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Original Article

Immune modulation and prognostic significance of MCM10 in pan-cancer: a comprehensive analysis

Mostafa A Abdel-Maksoud[1], Komal Iqbal[2], Sanobar Gull[3], Sanjay Kirshan Kumar[4], Maryam Mastoor[5], Saeedah Musaed Almutairi[1], Taghreed N Almanaa[1], Akram A Alfuraydi[1], Ayman Mubarak[1], Mohamed H Kotob[6], Muhammad Jamil[7], Mohamed Y Zaky[8]

[1]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [2]Basic Health Unit Hindwan, Department of Primary Secondary Health, Sahiwal, Sargodha 40210, Punjab, Pakistan; [3]Department of Biochemistry, Government College University Faisalabad, Faisalabad, Pakistan; [4]Bahria University Health Sciences, Karachi, Pakistan; [5]Department of Biochemistry, Amna Inayat Medical College, Lahore, Pakistan; [6]Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University of Vienna, Vienna, Austria; [7]PARC Arid Zone Research Center, Dera Ismail Khan 29050, Pakistan; [8]UPMC Hillman Cancer Center, Division of Hematology and Oncology, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA

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Abstract: Background: Oncogenic processes in cancer are often characterized by dysregulation of critical genes. Our study focused on the minichromosome maintenance 10 replication initiation factor (MCM10) gene's expression and its potential diagnostic and prognostic implications in pan-cancer. Method: Leveraging large-scale genomic datasets, and experimental validation we embarked on a comprehensive analysis to shed light on the diagnostic and prognostic role of MCM10. Results: Our findings underscore the wide-ranging up-regulation of MCM10 across 24 major cancer types, positioning it as a ubiquitous player in tumorigenesis. Significantly, MCM10 up-regulation was strongly associated with poorer overall survival in Kidney Renal Papillary Cell Carcinoma (KIRP), Liver Hepatocellular Carcinoma (LIHC), and Lung Adenocarcinoma (LUAD), emphasizing its potential as a valuable prognostic marker in these cancers. While genetic mutations often drive oncogenic processes, our mutational analysis revealed the relative stability of MCM10 in KIRP, LIHC, and LUAD. This suggests that epigenetic (hypomethylation) and non-mutational regulatory mechanisms predominantly govern MCM10 expression in these cancer types. Further analyses demonstrated positive correlations between MCM10 expression and immune cell infiltration, particularly CD8+ T cells and CD4+ T cells, offering insights into the gene's influence on the tumor immune microenvironment. Additionally, pathway enrichment analysis highlighted MCM10-associated genes' involvement in crucial signaling pathways, such as the cell cycle, DNA replication, and repair. Exploring the therapeutic potential, we examined important drugs capable of regulating MCM10 expression, opening doors to personalized treatment strategies. Conclusion: Our study elucidates the multifaceted roles of MCM10 in KIRP, LIHC, and LUAD. Its pervasive up-regulation, prognostic significance, epigenetic regulation, and influence on the immune microenvironment provide valuable insights into these cancers. This research contributes to the growing body of evidence surrounding MCM10 and invites further investigation, validation, and potential translational efforts to harness its clinical relevance.

Keywords: MCM10, cancer, diagnosis, prognosis

Introduction

The field of oncology has witnessed remarkable advancements in recent years, particularly in the identification of novel molecular biomarkers with diagnostic and prognostic significance [1-6]. One such promising candidate is the minichromosome maintenance 10 replication initiation factor (MCM10) gene, which has garnered increasing attention due to its potential implications in various cancer types [7-9]. The critical role of MCM10 in DNA replication, repair, and cell cycle control positions it as a prime candidate for exploring its diagnostic and prognostic role in cancer [10, 11].

Cancer remains a global health challenge, with early diagnosis and prognosis being pivotal fac-

tors in improving patient outcomes [12-17]. Therefore, understanding the molecular determinants that govern cancer development and progression is of paramount importance. In this context, our study embarks on a comprehensive investigation that combines in silico and molecular experiments to unravel the diagnostic and prognostic relevance of the MCM10 gene across a pan-cancer spectrum.

The present research is structured around a multifaceted approach. Through in silico analyses, we will leverage large-scale genomics datasets to explore the expression patterns and potential associations of MCM10 with various cancer types. Subsequently, we will complement these findings with rigorous molecular experiments, aiming to validate and extend our in silico discoveries. This integrated approach is envisioned to offer a holistic understanding of the MCM10 gene's involvement in cancer, shedding light on its potential as a diagnostic tool and prognostic indicator.

Our study carries the promise of not only contributing to the expanding knowledge of MCM10's roles in cancer but also of potentially paving the way for the development of innovative diagnostic and prognostic tools. By the close of this research, we aim to provide valuable insights into the clinical utility of the MCM10 gene, ultimately advancing personalized and effective cancer management.

Methodology

MCM10 expression profiling across various cancer types

UALCAN (https://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl) is a valuable bioinformatics platform that empowers researchers and clinicians in the field of oncology [18]. This resource provides accessible and user-friendly access to extensive cancer data, allowing for the exploration of gene expression, protein abundance, and clinical data from various cancer types. UALCAN offers a comprehensive and user-friendly interface, making it an indispensable tool for researchers seeking to investigate specific genes, assess their expression in different cancers, and analyze their potential clinical relevance. In the present study, UALCAN platform was used for the expression profiling of MCM10 gene expression across various cancer types.

