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Original Research

Pan-Cancer Multi-Omics Analysis of Minichromosome Maintenance Proteins (MCMs) Expression in Human Cancers

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Abstract

Background: Epigenetic modifications, such as transcription, DNA repair, and replication significantly influence tumour development. Aberrant gene expression and modifications can have a crucial impact on the initiation and progression of tumours. The minichromosome maintenance (MCM) protein family, which is responsible for DNA synthesis, plays a crucial role in tumorigenesis and chemotherapy resistance by regulating the cell cycle and DNA replication stress. Recent studies have shown that dysregulation of the MCMs can lead to these negative outcomes. This study aimed to examine the role of the MCM proteins in DNA synthesis in 33 types of cancers. **Methods**: Various public databases were used to examine the expression, methylation regulation, mutations, and functions of eight MCM proteins (MCM2–9) in pan-cancer. The study investigated the correlation between abnormal MCM expression and clinical outcomes, including prognosis and drug response. The microRNA–mRNA network upstream of the *MCM* genes and the downstream signalling pathways were extensively investigated to determine the molecular mechanisms that drive tumour development. **Results**: The study found that the *MCM* gene expressions differed depending on the type of cancer; high *MCM* gene expression was linked to poor overall survival in most cancers. Additionally, *MCM* gene expression was associated with various immunological features and drug sensitivity. These findings offer important insights for the development of targeted cancer therapies. **Conclusions**: Altogether, this study reveals that the *MCM* genes are differentially expressed across various cancers and are associated with clinical prognoses. These genes may influence the occurrence and development of tumours through several pathways, including the PI3K–AKT, PAS/MAPK and TSC/mTOR signalling pathways and immune-related pathways.

Keywords: minichromosome maintenance family; tumour immunity; methylation; pan-cancer; multi-omics; cancer biomarkers

1. Introduction

Abnormal DNA replication in cells is a crucial factor in the development of tumours, thereby making it a significant area of focus in cancer research. The first minichromosome maintenance (MCM) protein was identified in Saccharomyces cerevisiae and is considered crucial for maintaining extrachromosomal DNA replication [1]. The MCM2–7 complex possesses helicase activity, thereby playing a key role in the formation of the pre-replication complex. Additionally, it is responsible for recruiting DNA polymerase during DNA unwinding and eventually initiates both DNA replication and elongation [2]. The MCM2–7 complex is composed of six nuclear proteins that belong to the MCM family [3]. MCM8 and MCM9 are homologous to MCM2–7 and are capable of repairing DNA double-strand breaks in cells [4]. However, any changes in the MCM protein functions can contribute towards tumour development. Various studies have found that the *MCM* genes are significantly expressed in various types of cancers and result in disruptions to the cell cycle [5]. The aberrant expression of the *MCM* genes contributes to the initiation and progression of various types of cancers, such as gastrointestinal, lung, brain, kidney, breast, ovarian, and haematological malignancies [6–12]. MCM proteins play a significant role in the regulation of cell cycle progression and cell proliferation by interacting with various proteins. Specifically, the interaction between MCM7 and the retinoblastoma (Rb) protein controls cell cycle progression [13,14]. miR-885-5p has the ability to hinder the growth of neuroblastoma cells by binding to the MCM5 3′-untranslated region. Additionally, MCM7 has been found to enhance the spread and growth of tumours both in laboratory settings and in living organisms. However, there have been conflicting results when MCM7 has been suppressed. Furthermore, YAP/TAZ is known to encourage cell proliferation in non-small cell lung cancer (NSCLC), in a MCM7-dependent manner [15,16]. This study systematically evaluated the genomic and clinical characteristics of the MCM family members in 33 solid tumours and examined their potential as biomarkers for cancer diagnosis and monitoring. This study also analysed the influence of *MCM* gene expression on clinical prognosis and drug sensitivity and identified downstream signalling pathways that are potentially modulated by the MCM family. The findings suggest that MCM family members could be used as therapeutic targets for tumours, thereby providing an epigenetic theoretical basis for their application.

2. Materials and Methods

2.1 Data Acquisition

RNA sequencing and single nucleotide variation (SNV) data were extracted from The Cancer Genome Atlas (TCGA) database (https://gdc.cancer.gov/). In addition, publicly available data on copy number variation (CNV), methylation, and clinical characteristics of patients were collected. To perform differential expression analysis of MCM family members in various types of cancer, normalized and batch-corrected RSEM mRNA expression data were used. Analysis was conducted on over 10 paired tumours and normal samples from TCGA. Clinical data were obtained from tumour samples of nine cancer types and used to investigate gene expression changes associated with different subtypes. The gene set variation analysis (GSVA) score was used as a measure of gene set expression since it is positively correlated with gene expression. It was calculated using the GSVA R package (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria). To compare GSVA scores between groups, the Wilcoxon test was used in cases where the number of subtype groups was 2, and ANOVA was used in cases where the number of subtype groups exceeded 2. GSEA was implemented using the fgsea R package (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) to determine the overexpression levels of a gene set at the top or bottom of the list of genes. This analysis was based on the gene expression fold change (FC) values between the tumour samples and normal samples.

2.2 Single Nucleotide Variation Analysis and Copy Number Variation Analysis

The GSCALite platform was used for the genome-wide analysis of the *MCM* genes [17]. Differential expression of MCM family members was evaluated using RSEM-normalised RNA-seq data. A heatmap and waterfall plot were constructed to visualise the SNV and CNV frequencies of the *MCM* genes. The CNV data were obtained from 11,495 samples in the TCGA database, while GISTIC2.0 was used to identify regions of significant amplification or deletion in various patient groups [18].

2.3 Methylation Analysis

The DNA methylation data (Illumina Human Methylation 450k level 3) were extracted from the TCGA database for 14 cancer types. More than 10 pairs of tumours and adjacent normal tissues were analysed. Multiple methylation sites were typically present in a single gene, each with its tag storing the methylation level.

2.4 Survival Analysis

In this study, we collected clinical data from 33 different types of cancers for the purpose of conducting survival analysis. To integrate methylation and clinical survival data, sample barcodes were used, and tumour samples were categorized into high- and low-methylation groups based on the median methylation level. In this study, we utilized the survival package in R (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) to build Cox proportional hazards models and conduct log-rank tests on *MCM* genes across various types of cancers. A *p*-value of less than 0.05 was deemed statistically significant.

2.5 Pathway Activity Analysis

Pathway activity scores (PAS) for cancer-related pathways were calculated using RPPA data extracted from the TCPA (The Cancer Proteome Atlas, http://www.tcpaportal.org) database. In order to estimate differences in PASs between groups, the Student's *t*-test was utilized, with *p*-values being adjusted through the False Discovery Rate (FDR) method. Any FDRs that were equal to or less than 0.05 were considered to indicate significant differences [19,20].

2.6 Drug Sensitivity Analysis

The IC_{50} values of the small molecules and corresponding mRNA expression data were sourced from the Genomics of Cancer Drug Sensitivity (GDSC) and Genome Therapy Response Portal (CTRP). The drugs were ranked according to the correlation coefficients of the screened genes and the FDR comprehensive levels. Drug–gene pairs with absolute correlation coefficients of >0.1 and FDRs of <0.05 were retained, and each pair was assigned a score by multiplying their $-\log_{10}$FDRs and absolute correlation coefficients.

2.7 Analysis of Immune Cell Infiltration

The study utilized the immune infiltration and GSVA scoring modules to investigate the correlation between gene expression and immune cell infiltration. Gene set expression was assessed by estimating GSVA scores, which were positively correlated with gene set expression.

