CHOL		HCC		CHOL	
		Cor	P	Cor	P	R	P	R	P
	RORC	−0.254	**1.73e-06**	0.198	0.253	−0.15	**0.0041**	0.082	0.64
	STAT3	0.199	**1.99e-04**	0.234	0.175	0.21	**7e-05**	0.23	0.18
Treg	FOXP3	0.288	**5.19e-08**	−0.1	0.566	0.19	**3e-04**	−0.015	0.93
	STAT5B	0.29	**4.34e-08**	0.186	0.284	0.32	**1.6e-10**	0.28	0.1
	TGFB1	0.373	**7.26e-13**	0.294	0.087	0.26	**2.6e-07**	0.33	0.052
T cell exhaustion	PD−1	0.391	**4.67e-14**	0.294	0.086	0.3	**3e-09**	0.39	**0.019**
	TIM−3 (HAVCR2)	0.486	**8.02e-22**	−0.124	0.479	0.34	**2.5e-11**	0.055	0.75
	LAG3	0.36	**5.10e-12**	0.122	0.486	0.25	**8.9e-07**	0.2	0.24
	CTLA4	0.46	**1.85e-19**	−0.069	0.692	0.35	**2.1e-12**	0.062	0.72

HCC: hepatocellular carcinoma; CHOL: cholangiocarcinoma; DC: dendritic cells; TAM: tumor-associated macrophage; NK cell: natural killer cell; CTL: cytotoxic T lymphocyte; Th: T helper cell; Tfh: follicular helper T cell; Treg, regulatory T cell; Cor, R, P value of Spearman's correlation.

Bold values indicate P < 0.05.

Specially, our results demonstrated that DC markers such as CD11c (r = 0.481, P = 2.43e-21), NRP1 (r = 0.271, P = 3.32e-07), CD1C (r = 0.195, P = 2.73e-04), HLA-DPB1 (r = 0.295, P = 2.22e-08), HLA-DQB1 (r = 0.255, P = 1.64e-06), neutrophil markers such as CCR7 (r = 0.202, P = 1.61e-04), CD11b (r = 0.422, P = 2.69e-16) and CD66b (r = 0.136, P = 1.17e-02), monocyte markers such as CD115 (r = 0.311, P = 3.56e-09) and CD86 (r = 0.475, P = 8.27e-21), TAM markers such as CCL2 (r = 0.175, P = 1.10e-03), IL10 (r = 0.375, P = 5.68e-13), CD32 (r = 0.48, P = 2.70e-21), CD68 (r = 0.335, P = 1.62e-10), CD163 (r = 0.228, P = 1.95e-05) and VSIG4 (r = 0.221, P = 3.35e-05), NK cell markers such as CD56 (r = 0.266, P = 5.35e-07), CD16 (r = 0.428, P = 8.12e-17), KIR2DL3 (r = 0.202, P = 1.61e-04), KIR2DL4 (r = 0.234, P = 1.09e-05) and KIR3DL2 (r = 0.125, P = 2.06e-02), and B cell markers such as CD19 (r = 0.32, P = 1.21e-09), CD20 (r = 0.177, P = 9.66e-04) and CD79A (r = 0.269, P = 3.97e-07) showed significant correlation with MCM10 expression in HCC (Fig. 4). Furthermore, related marker genes of different subsets of T cells, including CTL markers, CD8A (r = 0.31, P = 4.02e-09), CD8B (r = 0.294, P = 2.50e-08) and EOMES (r = 0.266, P = 5.23e-07), Th1 markers, STAT4 (r = 0.308, P = 5.04e-09), TBX21 (r = 0.168, P = 1.76e-03), STAT1 (r = 0.435, P = 2.38e-17) and CXCR3 (r = 0.385, P = 1.28e-13), Th2 markers, GATA3 (r = 0.338, P = 1.16e-10), CCR4 (r = 0.309, P = 4.42e-09) and CXCR4 (r = 438, P = 1.28e-17), Tfh markers, IL21 (r = 0.201, P = 1.70e-04) and BCL6 (r = 0.17, P = 1.57e-03), Th17 markers, RORC (r=-0.254, P = 1.73e-06) and STAT3 (r = 0.199, P = 1.99e-04), Treg markers, FOXP3 (r = 0.288, P = 5.19e-08), STAT5B (r = 0.29, P = 4.34e-08) and TGFB1 (r = 0.373, P = 7.26e-13), and exhausted T cell markers, PD-1 (r = 0.391, P = 4.67e-14), TIM-3 (r = 0.486, P = 8.02e-22), LAG3 (r = 0.36, P = 5.10e-12) and CTLA4 (r = 0.46, P = 1.85e-19) were also associated with MCM10 expression. In short, these findings further corroborated the important role of MCM10 expression in HCC immune microenvironment.