Prognostic value of MCM10 across various cancer types

GEPIA2 (http://gepia.cancer-pku.cn/) is a powerful resource in the realm of cancer genomics, designed to facilitate gene expression analysis for researchers and scientists [19]. This web-based tool allows users to explore the diagnostic and prognostic potential of genes across numerous cancer types and normal tissues, aiding in the identification of potential biomarkers and therapeutic targets. With an intuitive interface and robust analytical features, GEPIA2 enables in-depth investigations into gene expression profiles and their correlations with patient survival outcomes. In the present study, GEPIA2 was utilized to explore the prognostic value of MCM10 gene expression across various cancer types.

Mutational profiling of MCM10

cBioPortal (https://www.cbioportal.org/) is an indispensable resource for cancer researchers, offering a user-friendly platform to explore genomic data from various cancer studies [20]. It provides a comprehensive view of genetic alterations in tumors, aiding in the identification of potential therapeutic targets and biomarkers. In the present study, cBioPortal database was used for the mutational profiling of MCM10 gene.

Promoter methylation profiling of MCM10

OncoDB (https://oncodb.org/index.html) is a valuable database for cancer genomics, offering a curated collection of oncogenes, tumor suppressor genes, and driver mutations [21]. Researchers can access comprehensive information on genetic alterations and their roles in cancer progression. This resource was used in the present study for the promoter methylation analysis of MCM10 genes.

Immune infiltration analysis

TIMER2 (http://timer.cistrome.org/) is an essential tool for understanding tumor immune interactions and their impact on cancer [22]. It provides comprehensive data on immune cell infiltration in various cancer types, enabling researchers to investigate the tumor microenvironment and its relevance to patient outcomes. In the present study, this database was used to identify correlation between MCM10 gene expression and immune cells.

Protein-protein interaction (PPI) network and pathway analysis of MCM10

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) and DAVID (Database for Annotation, Visualization, and Integrated Discovery) (https://string-db.org/) are indispensable resources in the realm of functional genomics [23]. STRING specializes in identifying protein-protein interactions, aiding researchers in understanding complex biological networks. DAVID, on the other hand, focuses on functional annotation of genes, offering insights into their roles in biological processes. In the present study, STRING was used for the construction of PPI, while DAVID was utilized for the pathway analysis.

Drug prediction analysis of MCM10

DrugBank (https://go.drugbank.com/) is a comprehensive pharmacological database, a vital resource for researchers and healthcare professionals [24]. It offers detailed information on drugs, their interactions, targets, and mechanisms of action. In the current study, this resource was used to explore MCM10-related drugs.

Collection of clinical samples and RNA extraction

Collection of LUAD and normal control tissue samples was carried out with the approval of the ethics committee of the concerned departments. A total of 15 pairs of LUAD tissues and their corresponding normal tissues were prospectively obtained from patients who visited Institute of Nuclear Medicine, Oncology, and Radiotherapy Hospital, and Ayub Medical Complex during the period from August 2022 to May 2023. Prior to their participation, all participants provided informed consent by the signing consent forms. All patients enrolled in the study were diagnosed with LUAD and had not received any therapy prior to their surgical procedures. RNA from LUAD and normal control tissue samples was extracted through TRIzol method [25].

Reverse transcription quantitative real-time PCR (RT-qPCR) validation

The specific protocols employed in this study were as follows: Firstly, the PrimeScript™ RT reagent kit (Takara, Japan) was used for reverse transcription of the extracted RNA from clinical LUAD and normal control samples to synthesize complementary DNA. Subsequently, RT-qPCR was conducted on an ABI ViiA 7 Real Time PCR System (Thermo Fisher, USA) using SuperReal SYBR Green Premix Plus (Tiangen Biotech, China) as the fluorescent dye. GAPDH was chosen as the internal reference in this study, and all experiments were performed independently in triplicate. The primer sequences for each hub gene are provided below. The 2-ΔΔCt method [26] was applied to assess the relative expression of each hub gene.

GAPDH-F 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R 5'-CTGTTGCTGTAGCCAAATTCG-3'; MCM10-F 5'-TCAAGGAACTGATGGACCTGCC-3', MCM10-R 5'-CTCCAACATCCGCTGCTTCTGT-3'.

Receiver operating curve (ROC) generation

Based on the RT-qPCR expression data, ROC curve of MCM10 expression was generated using the SRPLOT web source (https://bioinformatics.com.cn/srplot).

Statistics

The gene expression comparisons between cancer and normal sample groups were conducted using t-tests web resources. To evaluate the survival outcomes associated with gene expression levels in cancer patients, log-rank test was performed. Spearman test was employed for correlation analysis. A significance level of $P < 0.05$ was chosen as the cut-off criteria to determine statistically significant results. This commonly used threshold allowed us to distinguish between findings that were likely due to chance and those that were considered statistically significant.

Results

MCM10 expression profiling across various cancer types

In our research, we employed the pan-cancer analysis feature of the UALCAN platform to assess the expression of the MCM10 gene across a diverse spectrum of 24 cancer types. The results obtained from this comprehensive analysis were striking. In every single one of the examined cancer types, MCM10 exhibited a substantial (p-value < 0.05) up-regulation when compared to the corresponding normal controls (**Figure 1A**).