2.8 Statistical Analysis

The study utilized Student's *t*-test to determine variations in the MCM family expression and methylation levels in tumour tissues compared to their levels in corresponding normal tissues. The log-rank test was utilized to compare survival curves, while Pearson or Spearman analyses were utilized to estimate correlation coefficients. A *p*-value of less than 0.05 was deemed statistically significant. All statistical analyses were conducted using SangerBox database (http://www.sangerbox.com/, Sanger Box 3.0; Hangzhou, China) [21] and ChiPlot (https://www.chiplot.online/, Shantou, China), free online platforms for data analysis (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

3. Results

3.1 Abnormal Expression of MCM Family Members in Different Cancers

Paired tumour and normal tissue gene expression data from 33 types of cancers in the TCGA database were used to analyse the differential expression of the *MCM* genes. The results revealed that *MCM2*, *MCM3*, *MCM4*, *MCM5*, *MCM6*, and *MCM7* were upregulated in most tumour tissues, whereas *MCM8* and *MCM9* were downregulated in certain types of tumours. Specifically, *MCM8* was downregulated in kidney renal clear cell carcinoma (KIRC) and thyroid carcinoma (THCA), whereas *MCM9* was downregulated in kidney chromophobe (KICH), KIRC, and kidney renal papillary cell carcinomas (KIRP) (Fig. 1A). GSVA was used to determine the gene set expression. The GSVA scores for bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), oesophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), KICH, KIRC, KIRP, liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), stomach adenocarcinoma (STAD), and THCA were significantly higher than in the corresponding adjacent normal tissues (Fig. 1B). The expressions of *MCM2–8* have been found to be significantly correlated with BRCA subtypes. Notably, there was a significant difference in the expressions of MCM2–8 proteins between the subtypes of luminal A and luminal B, thereby suggesting that they could potentially be used as biomarkers to differentiate between the two subtypes (Fig. 1C, **Supplementary Fig. 1**). An assessment of the standardised enrichment scores (NESs) revealed that the *MCM* gene set was significantly enriched in several types of cancers, including HNSC, ESCA, COAD, LUSC, LIHC, BLCA, STAD, BRCA, and LUAD (Fig. 1D), especially in HNSC and ESCA (Fig. 1E,F). These findings indicate that irregular expression of the *MCM* genes plays a role in the onset of multiple forms of cancer.

3.2 Genetic Alterations of MCMs in Different Cancers

To identify the various genetic alterations in the *MCM* genes, the frequency and types of SNVs were evaluated across the 33 types of cancers. Uterine corpus endometrial carcinoma (UCEC) had the highest SNV frequency (198%), followed by skin cutaneous melanoma (SKCM) (110%), STAD (77%), LUAD (67%), COAD (66%), BLCA (63%), LUSC (56%), HNSC (40%), BRCA (32%), glioblastoma multiforme (GBM) (26%), LIHC (25%), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (22%), rectum adenocarcinoma (READ) (19%), OV (15%), KIRC (12%), prostate adenocarcinoma (PRAD) (12%), and brain low-grade glioma (LGG) (12%). The SNV frequency was $<$10% among the remaining 16 cancer types, and notably, no mutations were observed in the *MCM* genes in thymoma (THYM) (Fig. 2A). Conversely, genes with high SNV frequency included *MCM4* (21%), *MCM6* (19%), *MCM7* (18%), *MCM2* (17%), *MCM3* (16%), *MCM8* (16%), *MCM5* (15%), and *MCM9* (12%) (Fig. 2B). Furthermore, we evaluated the various mutation frequencies in the *MCM* genes. The study found that missense mutations were the most frequently identified mutation, with *MCM4* and *MCM6* having the highest mutation frequency. In particular, missense mutations were the most common type observed. Thereafter, a pie chart was created to provide a visual representation of the relationship between the different CNV types and mRNA expressions of the MCM members. Heterozygous amplification and heterozygous deletion were found to be the primary CNV types (Fig. 2C). Multiple cancer types exhibited heterozygous amplification of *MCM2*, *MCM3*, *MCM4*, *MCM5,* and *MCM6*. Specifically, a heterozygous amplification frequency of $>$25% was observed for *MCM2* in BLCA, CESC, ESCA, HNSC, KIRP, LUSC, OV, STAD, and uterine carcinosarcoma (UCS); *MCM3* in ESCA, LIHC, LUAD, LUSC, OV, READ, SKCM, UCS and uveal melanoma (UVM); *MCM4* in adrenocortical carcinoma (ACC), BLCA, BRCA, CESC, COAD, ESCA, HNSC, KICH, LIHC, LUAD, LUSC, OV, READ, sarcoma (SARC), SKCM, STAD, testicular germ cell tumours (TGCT), UCEC, UCS and UVM; MCM5 in KICH, LUSC, SKCM, and TGCT; *MCM6* in LUAD, LUSC, OV, TGCT, and UCS; *MCM7* in ACC, BLCA, BRCA, COAD, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, mesothelioma (MESO), OV, pancreatic adenocarcinoma (PAAD), READ, SARC, SKCM, STAD, TGCT, and UCS; *MCM8* in ACC, BLCA, BRCA, CESC, cholangiocarcinoma (CHOL), COAD, ESCA, GBM, HNSC, KICH, KIRP, LIHC, LUAD, LUSC, OV, READ, SKCM, STAD, and UCS; *MCM9* in UCS. Our research found a notable correlation between the mRNA expression of the MCM members and CNV frequency in the majority of cancer types ($p < 0.05$, Fig. 2D). These findings indicate that the *MCM* genes are subject to amplification and loss of heterozygosity mutations, which lead to abnormal expression, and ultimately, contribute to the development of cancer.

3.3 Epigenetic Alteration of MCMs in Various Cancers

The study found a strong negative correlation between the methylation levels and mRNA expression in most MCM family members. However, this correlation was not observed in *MCM7* in CHOL and *MCM3* in CESC, DLBC, KICH, KIRC, acute myeloid leukaemia (LAML), OV, PAAD, pheochromocytoma and paraganglioma (PCPG), PRAD, THYM, and UCS (Fig. 3A). Analysis of the differential methylation revealed that the methylation levels of the *MCM* genes were significantly lower in tumour tissues compared to the matched normal tissues: *MCM2* in BLCA, BRCA, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, THCA, and UCEC; *MCM3* in BLCA, BRCA, LIHC, LUSC, and PRAD; *MCM4* in BRCA, ESCA, KIRC,