Discussion

HCC is one of the highly aggressive malignant types of cancers globally[1]. Even though serum AFP is the most extensively applied indicator for HCC screening and diagnosis in clinical practice, on account of its poor sensitivity and specificity, its efficacy has been questioned, especially in early stage of HCC[6, 36, 37]. Therefore, novel molecular biomarkers for HCC progression and prognosis in the era of precision medicine are urgently required. In the present study, we concentrated on MCM10 gene which was not detailedly investigated in HCC, and found that MCM10 mRNA expression was commonly overexpressed in cancer specimens compared to paracarcinoma tissues. A higher expression level of MCM10 was associated with adverse clinical outcomes in certain cancers. Of note, increased MCM10 expression was observed to be related to shorter OS and PFS in early stage of HCC, and it served as an independent prognostic indicator for unfavorable OS and DFS of HCC patients. Furthermore, we for the first time reported that MCM10 expression was associated with diverse immune infiltrating levels in HCC immune microenvironment. Our findings highlighted the potential prognostic role of MCM10 gene expression in HCC.

DNA synthesis and replication, the vital event during cell proliferation, is a highly regulated process that ensures faithful replication of the eukaryotic genome and subsequent cell division only once per cell cycle. Dysregulation of DNA duplication could contribute to aberrant cell proliferation and account for tumorigenesis[38, 39]. During the initiation and elongation of genome duplication, the MCM complex functions as DNA helicases in melting origin DNA and unwinding replication forks[9, 40]. MCM10, a replication licensing factor, is bound to chromatin by interacting with the MCM2-7 helicase and the DNA polymerase alpha/primase complex[16−18]. Recent researches have unveiled that MCM10 plays a key functional role as a DNA binding scaffold for guarding against replication stress in replication, and is involved in maintaining genome integrity, indicating that its dysregulation may contribute to genomic instability, which can induce abnormal proliferation and cancer progression in turn[41, 42]. Over-expression of MCM10 had been found in a variety of malignancies, such as prostate[19], breast[20], pancreatic[21], lung[43] and cervical[44] cancers. In cancer cell lines, knockdown of MCM10 expression showed noticeably decreased tumor properties such as proliferation, migration and colony formation, but increased cell apoptosis[19, 20]. Several lines of evidence showed that MCM10 was of prognostic relevance for predicting survivals in malignant tumors[19−21]. Even although results from Liu et al. have reported MCM10 over-expression in HCC[23], the functional role and prognostic implication of MCM10 in HCC remains obscure.

In the current study, MCM10 expression level and its prognostic effects on survivals in HCC were elucidated using the publicly available databases. Results from our study demonstrated that MCM10 exhibited a significantly overexpressed pattern in most cancers. Nevertheless, MCM10 expression varied in different cancers, for which different data collection methods and potential pathogenetic mechanisms may account. Consistently, across different databases, MCM10 expression was remarkably up-regulated in HCC samples. Survival analyses in Kaplan-Meier Plotter revealed that higher MCM10 expression was related to adverse prognosis in several cancer types, including HCC, breast cancer, lung cancer, ovarian cancer, pancreatic ductal adenocarcinoma, kidney renal papillary cell carcinoma, pheochromocytoma and paraganglioma, sarcoma, thyroid carcinoma, as well as uterine corpus endometrial carcinoma. Additionally, survival analyses in GEPIA showed that high MCM10 expression predicted dismal outcomes in HCC, KICH, LUAD, MESO, PCPG, SKCM, PRAD, THCA, UVM, ACC, KIRP, LGG, PAAD and SARC. Notably, in HCC, increased MCM10 expression had a close relationship with shorter OS and PFS in males, Asians, non-alcoholics, patients without hepatitis virus, and patients in early stage (stage 1 + 2 or grade 2). Moreover, MCM10 was found to be an independent prognostic indicator for unfavorable OS and DFS of HCC patients by multivariate analysis. These results strongly suggested a use of MCM10 gene as an informative tumor biomarker in HCC.