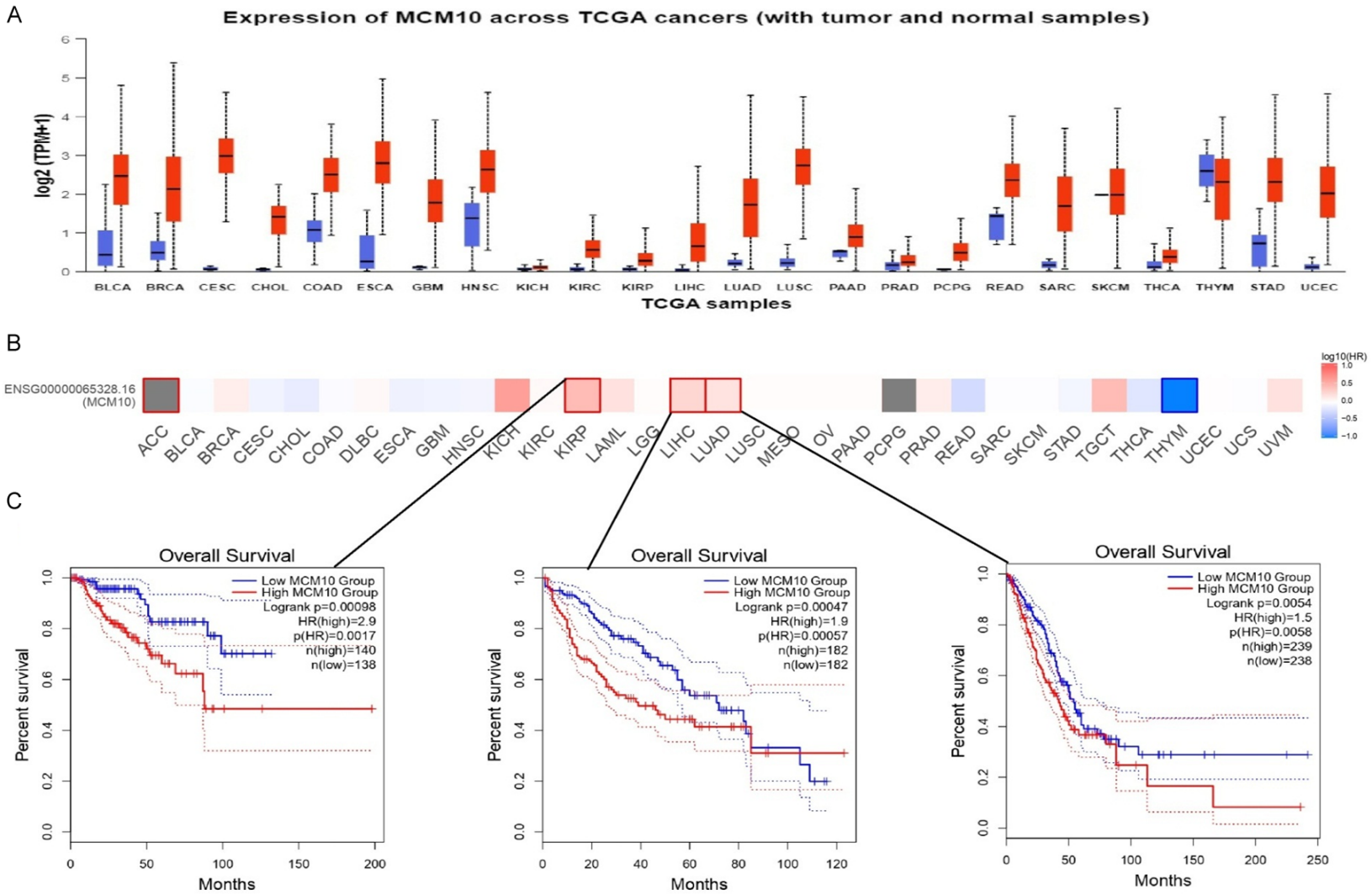

Figure 1. Comprehensive analysis of MCM10 gene in various cancers using UALCAN and GEPIA2 databases. (A) Examination of Pan-Cancer Outcomes of MCM10 Gene in UALCAN, (B) Visualization of MCM10 Gene's Survival Patterns Across a Spectrum of Cancers Utilizing GEPIA2, and (C) Survival Analysis Results Focused on MCM10 in KIRP, LIHC, and LUAD. A *p*-value < 0.05 was considered significant. Kidney Renal Papillary Cell Carcinoma = KIRP, Liver Hepatocellular Carcinoma = LIHC, Lung Adenocarcinoma = LUAD, Minichromosome maintenance 10 replication initiation factor = MCM10.

This uniform pattern of up-regulation across such a wide array of cancer types is of paramount significance. It suggests that the MCM10 gene may play a pivotal role in the molecular processes underpinning cancer development and progression. The consistent overexpression of MCM10 could potentially make it a valuable candidate for further exploration as a diagnostic marker or therapeutic target in multiple cancer contexts.

Prognostic value of MCM10 across various cancer types

By performing survival analysis of MCM10 among the diverse 24 cancer types mentioned in **Figure 1A**, it was observed that higher MCM10 expression levels were significantly (p-value < 0.05) correlated with poorer overall survival (OS) in three specific cancer patients: Kidney Renal Papillary Cell Carcinoma (KIRP), Liver Hepatocellular Carcinoma (LIHC), and Lung Adenocarcinoma (LUAD) (**Figure 1B**, **1C**). These results indicate that an elevated MCM10 expression may serve as a prognostic factor in these particular cancer types. These findings may ultimately contribute to more accurate patient risk stratification and the development of tailored treatment strategies KIRP, LIHC, and LUAD patients.