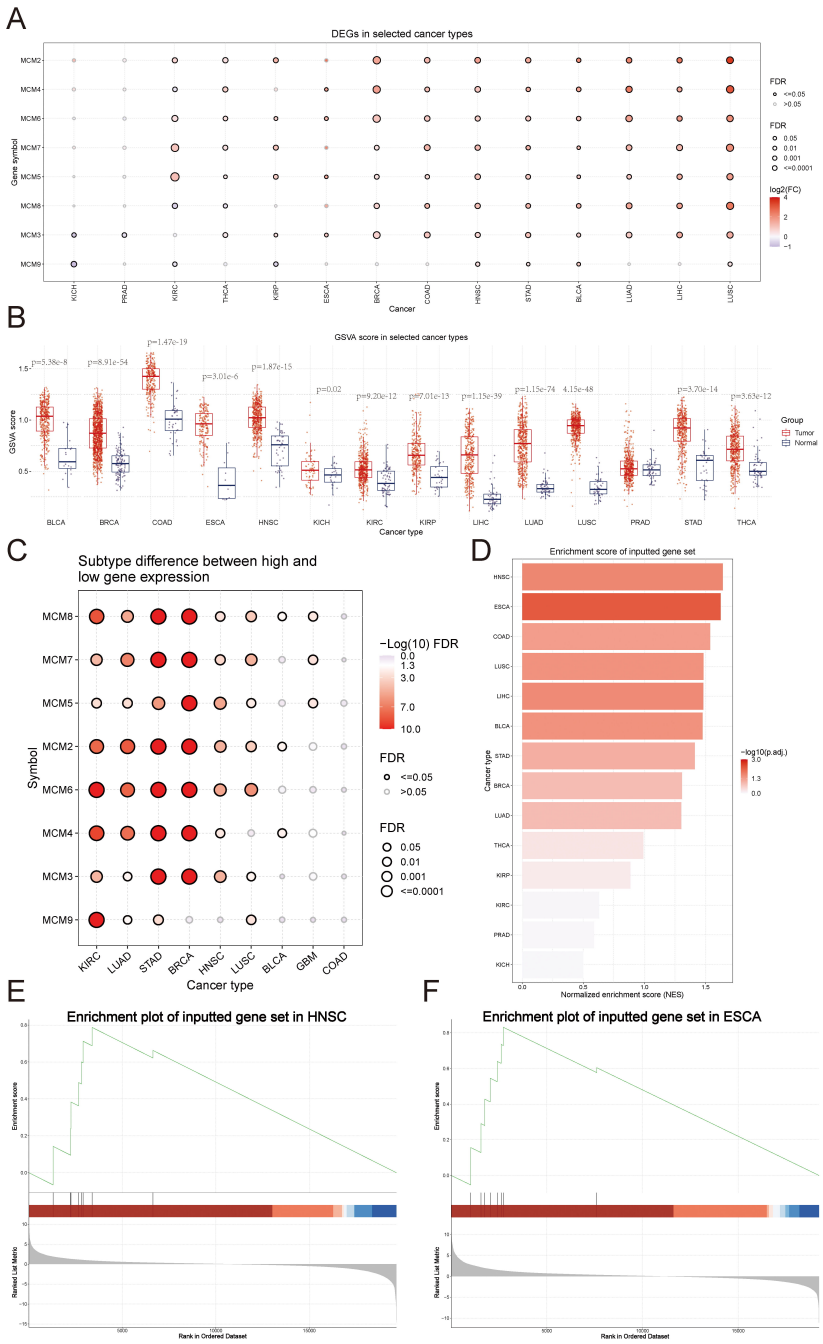

Fig. 1. Comparison of the Minichromosome Maintenance Proteins (MCM) family mRNA expression in various types of cancer. (A) Differential expression of *MCM* genes between 14 paired normal and tumour tissues. Purple to red represents the fold change between tumour tissues and normal tissues. Red dots indicate that gene expression is higher in tumour tissues than in normal tissues, and blue dots indicate that gene expression is lower in tumour tissues than in normal tissues. The size of the dots indicates significance based on the False Discovery Rate (FDR), while the FDR value of each dot is shown on the right. (B) The gene set variation analysis (GSVA) score was calculated by comparing the molecular characterization maps (MCMs) between 14 paired normal and tumour tissues. (C) Relationship between the *MCM* gene expressions and subtypes. FDR values are represented by the bubble's colours and sizes in the graph. Rows represent gene set symbols, whereas columns represent selected cancer types. Significance based on FDR values is indicated by the colour of the bubbles, with white indicating low significance and red indicating high significance. Bubble size positively correlates with significance. (D) Summary of the enrichment scores (ES) of *MCM* genes in the selected cancers. The score indicates the extent to which a set of genes is overrepresented at either the top (ES >0) or bottom (ES <0) of a gene ranking list. (E,F) *MCM* gene sets were found to be significantly enriched in head and neck squamous cell carcinoma (HNSC) and oesophageal carcinoma (ESCA). In the plot, the running ES for the gene set is displayed as the analysis descends the ranked list. DEGs, Differentially expressed genes; KICH, kidney chromophobe; PRAD, prostate adenocarcinoma.

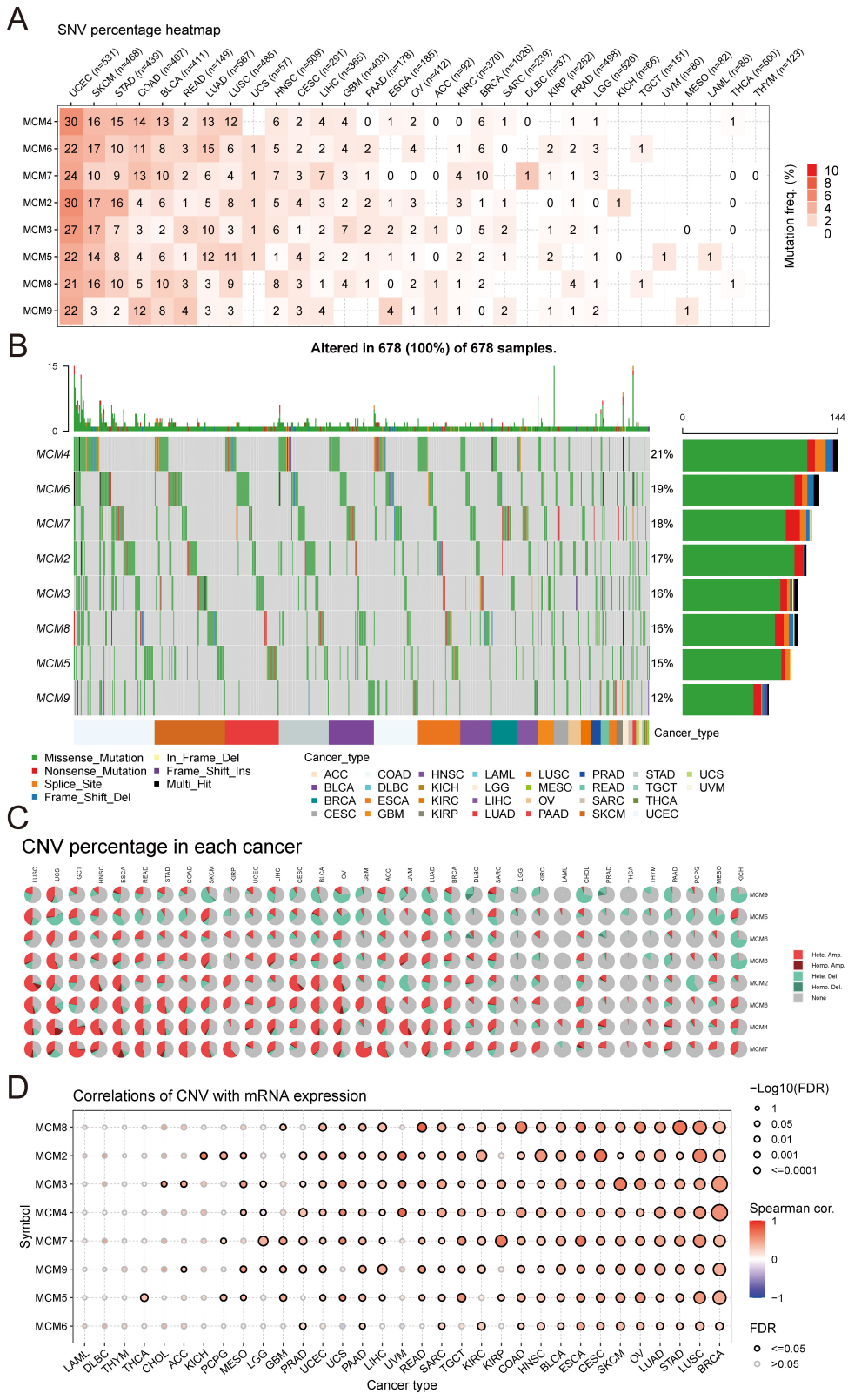

Fig. 2. Relationship between the *MCM* gene expressions and genomic alterations. (A) Heatmap demonstrating the SNV frequency in *MCM2–9* genes in tumours. (B) Waterfall plot demonstrating SNV distribution and its classification in *MCM* genes. (C) Pie chart demonstrating the proportion of different types of copy number variations (CNVs) in each gene across various cancer types. (D) Bubble plots demonstrating the relationship between mRNA expression and CNV frequency. The colour of the bubbles indicates the strength of correlations, with blue bubbles representing negative correlations and red bubbles representing positive correlations. The bubble sizes are proportional to the significance, based on the FDR values. Black borders indicate an FDR of $\leq$0.05.

