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Original Research

The expression and prognostic value of minichromosome maintenance markers in human breast cancer: a comprehensive analysis

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Abstract

Objective: Breast cancer (BC) is one of the most health-threatening neoplasms for women worldwide. Despite advances in detection and treatment strategies over the past few decades, the current biomarkers of BC are less than satisfactory for effective prognosis and individualized treatment. This study aimed to investigate the new biomarkers to meet this urgent demand. **Methods**: The current study investigated the transcriptional levels of minichromosome maintenance genes (MCMs) in BC patients from the Oncomine, UALCAN database, and Gene Expression Profiling Interactive Analysis (GEPIA); protein expression levels of MCM proteins in BC patients were derived from the Human Protein Atlas (HPA) database. Further, survival analysis was evaluated with Kaplan-Meier Plotter. BC genome atlas data were obtained from cBioPortal databases. Gene regulatory network analysis was performed using the STRING online tool, and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using DAVID. **Results**: Based on multiple database analysis, mRNA and protein levels of *MCM2*, *MCM4* and *MCM10* were much higher in BC patient, and survival analysis showed that high transcription levels of most MCMs were found to be associated with poor prognosis for BC patients; moreover, the MCMs genetic alterations, especially of *MCM2*, *MCM4* and *MCM10*, were found in 45% of BC patients. In addition, dysregulation of MCMs was considered to possibly affect DNA damage/repair, cell cycle dysregulation and chromosome instability. **Conclusions**: In summary, this study indicated that *MCM2*, *MCM4*, and *MCM10* are potential prognostic markers and therapeutic targets for BC.

Keywords: Minichromosome maintenance gene family; Breast cancer; mRNA expression level; Prognostic marker; Bioinformatics analysis

1. Introduction

Breast cancer (BC) is the most common type of malignancy and the second leading cause of cancer-related mortality in women worldwide [1,2]. Despite advances in detection and treatment strategies over the past few decades, the prognosis for BC patients is still suboptimal, with only one-fifth surviving for 5 years [3–6]. The current biomarkers of BC are less than satisfactory for effective prognosis and individualized treatment; thus, new biomarkers for BC are urgently needed.

DNA replication is involved in biological processes such as development, aging and cancer etiology [7]. The minichromosome maintenance gene (MCM) family members are involved in cell cycle processes and DNA replication [8]. This gene family comprises 10 members: serum response factor (*SRF*, as know as *MCM1*), *MCM2-MCM10* [9]. Assembled by six subunits (protein MCM2-MCM7), the MCM complex exhibits helicase activity and participates in DNA replication initiation [10–13]. Although MCM1, MCM8, MCM9 and MCM10 protein are not included in the MCM complex, they are also indispensable for DNA replication [14,15].

Mounting evidence has shown that MCM family members are dysregulated in various malignant tumors, and can be used predict tumor progression and prognosis [16–23]. High mRNA expressions of *MCM2*, *MCM3* and *MCM7* are of great relevance as they indicate poor prognosis for patients with glioma [20]. MCM6 protein has been identified as a driver of S/G2 cell cycle progression; thus, the upregulation of *MCM6* transcriptional level is indicative of adverse tumor features and poor outcomes in hepatocellular carcinoma [21]. The upregulation of *MCM10* transcriptional level is strongly linked to poor overall survival (OS) for lung cancer [22]. Further, the upregulation of *MCM2*, *MCM4*, *MCM6* and *MCM10* in pancreatic cancer correlate with poor prognosis [23]. MCMs play complex and distinct roles in human BC [24,25]. However, the clinical significance and the particular functions of MCMs in BC have not yet been fully elucidated, and required further exploration.

Therefore, we performed our study to analyze the expression levels and genetic alterations of MCMs in BC patients in detail, to determine the expression patterns, potential functions and prognostic values of these markers.