Expression analysis of MCM10 in different stages of KIRP, LIHC, and LUAD

We delved into the expression of the MCM10 gene across various stages of KIRP, LIHC, and LUAD. The results unveiled a notable pattern in the expression levels of MCM10 across the different cancer stages. Comparing the MCM10 expression in these cancers to that in the first stage, it was evident that in the second, third, and fourth stages of KIRP, LIHC, and LUAD, the expression of MCM10 was consistently higher (**Figure 2A**). This trend of increasing expression as cancer progresses through stages implies a potential role for MCM10 in cancer development and progression. These findings underline the possibility that MCM10 may contribute to the biological processes associated with cancer advancement.

Promoter methylation analysis of MCM10

In our quest to understand the underlying reasons for MCM10 dysregulation, we conducted an analysis of the promoter methylation status of the MCM10 gene in KIRP, LIHC, and LUAD samples. Our investigation was facilitated through the OncoDB database. The results of this analysis were intriguing and indicative of a potential mechanism contributing to MCM10 dysregulation. It was notably observed that the promoter region of the MCM10 gene exhibited significant (p-value < 0.05) hypomethylation in KIRP, LIHC, and LUAD samples when compared to controls (**Figure 2B**). Promoter hypomethylation is often associated with increased gene expression, which aligns with the up-regulation of MCM10 observed in our earlier analysis. These findings suggest that the reduced methylation of the MCM10 promoter may be a driving factor in the higher expression of MCM10 in these cancers.

Mutational profiling of MCM10

Next, we extended our investigation to explore the mutational landscape of the MCM10 gene in KIRP, LIHC, and LUAD using the cBioPortal database. The mutational analysis yielded intriguing results. First and foremost, it became evident that mutations in the MCM10 gene were relatively rare in the analyzed samples across these three cancer types. For example, MCM10 mutations were observed only in 0.7%, 2.5%, and 3% cases of the analyzed KIRP, LIHC, and LUAD cohorts, respectively (**Figure 3**). This observation suggests that, while MCM10 expression is associated with adverse survival outcomes in KIRP, LIHC, and LUAD, mutations in the gene itself are not a prevalent feature in the majority of cases. Furthermore, the type of mutations observed in MCM10 predominantly consisted of missense mutations. This particular mutation type involves changes in a single nucleotide, potentially resulting in alterations to the amino acid sequence of the encoded protein. The presence of missense mutations underscores the significance of examining the functional implications of these genetic changes in MCM10 and how they may contribute to the pathogenesis of these cancers.

Immune infiltration analysis

Immune cells play a pivotal role in orchestrating the anti-cancer immune response, serving as the foundation for contemporary cancer immunotherapies [27-29]. In this investigation,