LUAD, and THCA; *MCM5* in BLCA, BRCA, COAD, KIRC, LIHC, LUAD, LUSC, PAAD, PRAD, and UCEC; *MCM6* in BLCA, BRCA, HNSC, KIRC, LIHC, LUAD, LUSC, THCA, and UCEC; *MCM7* in KIRP, LIHC, LUAD, PRAD, and THCA; *MCM8* in BRCA and PRAD; *MCM9* in BLCA, KIRP, LIHC, LUAD, LUSC, PRAD, and UCEC. However, the methylation levels of the following genes were higher in the tumour tissues than in the matched normal tissues: *MCM3* in KIRC, KIRP, and THCA; *MCM4* in KIRP; *MCM5* in ESCA; *MCM6* in PRAD; *MCM7* in BRCA and LUSC; *MCM8* in KIRC, LUSC, and THCA; *MCM9* in BRCA, KIRC, and PAAD (Fig. 3B). Hypomethylation of *MCM2–9* in various types of cancers was associated with an increased risk of mortality (Fig. 3C). The study found that hypermethylation of *MCM2*, *MCM3*, *MCM7*, and *MCM8* was a significant risk factor for survival in certain types of cancers, including UVM, LGG, CESC, and ACC (Fig. 3D–H). Altogether, these results suggest that abnormal DNA methylation regulates abnormal expression of MCMs, thereby influencing tumour progression.

3.4 MCMs were Significantly Associated with Survival

The survival analysis results indicate that in most cancer types, a high expression of *MCM2–9* was linked to a poor prognosis. However, a high expression of certain *MCM* genes was associated with a better prognosis in specific cancer types: *MCM2* in CESC, STAD, and UVM; *MCM3* in CESC, OV, STAD, and THCA; *MCM4* in READ and THYM; *MCM5* in CESC and THCA; *MCM6* in CESC, READ, and THYM; *MCM7* in DLBC and THCA; *MCM9* in THYM (Fig. 4A–C). Various tumours were found to be influenced by the *MCM2–9* genes, in terms of their pathological staging. Specifically, *MCM4*, *MCM6*, and *MCM2* were associated with KIRP; *MCM7, MCM5*, *MCM6*, and *MCM2* were associated with KIRC; *MCM3* and *MCM2* were associated with BRCA; *MCM5* and *MCM6* were associated with THCA; *MCM7* was associated with KICH; *MCM8* was associated with SKCM; *MCM3* was associated with TGCT (Fig. 4D). According to the findings for KICH, a positive correlation existed between *MCM2* expression and the pathological stage. This suggests that tumours with higher stages tend to exhibit higher levels of *MCM2* expression (Fig. 4E). Furthermore, the *MCM2–9* genes exhibited varying levels of expression in different clinical stages of tumours. Specifically, *MCM4*, *MCM6*, and *MCM2* were differentially expressed in different clinical stages of KIRP (Fig. 4F). While the expression of *MCM3* increased with the clinical stage in TGCT (Fig. 4G). This study suggests that the abnormal expression of *MCM* genes is closely linked to cancer prognosis.

3.5 Correlation between Immune Cell Infiltration and MCM Gene Expression

This study investigated the correlation between *MCM* gene expressions and immune cell infiltration in the tumour microenvironment (TME). *MCM2* expression significantly correlated with the immune, stromal, and microenvironmental scores. A negative correlation was observed between the immune scores and *MCM2* expression in cancers, including GBM, UCEC, ESCA, stomach and oesophageal carcinoma (STES), SARC, KIRP, LUSC, high-risk Wilms tumour (WT), neuroblastoma (NB), TGCT, and PCPG (Fig. 5A). In this study, we investigated the relationship between the infiltration levels of 24 immune cell types and GSVA scores. Our findings suggest the existence of two distinct clusters of cancer types based on this correlation (Fig. 5B). The two clusters exhibited different patterns of immune cell infiltration. The *MCM* gene expressions positively correlated with the infiltration levels of the immunosuppressive cells in cancers, including the natural regulatory T (nTreg) cells, induced regulatory T (iTreg) cells, exhausted, dendritic cells (DCs), and macrophages. However, the expression of the MCMs was significantly negatively correlated with the infiltration levels of the immune effector cells, including the natural killer (NK) cells, $CD8^+$ T cells, follicular helper T (Tfh) cells and $CD4^+$ T cells, in cancers, such as in TGCT, THCA, HNSC, LUAD, STAD, CESC, PCPG, THYM, GBM, and KIRP. These findings suggest that the upregulation of the *MCM* gene expressions is associated with the immune microenvironment of these tumours. Therefore, targeting the MCM family could have significant implications for improving the effectiveness of immunotherapy in cancer patients.

3.6 Correlation between MCM Expression Levels and Drug Treatment Sensitivity

To investigate the impact of the *MCM* genes on drug efficacy, the study analysed the correlation between gene expression and drug sensitivity using data obtained from the GDSC and CTRP databases. The results indicated that the increased expression of the *MCM2–9* genes was linked to heightened resistance to drugs, such as 17-AAG, RDEA119, trametinib, and selumetinib (Fig. 6A). Moreover, the study found that there was a negative correlation between the expressions of *MCM2–9* and drug sensitivity. This correlation was validated by analysing the IC_{50} values of the drugs (Fig. 6B). These findings suggest that the overexpression of the MCMs results in a resistance to chemotherapy and targeted drug therapy, meaning that MCMs are useful predictors of drug efficacy and potential therapeutic targets for cancer.

3.7 Potential Molecular Mechanisms of MCMs.

The miRNA–gene network analysis revealed that all eight *MCM* genes were regulated by multiple miRNAs (Fig. 7A). The expressions of the *MCM* genes were regulated by a complex network of miRNAs, which play an essential role in tumour development and progression. Pathway enrichment analysis revealed that the *MCM2–9* genes were associated with several pathways, including