To our best knowledge, no research has extensively explored whether MCM10 could elicit immune responses in patients with HCC or any other type of cancer. We for the first time reported the associations of MCM10 expression with the status of different immune infiltrating cells. Our findings showed that in HCC, MCM10 expression had a positive relationship with the immune infiltrating levels of B cells, CD8$^+$ T cells, CD4$^+$ T cells, macrophages, neutrophils, and DCs. Accordingly, the correlations between MCM10 expression and specific marker genes of various immune cells (DCs, neutrophils, monocytes, TAMs, NK cells, B cells, and T cells) in HCC, which was analyzed using the TIMER and verified by the GEPIA, showed a statistical significance. Meanwhile, our study also demonstrated that MCM10 expression was correlated with related marker genes of different T cell subsets (CTLs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, Tregs, and exhausted T cells). Studies carried by Ariane et al. found that *MCM3*, a

member of the prereplicative factors, could elicit a cancer-restricted high-titer immunoglobulin G response in vitro in cases with astrocytic tumors[45]. Additionally, Celso et al. demonstrated that high expression levels of *MCM6* and *MK167* were both negatively related to immune response, and high-index anaplastic oligodendroglioma could down-regulate the immune response[46]. In this study, we revealed the significant correlations between MCM10 expression and the abundance of immune infiltrating cells according to the levels of specific marker genes for different types of immune cells in HCC, indicating the potential immunogenic role of MCM10 in HCC immune microenvironment.

There are some limitations that should be considered. Firstly, our findings of prognostic prediction for MCM10 expression in tumors was mainly retrieved from published literature in the ONCOMINE, Kaplan-Meier Plotter, cBioPortal, TIMER, as well as GEPIA databases. Therefore, the quality of the present data may affect our results. Examining our own patients will promote clinical application of our findings. Secondly, the sample sizes of certain tumors in the above databases were small. Further researches enrolling larger sample sizes are warranted for more reliable interpretation of data in this case. Finally, we did not explore the potential mechanism of MCM10 in HCC and its correlations with immune infiltrating cells in HCC microenvironment. Further experiments in vivo and vitro such as knockdown or over-expression of MCM10 on cell or animal models are needed to reveal the mechanism of MCM10 in HCC.

Conclusion

The current study conducted a systemic analysis on MCM10 referring to expression, prognostic significance and potential immunogenic role in HCC patients. We showed that MCM10 was commonly overexpressed in cancer specimens and was correlated with adverse prognosis in HCC and other certain cancers. MCM10 expression was also associated with the abundance of immune infiltrating cells according to the levels of specific marker genes for different types of immune cells in HCC. Thus, MCM10 could be exploited as a potential independent prognostic indicator as well as therapeutic target for HCC patients.

Abbreviations

AFP
alpha-fetoprotein; CI:confident interval; CTL:CD8 + cytotoxic T lymphocyte; DC:dendritic cell; DMFS:distant metastasis-free survival; DSS:disease-specific survival; FP:first progression; HCC:hepatocellular carcinoma; HR:hazard ratio; MCM10:minichromosome maintenance protein 10; NK cell:natural killer cell; OS:overall survival; PFS:progression-free survival; PPS:post-progression survival; RFS:relapse-free survival; TAM:tumor-associated macrophage; TCGA:the cancer genome atlas; Tfh cell:follicular helper T cell; Th cell:T-helper cell; Treg:regulatory T cell.

Declarations

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Author contributions

Sen-Lin Zhu contributed to the study design and critical revision of the manuscript. Yi-Ru Chen, Yi-Ting Li and Xi-Jie Chen carried out the study and drafted the manuscript. Yi-Ru Chen and Yan-Ling Chen analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials

TCGA cohort data was downloaded from cBioPortal (https://www.cbioportal.org).