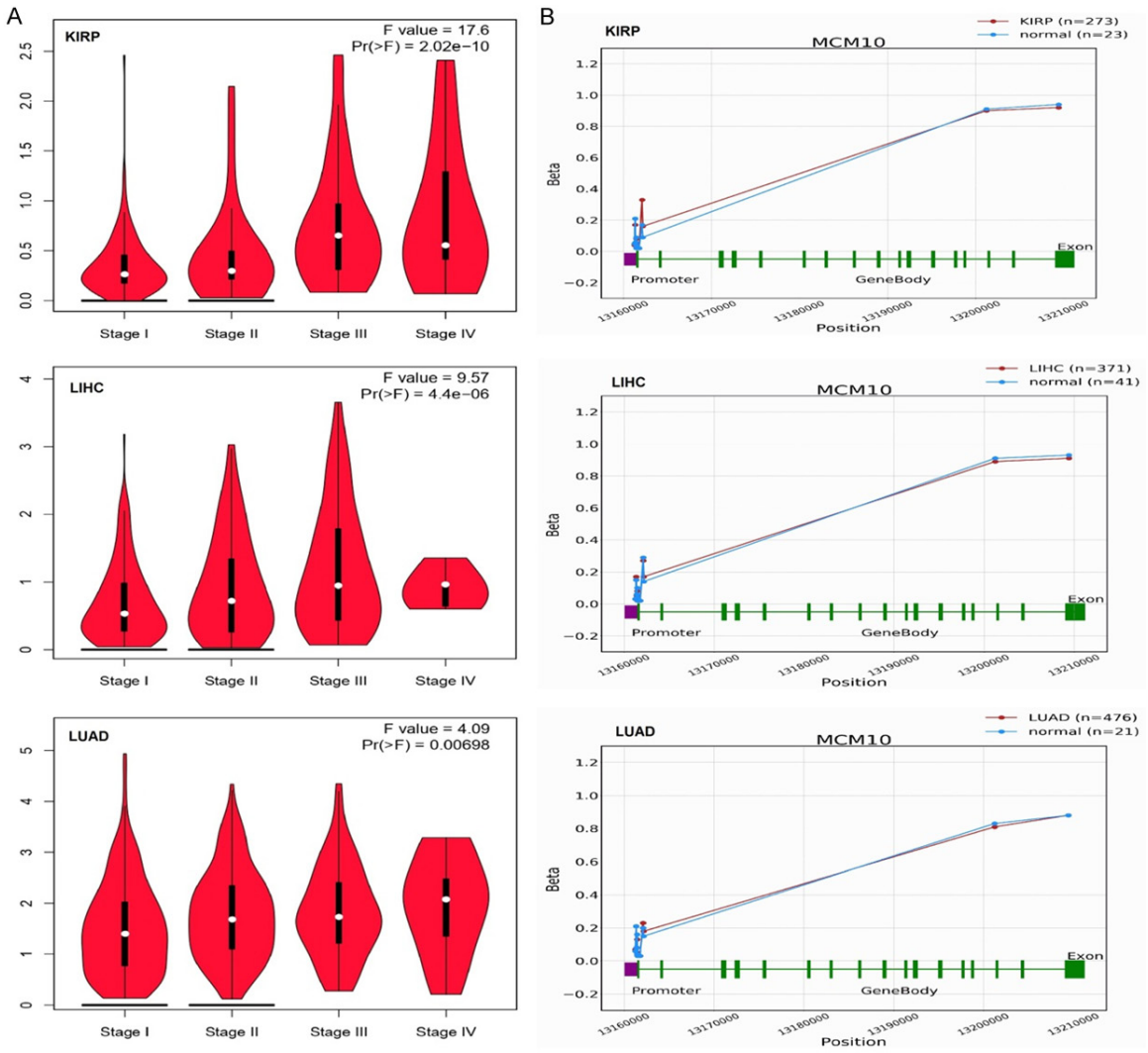

Figure 2. Evaluation of MCM10 expression at varied cancer stages and investigation of MCM10 gene promoter methylation. (A) Assessment of MCM10 Expression at Diverse Stages in KIRP, LIHC, and LUAD Using GEPIA2 and (B) Examination of MCM10 Promoter Methylation Status in KIRP, LIHC, and LUAD samples and corresponding controls via the OncoDB. A *p*-value < 0.05 was considered significant. Kidney Renal Papillary Cell Carcinoma = KIRP, Liver Hepatocellular Carcinoma = LIHC, Lung Adenocarcinoma = LUAD, Minichromosome maintenance 10 replication initiation factor = MCM10.

we leveraged TIMER2 to assess the Spearman correlations between immune cell infiltration, including CD8+ T cells and CD4+ T cells, and MCM10 expression in KIRP, LIHC, and LUAD. Our analysis unveiled positive correlations between MCM10 expression and the infiltration of CD8+ T cells and CD4+ T cells in these specific cancers, as depicted in **Figure 4**.

Protein-protein interaction (PPI) network and pathway analysis of MCM10

Subsequently, we utilized the STRING database to construct a PPI network centered around MCM10, aiming to identify its interacting genes. The network revealed a total of 10 genes closely associated with MCM10, as visually represented in **Figure 5A**. To gain deeper insights into the functional roles of these MCM10-associated genes, we subjected them to pathway enrichment analysis. Remarkably, the results of this analysis unveiled that genes associated with EAF2 were significantly implicated in four distinct pathways, encompassing processes such as 'Pre-replicative complex assembly in nuclear cell cycle DNA replication', 'Double strand break repair via break-induced replication', and 'Mitotic DNA replication', etc. (**Figure 5B**).