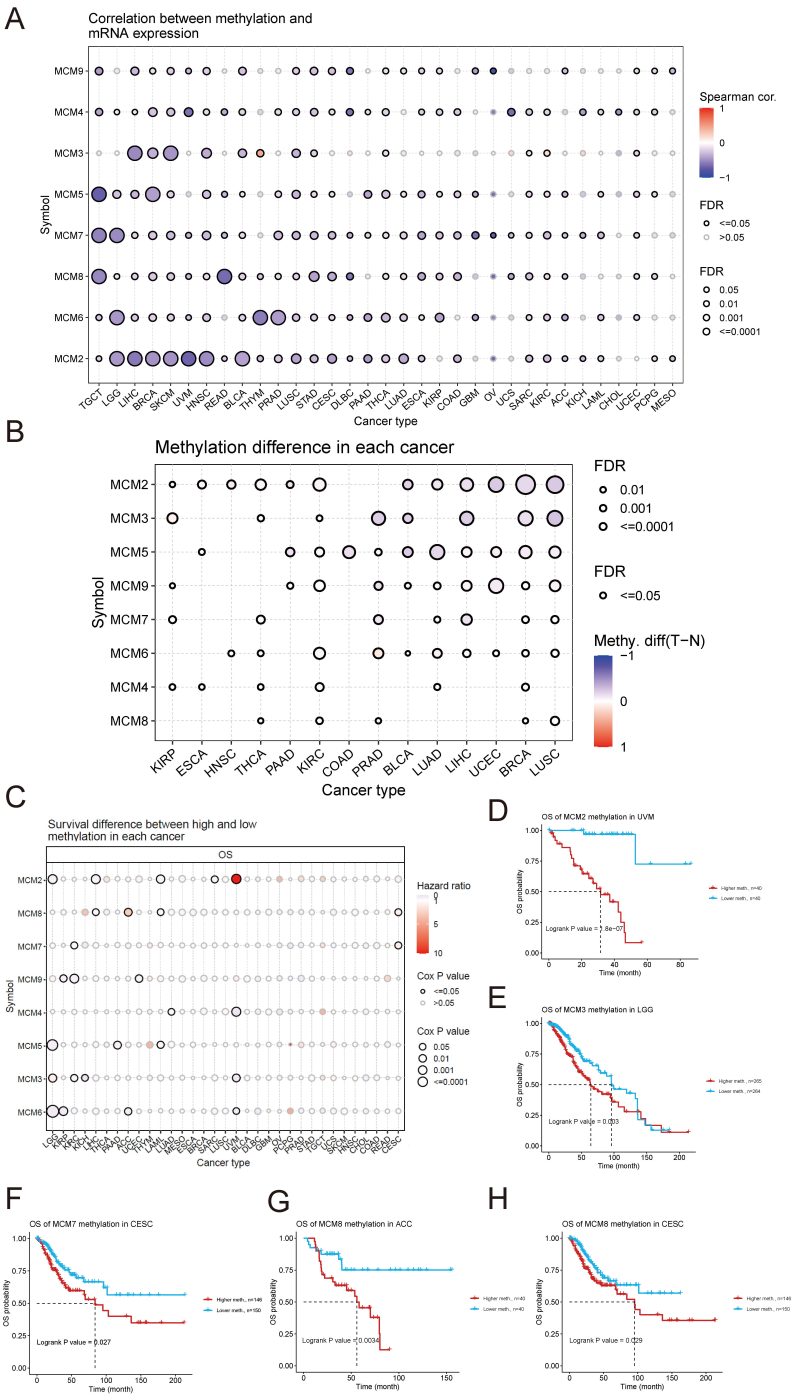

Fig. 3. Methylation alterations in *MCM2–9* genes and overall survival (OS). (A) Correlation between the mRNA expression and methylation levels of *MCM* genes. Negative correlations are represented by blue bubbles, whereas positive correlations are represented by red bubbles. The intensity of the colour reflects the strength of the correlation, with darker colours indicating a stronger correlation. The size of the bubbles is positively correlated with significance, based on FDR values. Black borders indicate an FDR of ≤0.05. (B) Differential methylation of *MCM* genes between 14 paired normal and tumour tissues. The colour and size of the bubbles represent fold change values and FDRs, respectively. Rows represent gene symbols, whereas columns represent the selected cancer types. (C) Differences in overall survival between hypermethylated and hypomethylated groups of *MCM* genes in specific cancers. The plot displays hazard ratios and *p*-values (Cox regression analysis) for various cancer types and gene symbols. The colour of the bubbles indicates the hazard ratio from low to high, whereas the size of the bubbles represents a positive correlation with the *p*-value. Bubbles with black borders signify *p*-values of ≤0.05. (D–H) KM plots (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) demonstrating differences in overall survival between patients with low and high hypermethylation groups for *MCM2* in uveal melanoma (UVM), *MCM3* in brain low-grade glioma (LGG), *MCM7* in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), and *MCM8* in adrenocortical carcinoma (ACC) and CESC.