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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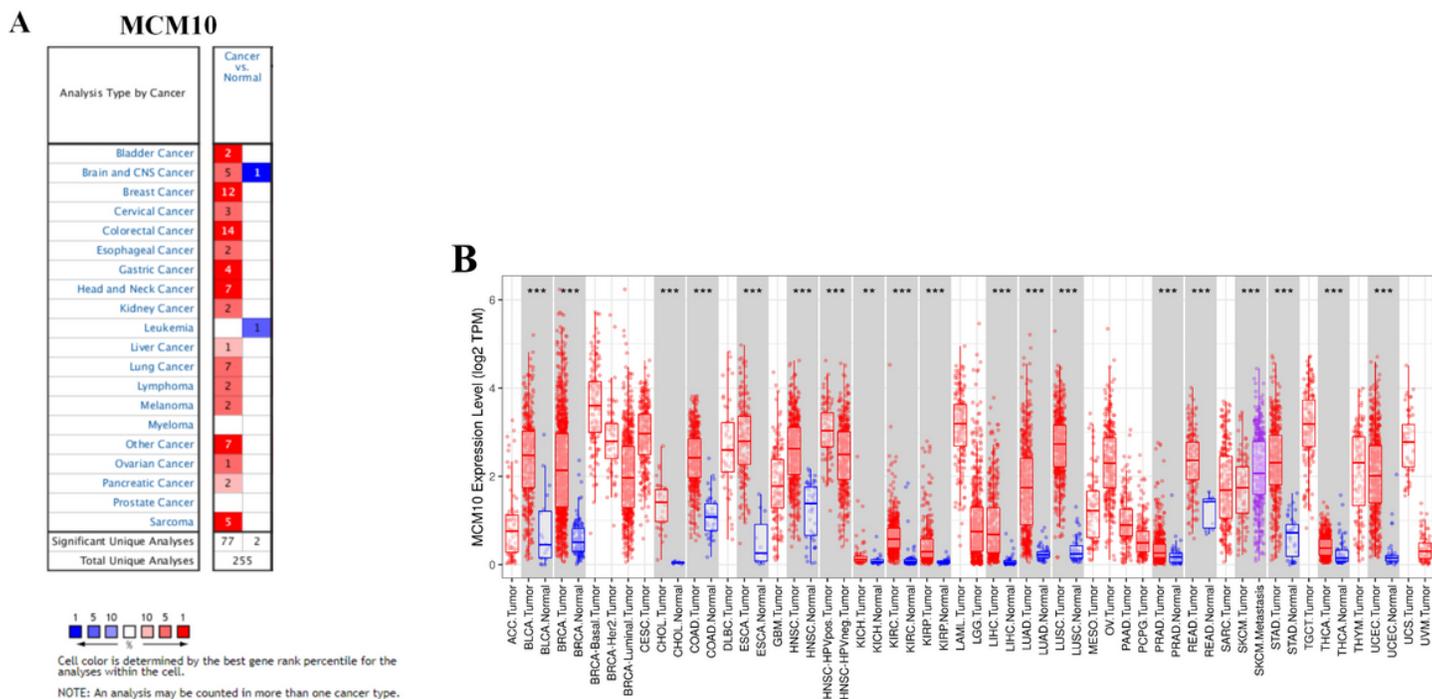
Figures

Figure 1

The transcriptional expression of MCM10 in different types of cancer diseases. (A) The transcription level of MCM10 in different human cancer tissues compared with match normal tissues in ONCOMINE. (B) The expression level of MCM10 in different tumor types in TIMER. *P<0.05, **P<0.01, ***P<0.001.
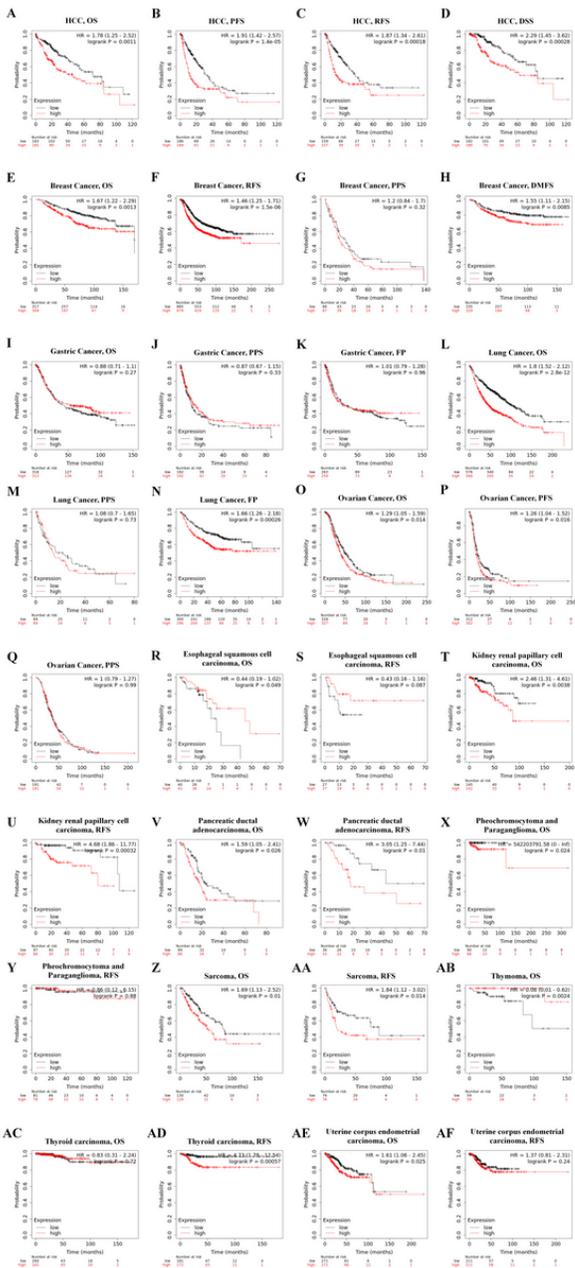
Figure 2