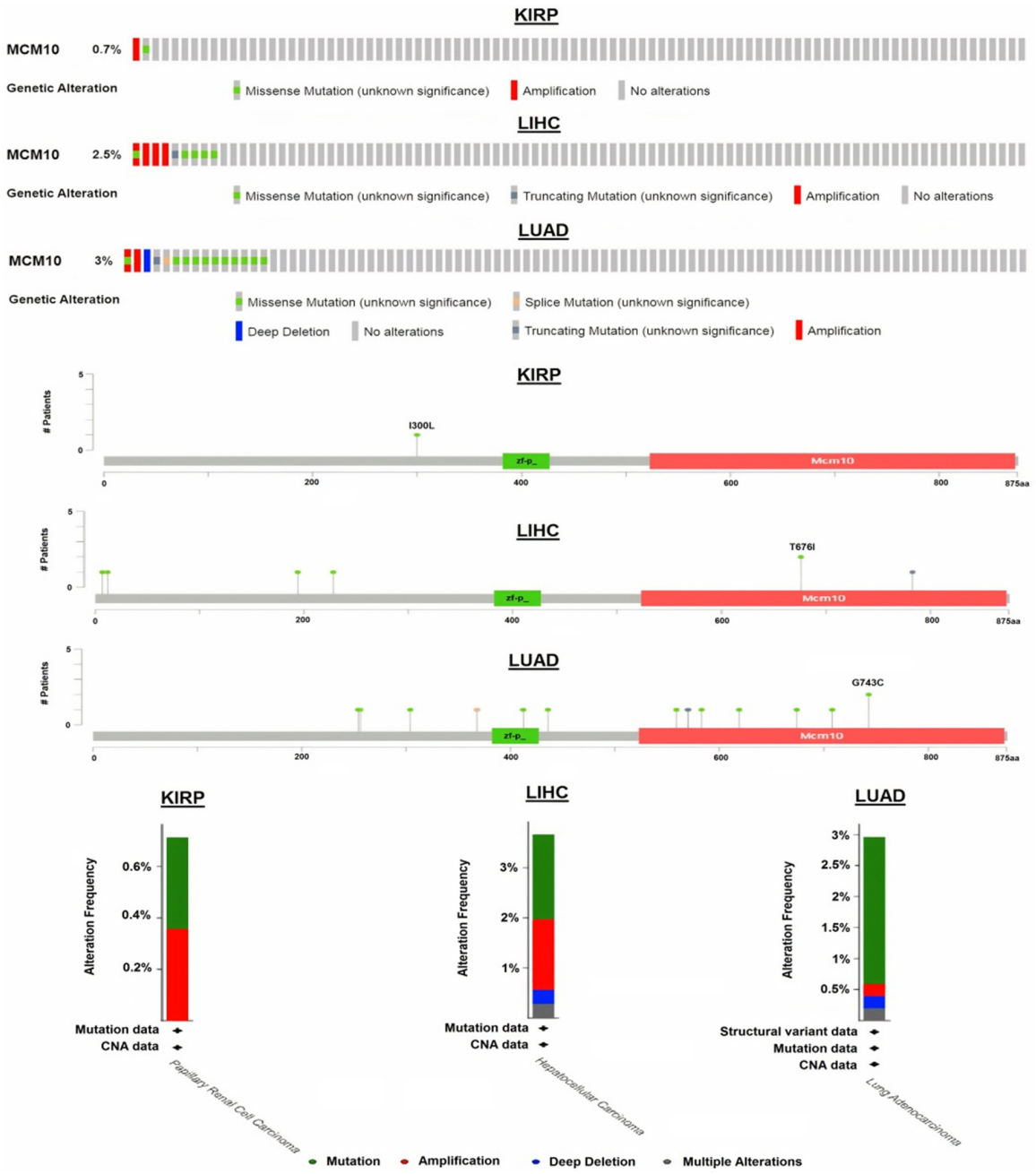

Figure 3. Investigation of MCM10 mutation patterns in KIRP, LIHC, and LUAD cohorts utilizing the cBioPortal database. Kidney Renal Papillary Cell Carcinoma = KIRP, Liver Hepatocellular Carcinoma = LIHC, Lung Adenocarcinoma = LUAD, Minichromosome maintenance 10 replication initiation factor = MCM10.

RT-qPCR-based expression validation of MCM10 in LUAD clinical samples

In the subsequent phase of our investigation, we sought to validate the findings obtained from TCGA datasets by conducting a rigorous analysis of MCM10 expression in clinical LUAD tissue samples using RT-qPCR. The results of this experimental validation were enlightening and further affirmed the previous observations. Upon analysis of LUAD clinical tissue samples, we observed a consistent pattern: MCM10 expression was significantly up-regulated in these samples in comparison to the control samples (**Figure 5C**). This congruence with the TCGA dataset results reinforced the robustness