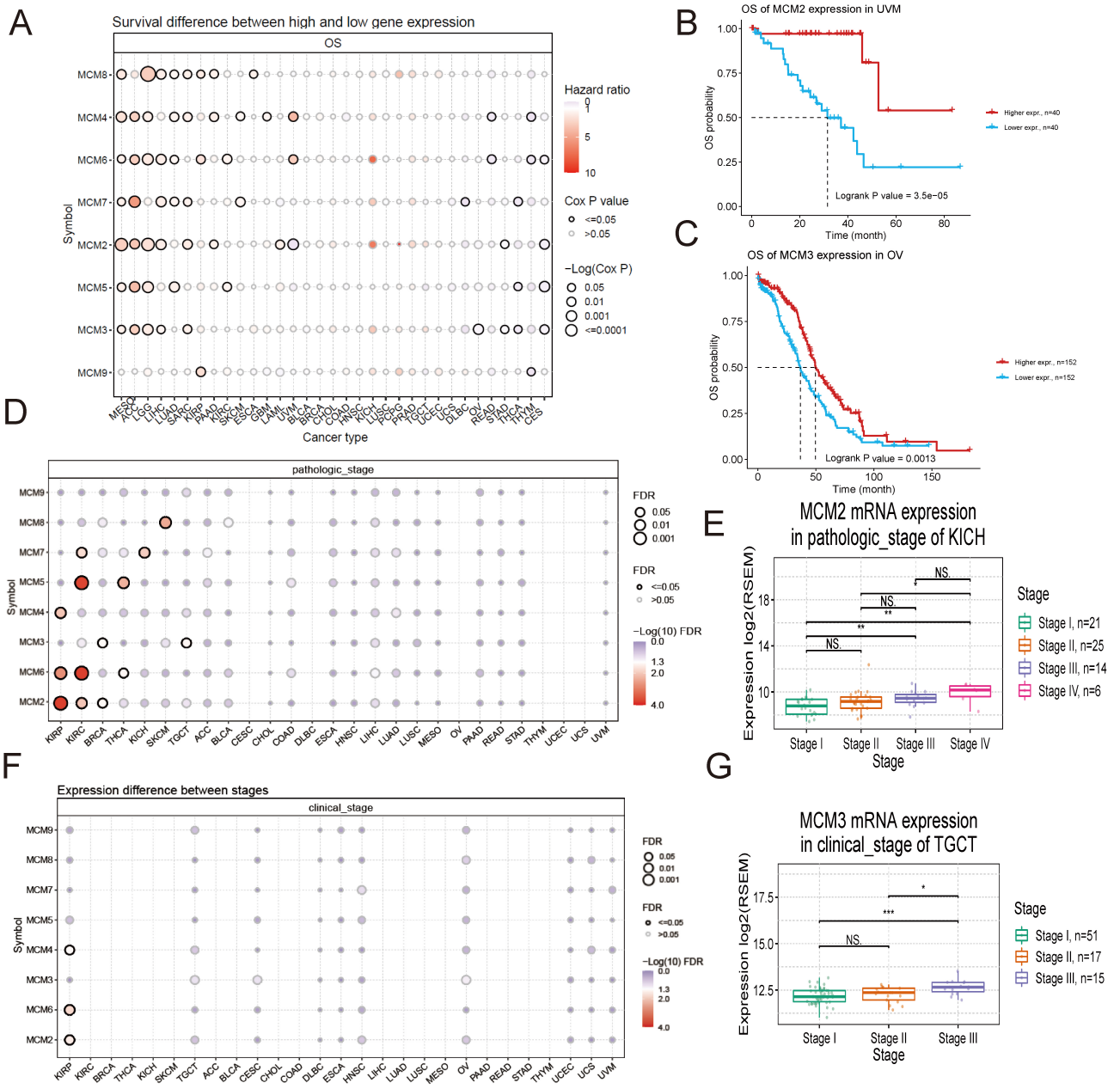

Fig. 4. Relationship between *MCM* gene expressions and overall survival (OS) in pan-cancer. (A) Heatmap demonstrating the univariate Cox proportional-hazards regression analysis $-\log_{10}$ value (*p*-value). The colour of the bubbles from blue to red represents the low to high hazard ratios, respectively, whereas the size of the bubbles positively correlates to the *p*-value. Bubbles with black borders have *p*-values of ≤ 0.05. (B,C) KM plots demonstrate differences in overall survival between the low and high *MCM* expression groups in UVM and OV. Statistical analysis was performed using the log-rank test, with *p*-values of <0.05 indicating significance. (D) Differences in the mRNA expression of *MCM* genes among different pathological stages of specific cancers. (E) Boxplot demonstrating *MCM2* expression in different pathological stages of KICH. (F) Differences in the mRNA expression of *MCM* genes among different clinical stages in specific cancers. (G) Boxplot demonstrating *MCM3* expression in different clinical stages of TGCT. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, no significance.

the TSC/mTOR signalling, cell cycle-related, PI3K/AKT signalling, RTK signalling, RAS/MAPK signalling, and apoptosis-related pathways (Fig. 7B). The *MCM* genes had a positive impact on specific pathways in several cancers, whereby *MCM7* and *MCM5* were primarily involved in the activation of Androgen Receptor (AR) and Epithelial–Mesenchymal Transition (EMT), respectively (Fig. 7C,D). The GSVA demonstrated that the expressions of the *MCM* gene sets were linked to cell cycle regulation and apoptosis in cancer (Fig. 7E). This finding suggests that the MCMs have a significant role in controlling cancer-related pathways.

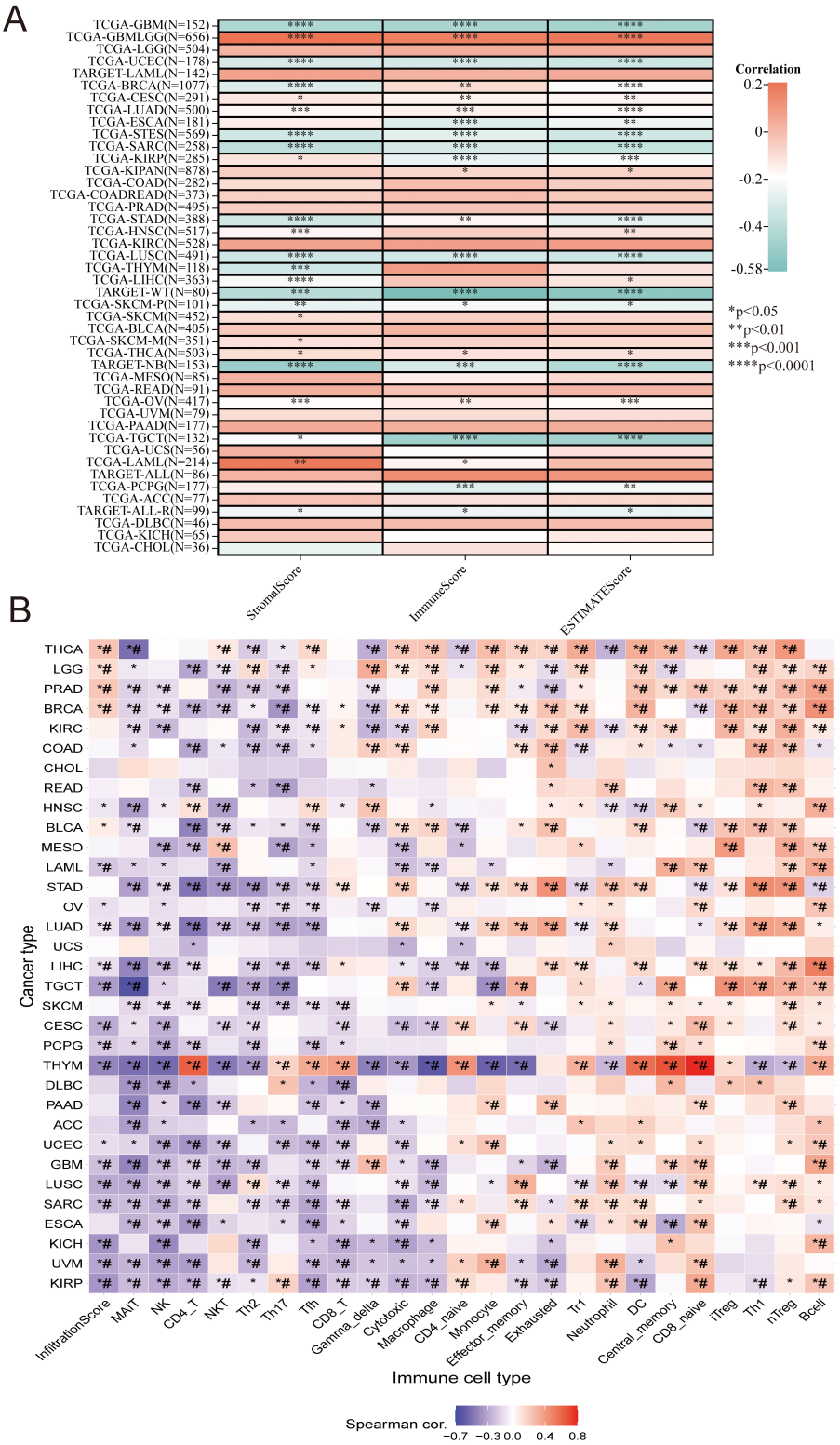

Fig. 5. Relationship between the *MCM* gene expressions and the tumour microenvironment. (A) Heatmap demonstrating the correlation between *MCM2* expression and immune, stromal, and microenvironmental scores. (B) Relationship between the *MCM* gene expressions and immune cell infiltration. The heatmap summarises the significant correlations based on *p*-values and FDRs from Spearman correlation analysis of GSVA scores and immune cell infiltration. Blue represents the negative correlations, whereas red represents positive correlations. Note: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; #, FDR <0.05.