The prognostic value of MCM10 expression in different cancer types (Kaplan-Meier Plotter). (A-D) OS, PFS, RFS and DSS survival curves of HCC (n = 364, n = 370, n = 316, n = 362). High MCM10 expression was related to shorter OS, PFS, RFS, DSS in HCC patients. (E-H) OS, RFS, PPS and DMFS survival curves of breast cancer (n = 626, n = 1764, n = 173, n = 664). High MCM10 expression was related to shorter OS, RFS and DMFS in breast cancer patients. (I-K) OS, PPS and FP survival curves of gastric cancer (n = 631, n = 384, n = 522). (L-N) OS, PPS and FP survival curves of lung cancer (n = 1144, n = 138, n = 596). High MCM10 expression was related to shorter OS and FP in lung cancer patients. (O-Q) OS, PFS and PPS survival curves of ovarian cancer (n = 655, n = 614, n = 382). High MCM10 expression was related to shorter OS and PFS in ovarian patients. (R, S) OS and RFS survival curves of esophageal squamous cell carcinoma (n = 81, n = 54). High MCM10 expression was related to better OS in esophageal squamous cell carcinoma patients. (T, U) OS and RFS survival curves of kidney renal papillary cell carcinoma (n = 287, n = 183). High MCM10 expression was related to shorter OS and RFS in kidney renal papillary cell carcinoma patients. (V, W) OS and RFS survival curves of pancreatic ductal adenocarcinoma (n = 177, n = 69). High MCM10 expression was related to shorter OS and RFS in pancreatic ductal adenocarcinoma patients. (X, Y) OS and RFS survival curves of pheochromocytoma and paraganglioma (n = 178, n = 159). High MCM10 expression was related to shorter OS in pheochromocytoma and paraganglioma patients. (Z, AA) OS and RFS survival curves of sarcoma (n = 259, n = 152). High MCM10 expression was related to shorter OS

and RFS in sarcoma patients. (AB) OS survival curves of thymoma (n = 118). High MCM10 expression was related to better OS in thymoma patients. (AC, AD) OS and RFS survival curves of thyroid carcinoma (n = 502, n = 353). High MCM10 expression was related to shorter RFS in thyroid carcinoma patients. (AE, AF) OS and RFS survival curves of uterine corpus endometrial carcinoma (n = 542, n = 422). High MCM10 expression was related to shorter OS in uterine corpus endometrial carcinoma patients.
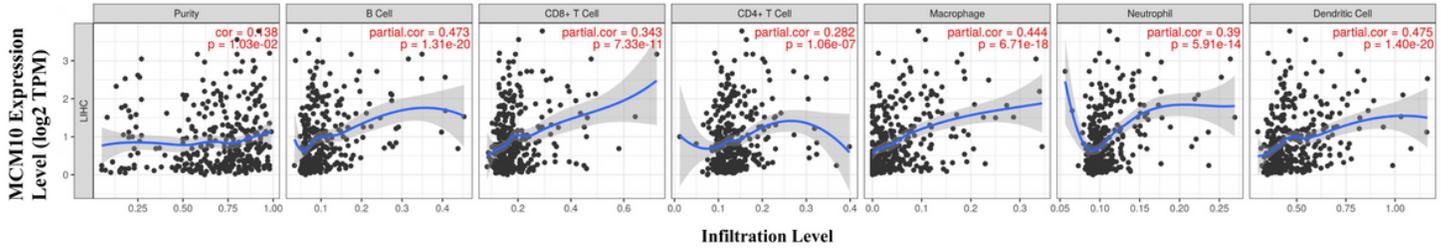