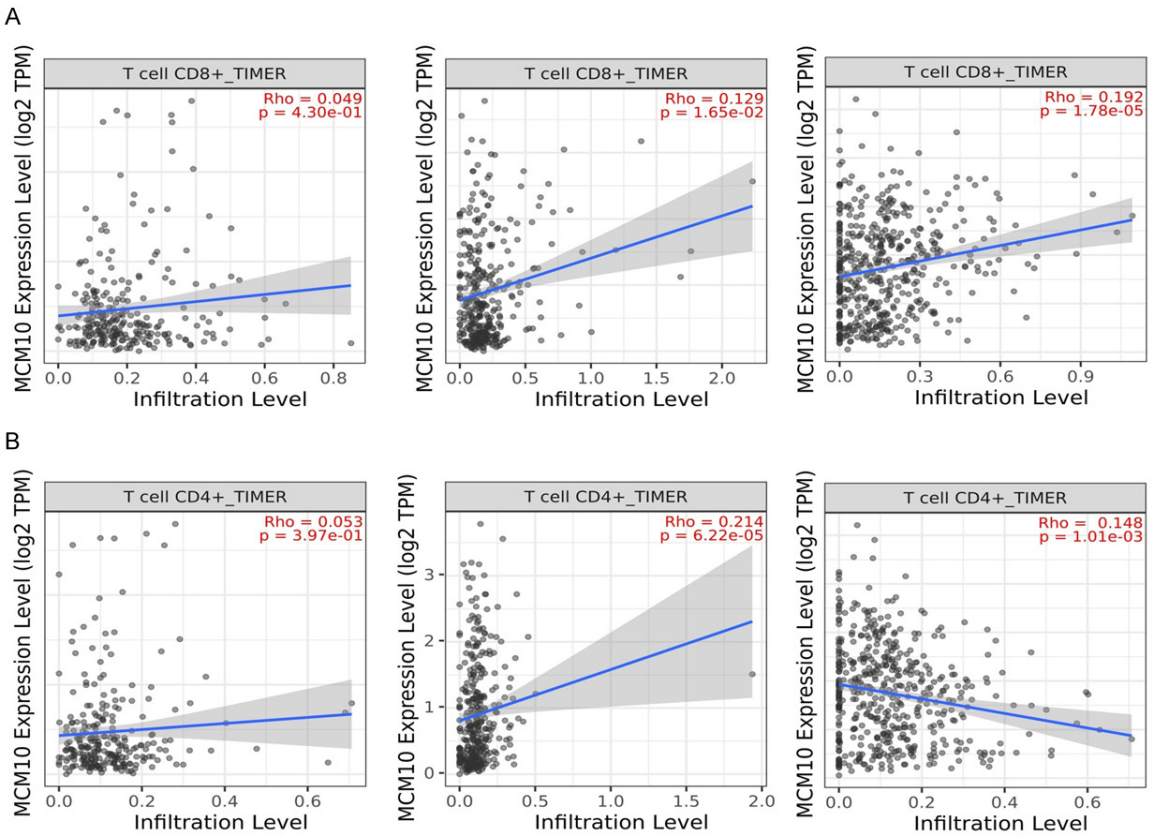

Figure 4. Results of spearman correlation analysis, demonstrating the relationship between MCM10 and infiltration levels of CD8+ T and CD4+ T cells in KIRP, LIHC, and LUAD using TIMER2. (A) Correlation Analysis Results for MCM10 and CD8+ T Cell Infiltration, and (B) Correlation Analysis Results for MCM10 and CD4+ T Cell Infiltration. A *p*-value < 0.05 was considered significant. Kidney Renal Papillary Cell Carcinoma = KIRP, Liver Hepatocellular Carcinoma = LIHC, Lung Adenocarcinoma = LUAD, Minichromosome maintenance 10 replication initiation factor = MCM10.

and reliability of our initial findings. These results provide compelling evidence that MCM10 is indeed overexpressed in LUAD clinical tissue samples, and this up-regulation is not limited to the data obtained from bioinformatics analysis but is also demonstrable in real clinical specimens. Moreover, the receiver operating curve (ROC) for MCM10 (AUC: 0.910, *p*-value < 0.05) exhibited significant diagnostic potential, sensitivity, and specificity (**Figure 5D**).

MCM10-associated drugs

In our investigation, we delved into the DrugBank database to identify drugs with potential regulatory effects on MCM10 expression in the context of KIRP, LIHC, and LUAD. The results of this analysis unveiled several noteworthy pharmaceutical candidates. Specifically, Acetaminophen, Acteoside, Cyclosporine, Polydatin, Estradiol, and Panobinostat emerged as significant drugs with the capacity to modulate MCM10 expression in these cancers (**Table 1**).

These findings hold promise for the development of personalized therapeutic strategies for KIRP, LIHC, and LUAD patients. Acetaminophen, a widely used analgesic, Acteoside, a natural compound with potential health benefits, Cyclosporine, an immunosuppressive medication, Polydatin, a polyphenolic compound, Estradiol, a sex hormone, and Panobinostat, a histone deacetylase inhibitor, offer diverse avenues for potential intervention. Their ability to influence MCM10 expression highlights their relevance in the context of these specific cancers. While these results are intriguing, further experimental validation and clinical studies are necessary to assess the safety and efficacy of these drugs in the context of cancer treatment.