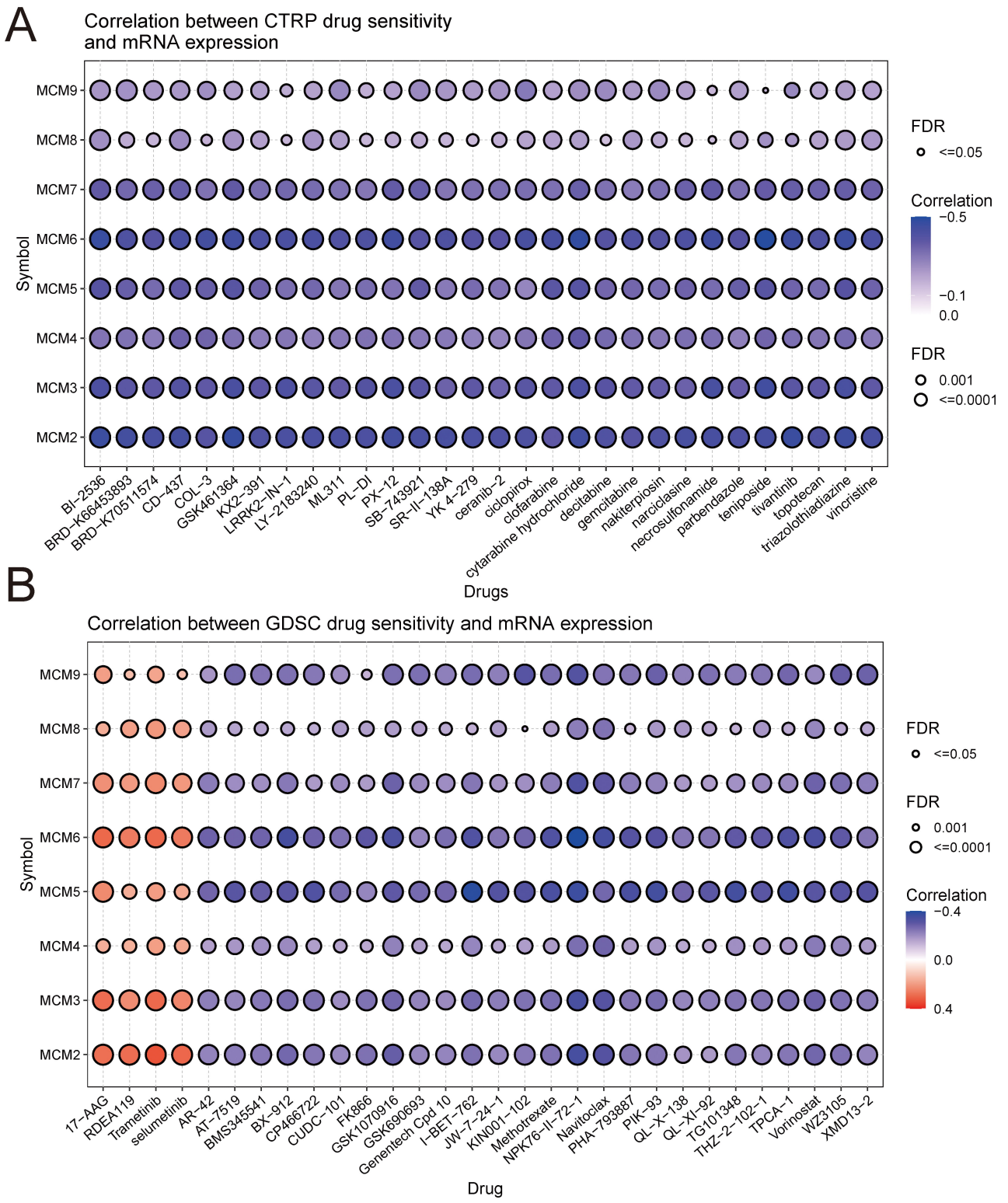

Fig. 6. Relationship between the *MCM* gene expressions and drug sensitivity. (A) Correlation between the expressions of the *MCM* genes and drug sensitivity based on Genome Therapy Response Portal (CTRP) data. (B) Correlation between the expressions of the *MCM* genes and drug sensitivity based on GDSC data. In the scatterplot, blue bubbles indicate negative correlations and red bubbles indicate positive correlations, while the bubble size positively correlates to significance based on FDR values. Darker colours indicate stronger correlations. Additionally, black borders indicate FDR values of $\leq$0.05.

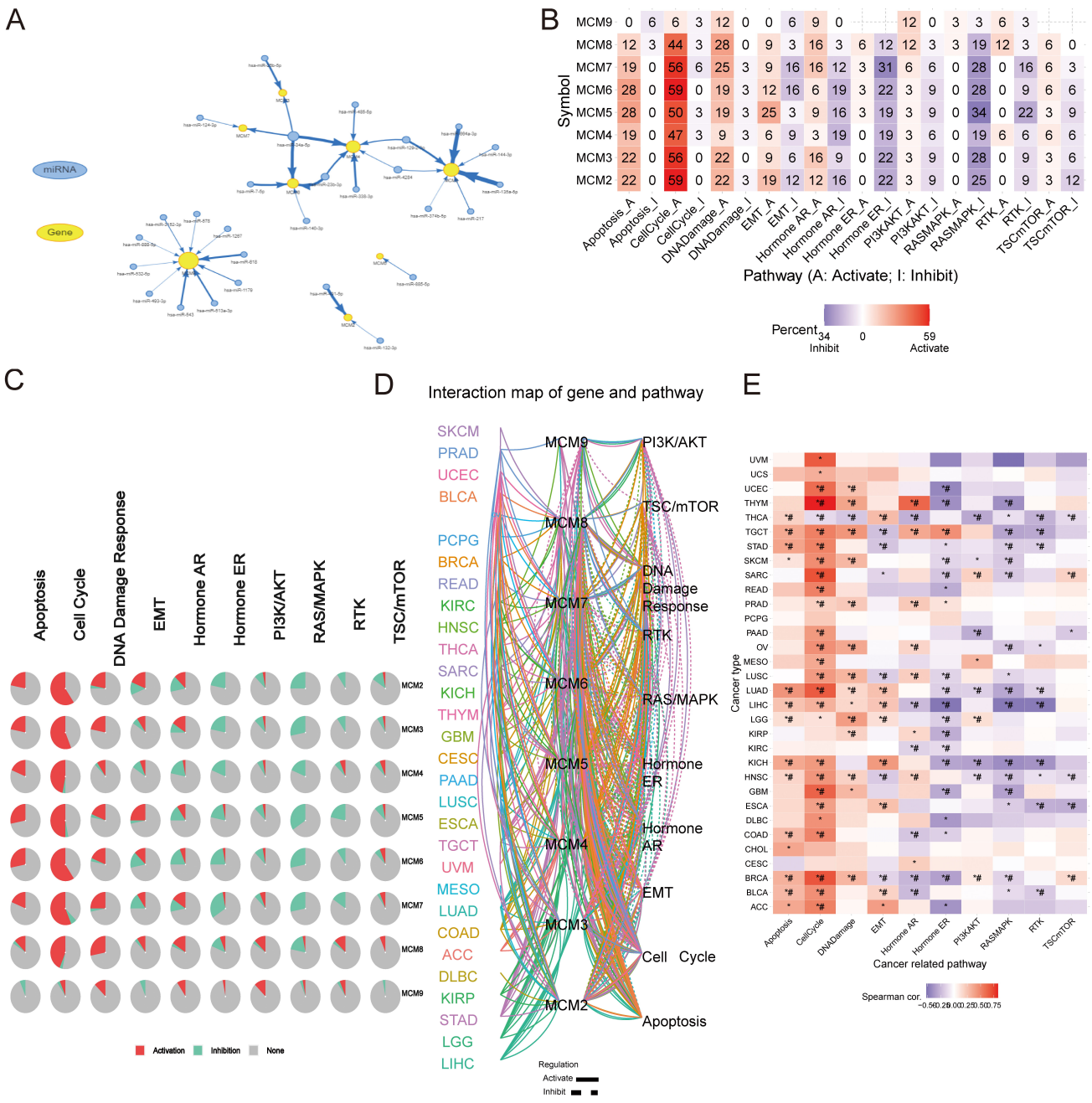

Fig. 7. Potential molecular mechanisms underlying altered *MCM* gene expressions. (A) The miRNA regulatory network is representative of miRNAs and target genes, whereby nodes represent miRNAs or target genes, edges represent the miRNA-to-gene conversion regulation, and edge widths indicate the absolute correlation coefficients. (B) The combined percentage of the effects of the MCM complex on pathway activity. (C) Pie chart demonstrating the proportion of genes contributing to the pathway activity across 32 cancer types. (D) A network demonstrating the connection between genes and pathways using straight lines. Activation is represented by solid lines, whereas inhibition is denoted by dashed lines. The line colours are used to indicate different cancer types. (E) Association between GSVA scores and cancer-related pathway activity. *, p-value $\leq$ 0.05; #, FDR $\leq$0.05.