Figure 3

Correlation of MCM10 expression with immune infiltration levels in HCC (TIMER). MCM10 expression had significant positive relationship with immune infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells (n =371).
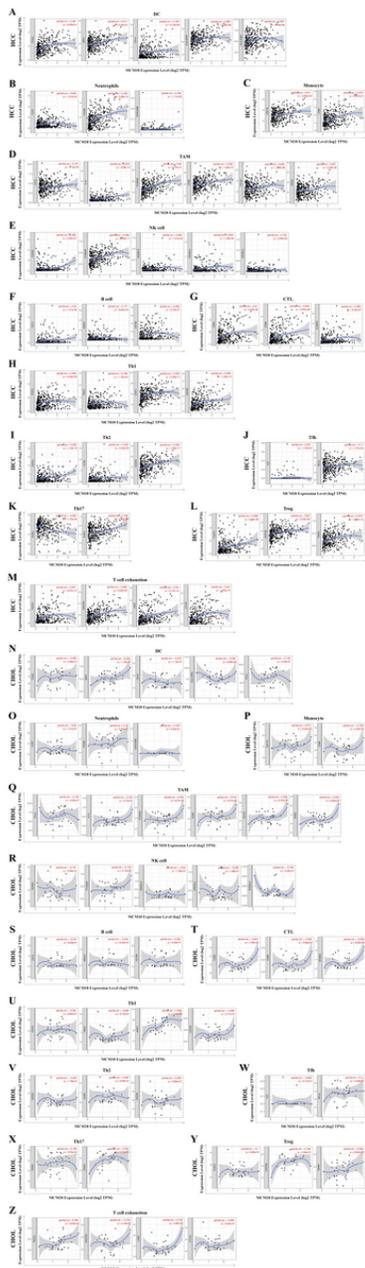
Figure 4

Correlation analysis of MCM10 expression with the expression of related marker genes of immune cells in HCC (A-M) and CHOL (N-Z) in TIMER. (A-M) The scatter plots displayed correlation between MCM10 expression and the gene markers of (A) DCs (CD11c, NRP1, CD1C, HLA-DPB1 and HLA-DQB1); (B) Neutrophils (CCR7, CD11b and CD66b); (C) Monocytes (CD115 and CD86); (D) TAMs (CCL2, IL10, CD32, CD68, CD163 and VSIG4); (E) NK cells (CD56, CD16, KIR2DL3, KIR2DL4 and KIR3DL2); (F) B cells (CD19, CD20 and CD79A); (G) CTLs (CD8A, CD8B and EOMES); (H) Th1 cells (STAT4, TBX21, STAT1 and CXCR3); (I) Th2 cells (GATA3, CCR4 and CXCR4); (J) Tfh cells (IL21 and BCL6); (K) Th17 cells (RORC and STAT3); (L) Tregs (FOXP3, STAT5B and TGFB1); and (M) Exhausted T cells (PD-1, TIM-3, LAG3 and CTLA4) in HCC samples (n = 371). (N-Z) The scatter plots displayed correlation between MCM10 expression and the gene markers of (N) DCs (CD11c, NRP1, CD1C, HLA-DPB1 and HLA-DQB1); (O) Neutrophils (CCR7, CD11b and CD66b); (P) Monocytes (CD115 and CD86); (Q) TAMs (CCL2, IL10, CD32, CD68, CD163 and VSIG4); (R) NK cells (CD56, CD16, KIR2DL3, KIR2DL4 and KIR3DL2); (S) B cells (CD19, CD20 and CD79A); (T) CTLs (CD8A, CD8B and EOMES); (U) Th1 cells (STAT4, TBX21, STAT1 and CXCR3); (V) Th2 cells (GATA3, CCR4 and CXCR4); (W) Tfh cells (IL21 and BCL6); (X) Th17 cells (RORC and STAT3); (Y) Tregs (FOXP3, STAT5B and TGFB1); and (Z) Exhausted T cells (PD-1, TIM-3, LAG3 and CTLA4) in CHOL samples (n = 36).

Supplementary Files

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