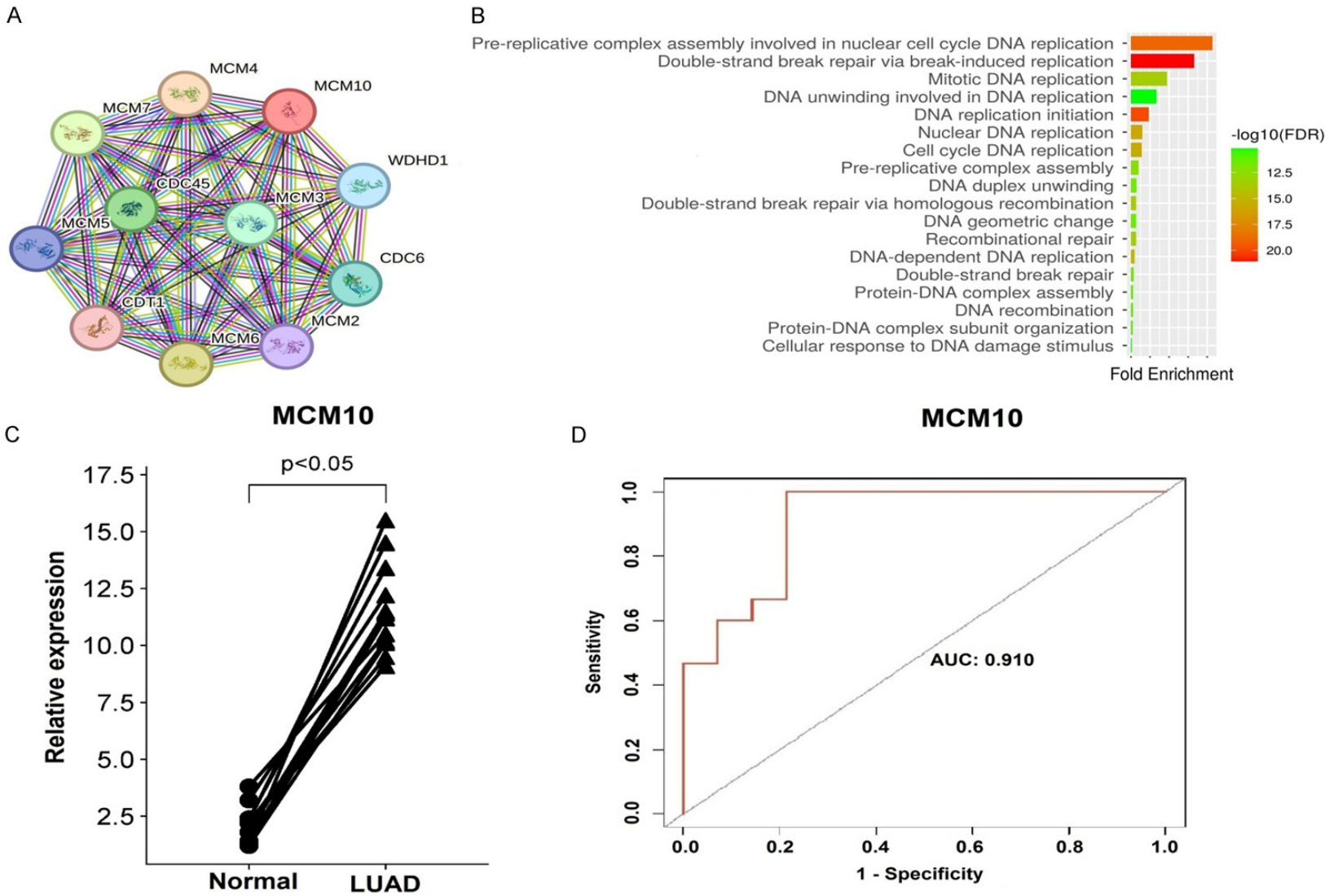

Figure 5. Creation of a protein-protein interaction (PPI) network involving MCM10-associated genes and analysis of pathways associated with these genes. (A) Establishment of the PPI Network of MCM10-Associated Genes Using STRING, and (B-D) Results of Pathway Analysis for Genes Closely Linked to MCM10. A *p*-value < 0.05 was considered significant. Minichromosome maintenance 10 replication initiation factor = MCM10.

Table 1. DrugBank-based MCM10-associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	MCM10	Acetaminophen	Decrease expression of MCM10 mRNA	A20426	Approved
		Acteoside		A20456	
		Cyclosporine		A20661	
		Polydatin		A20456	
		Estradiol		A21424	
		Panobinostat		A21037	

MCM10 = Minichromosome maintenance 10 replication initiation factor.

Discussion

The study builds upon a foundation of previous research that has identified MCM10 as a crucial player in cancer biology [30, 31]. The collective findings from this study further reinforce the notion that MCM10 is a gene of significant interest in multiple cancer types. Previous studies have consistently reported the up-regulation of MCM10 in various cancer types, mirroring the findings of this research [32-34]. The widespread overexpression of MCM10 is in line with the established role of this gene in promoting oncogenic processes.

However, in terms of the distinctive attributes of this study, a significant link was discovered between the up-regulation of MCM10 and a decrease in overall survival (OS) among patients with KIRP, LIHC, and LUAD. While it's noteworthy that various studies in medical literature have also recognized the prognostic significance of MCM10 in different contexts [35-37], our research contributes unique findings to the body of evidence, further solidifying MCM10's role as a valuable prognostic indicator.

The results from the analysis of MCM10 promoter methylation in this study revealed that KIRP, LIHC, and LUAD exhibited hypomethylation of the MCM10 promoter. To the best of our knowledge, this study is the pioneer in reporting promoter hypomethylation as a contributing factor to MCM10 dysregulation in KIRP, LIHC, and LUAD. In contrast, previous investigations across other cancer types have consistently emphasized the role of promoter hypomethylation in driving up-regulation of gene expression [38, 39].

The findings of this study showed that MCM10 gene was not frequently mutated in KRP, LIHC, and LUAD. Therefore, the stability of MCM10 in terms of mutational frequency in KIRP, LIHC, and LUAD patients echoes previous research suggesting that MCM10 is more often regulated by epigenetic alterations than genetic mutations [40, 41]. While genetic mutations play a crucial role in many oncogenic processes, the relative stability of MCM10 in this regard suggests the prominence of epigenetic modifications and other non-mutational drivers in shaping MCM10's expression patterns in these specific cancer types.

Our research also demonstrated a positive correlation between increased MCM10 expression and heightened levels of CD8+ T and CD4+ T immune cells in the KIRP, LIHC, and LUAD cases. Previous studies have consistently reported similar associations between gene expression and immune responses in various cancer contexts [42, 43]. The impact of MCM-10 on immune infiltration accentuates its potential significance within the tumor immune microenvironment, thus bearing implications for the development of immunotherapeutic strategies.

The participation of MCM10-associated genes in critical signaling pathways, such as 'Pre-replicative complex assembly in nuclear cell cycle DNA replication', 'Double strand break repair via break-induced replication', and 'Mitotic DNA replication', etc. resonates with established research on the multifaceted roles of these pathways in cancer development and progression [44-46]. This study contributes to the understanding of how MCM10 fits into the intricate network of cancer-related pathways. Finally, the exploration of different drugs (Acetaminophen, Acteoside, Cyclosporine, Polydatin, Estradiol, and Panobinostat) based interventions for modulating MCM10 expression aligns with the broader field of precision medicine and targeted therapies in cancer treatment [47, 48].

Conclusion

In conclusion, our pan-cancer analysis of MCM10 has revealed its consistent up-regulation in various cancer types. Notably, in KIRP, LIHC, and LUAD, MCM10 up-regulation was associated with poorer overall survival. We found that hypomethylation, rather than mutations, plays a crucial role in MCM10 dysregulation in these cancers. Additionally, MCM10's impact on immune infiltration and involvement in key signaling pathways underscores its significance. Exploring drug interventions adds a therapeutic dimension. This study enhances our understanding of MCM10's diverse roles and potential clinical implications in specific cancers. Further research is warranted to validate these findings and unlock therapeutic possibilities.

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Disclosure of conflict of interest

None.

Address correspondence to: Muhammad Jamil, PARC Arid Zone Research Center, Dera Ismail Khan 29050, Pakistan. E-mail: jamilmatrah@parc.gov.pk

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