4. Discussion

Recently, DNA replication has emerged as a major research focus for investigating the occurrence and development of tumours. The MCM family comprises replicative DNA helicases that play an indispensable role in DNA replication and perform essential functions during all stages of the cell cycle [2]. Recent studies have demonstrated the significant roles MCM family members play in carcinogenesis. Abnormal expressions of the MCMs have been observed in various malignant tumours, such as cervical cancer, breast cancer, and human glioma. These findings suggest that the MCMs can be used to predict tumour devel-

opment and prognosis [22–24]. This study investigated the correlation between *MCM* gene expressions and genomic alterations, TME features, prognosis, and drug sensitivity. It also identified the potential mechanisms through which the MCM family members contribute to tumour development. The results indicate that abnormal expressions of the MCMs could be a useful marker in early tumour diagnoses and in predicting treatment effectiveness.

The expressions of *MCM2–9* were found to be elevated in most types of cancer. However, the expression of MCM3 was significantly downregulated in KICH and PRAD. The GSVA scores for the BLCA, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, STAD, and THCA tissues were significantly higher than for para-carcinoma tissues. Furthermore, survival analysis revealed that the high expressions of *MCM2–9* were associated with a poorer prognosis in most tumour types, suggesting that the *MCM2–9* genes play important roles in the pathological and clinical staging in the majority of tumours. These findings are consistent with those from previous studies, which suggested that abnormal *MCM* gene expressions were closely related to patient prognoses [25–29]. Breast cancer is a complex illness that can be classified into different subtypes, depending on gene expression patterns. An in-depth understanding of these subtypes may help to develop targeted therapies for each subtype, which can lead to more effective and individualised treatments [30,31]. In this study, a strong correlation was observed between the expressions of *MCM2–8* and the different BRCA subtypes. Specifically, the expressions of *MCM2–8* were significantly different between luminal A and luminal B subtypes, indicating that the *MCM2–8* genes are potential biomarkers for the stratification of patients with these two subtypes. In addition, the findings of this study suggest that the *MCM* genes are reliable predictors of prognosis in various types of cancer. Genomic instability is a major cause of cancer development. Although most cancer genomes undergo regular changes in chromosomal regions, the exact regulatory mechanisms that promote copy number alterations in specific regions of the genome remain unclear [32,33]. Various mechanisms underlying the generation of CNVs have been proposed, including the stalling of the replication forks or the induction of double strand breaks during DNA replication. Another mechanism that leads to the generation of CNVs is the use of damaged intermediates as primers, which re-fuse specific segments of DNA back into the genome, ultimately, causing gene duplication or deletion [34]. In this study, frequent variations in the gene copy numbers were observed in the MCM complex. Our study revealed a significant positive correlation between the frequency of the copy number variations (CNVs) and the mRNA expression of the MCM members. Among the *MCM* genes, *MCM4* and *MCM6* had the highest mutation frequencies, with missense mutations being the primary type. These results indicate that alterations in copy number can have an impact on the expressions of the *MCM* genes, which could potentially lead to the development of tumours.

This study provides evidence that the expression of the MCM family members is significantly influenced by complex regulations at both the genomic and epigenomic levels. The methylation patterns of the *MCM* genes vary greatly among different types of cancer. However, with the exceptions of *MCM7* in CHOL and *MCM3* in CESC, DLBC, KICH, KIRC, LAML, OV, PAAD, PCPG, PRAD, THYM, and UCS, the expression of most *MCM* genes correlated negatively with their methylation levels. The study found that hypomethylation of *MCM2–9* was generally associated with an increased risk of mortality in most types of cancer. However, in UVM and LGG, hypermethylation of *MCM2* and *MCM3*, respectively, was found to be a risk factor. In CESC, hypermethylation of *MCM7* was identified as a risk factor, while in ACC and CESC, hypermethylation of *MCM8* was found to be a risk factor. Upregulated *MCM2* expression has been associated with promoter methylation and clinical characteristics of patients [35]. Therefore, the aberrant expression of the *MCM* genes in tumours may be influenced by abnormal DNA methylation, which eventually affects the prognosis.

Targeting the TME has become a promising approach for cancer treatment in recent years and our study found a significant correlation between *MCM2* expression and immune, stromal, and microenvironmental scores. We also evaluated the correlation between the infiltration levels of 24 different types of immune cells and GSVA scores [36]. The *MCM* gene expressions correlated positively with the infiltration levels of the immunosuppressive cells in ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, HNSC, and KIRC. However, it correlated negatively with the infiltration levels of NK cells, $CD8^+$ T cells, Tfh cells, and $CD4^+$ T cells in TGCT, THCA, HNSC, LUAD, STAD, CESC, PCPG, THYM, GBM, and KIRP. These findings indicate that targeting the MCM family represents a potential strategy for reversing the cold immune microenvironment and improving the tumour-killing ability of immune cells.

The increased expressions of the *MCM* genes can lead to apoptosis, hinder cell cycle progression, and trigger the DNA damage response in different types of cancer [15,16,37,38]. In addition, the *MCM* genes affect tumour invasion and metastasis [39,40]. The study highlights the crucial role the *MCM2–9* genes play in regulating cancer-related pathways, such as PI3K/AKT signalling, RTK signalling, RAS/MAPK signalling, and apoptosis-related pathways. Drug sensitivity analysis revealed that the *MCM* gene expressions correlated with the resistance to 17-AAG, RDEA119, trametinib, and selumetinib. However, the mechanisms through which these drugs affect the expressions of the *MCM* genes and tumour development require further investigation.

Despite the significant advancements in cancer treatment, there are still several crucial matters that need to be resolved. Specifically, future studies should focus on elucidating the specific mechanisms underlying the high expression of MCM proteins in certain cancer types and investigating the associated genetic and epigenetic changes. In addition, the mechanisms through which the MCM family is regulated in malignant tumours warrant further investigation. To improve therapeutic strategies for cancer, it is crucial to identify potential upstream regulators of the MCM members and understand the mechanisms through which they affect them at the transcriptional and post-transcriptional levels. A comprehensive understanding of the MCM family can aid in the development of more effective treatments for cancer.

5. Conclusions

In this study, our focus was to investigate the expression and function of eight key MCMs within tumors and the TME. We explored genomic alterations and the miRNA network to uncover additional mechanisms that contribute to the dysregulation of MCMs in cancer. Our findings are consistent with previous research and also provide new insights for future investigations. Additionally, we discovered that these genes have significant effects on the TME and drug resistance, which can offer valuable insights for developing cancer treatment strategies. These findings may open up possibilities for alternative approaches in managing clinically refractory cancers.

Abbreviations

ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; COADREAD, colon adenocarcinoma/rectum adenocarcinoma oesophageal carcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, oesophageal carcinoma; FPPP, FFPE Pilot Phase II; GBM, glioblastoma multiforme; GBMLGG, glioma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIPAN, pan-kidney cohort (KICH + KIRC + KIRP); KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukaemia; LGG, brain low-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OVO, Varian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; SKCM, skin cutaneous melanoma; STES, stomach and oesophageal carcinoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; OS, osteosarcoma; ALL, acute lymphoblastic leukaemia; NB, neuroblastoma; WT, high-risk Wilms tumour.

Availability of Data and Materials

All raw data can be provided upon request.

Author Contributions

LW conceived the study, drafted the manuscript, and performed the analysis. XL analyzed and interpreted the data and ultimately reviewed the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

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