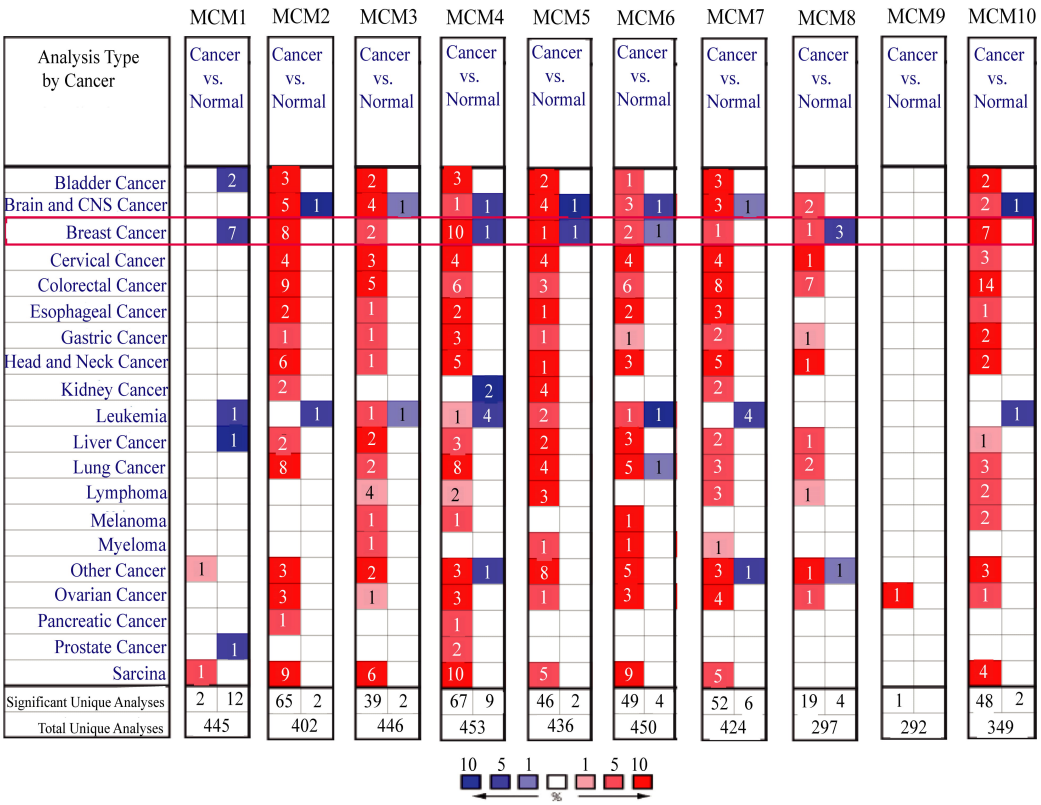

Fig. 1. The mRNA Expression Levels of MCMs in Different Types of Cancers (Oncomine). Red indicates increased expression and blue represents decreased expression. The values in the grid indicate the number of databases. MCM, minichromosome maintenance gene.

2. Material and methods

2.1 MCM-expression analysis

The Oncomine database (http://www.oncomine.org) was used to analyze the mRNA expression levels of MCMs. The comparison of mRNA expression levels of MCMs between BC and normal samples was conducted using the Student's *t*-test. The cut-off values for the *p*-value and fold-changes were as follows: *p*-value < 0.01, fold changes >2. GEPIA (http://gepia.cancer-pku.cn/) was used to validate the transcription levels of MCMs in BC tissues and different pathological stages, as well as to perform the correlation analysis of MCMs with each other. The UALCAN database (http://ualcan.path.uab.edu/) was used to detect the transcriptional expression levels of MCMs in different BC subtypes.

2.2 Kaplan-Meier plotter analysis

The Kaplan-Meier plotter (http://kmplot.com/analysis/) was used to evaluate the correlation between MCM expression and prognosis in BC. BC patients were divided into high and low expression groups according to the median value, and the OS, relapse-free survival (RFS) and distant metastasis-free survival (DMFS) of BC patients were assessed using Kaplan-Meier survival plots. The hazard ratios (HRs), 95% confidence intervals (CIs) and log rank *p*-values less than 0.05, were retrieved from the Kaplan-Meier plotter.

2.3 Immunohistochemistry analysis

The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/) is a valuable tool for studying protein expression profiles in normal tissues, pathological tissues and cell lines [26]. MCM protein expression of BC and normal tissues was determined from the HPA database.

2.4 Mutation and copy-number alteration analysis

The Breast Invasive Carcinoma (TCGA, Firehose Legacy) database, involving 1101 cases, was selected for further analyses of MCMs using cBioPortal (http://www.cbioportal.org). Mutations, putative copy number alterations (CNAs) from the genomic identification of significant targets in cancer (GISTIC), mRNA expression Z scores relative to diploid samples (RNA-seq v.2 RSEM) and protein expression Z scores (reverse phase protein array [RPPA]) were included in the genomic profiles.

2.5 Gene regulatory network analysis

To explore the interaction among MCMs at the protein levels, the STRING online tool (https://string-db.org/) was used to construct a protein-protein interaction (PPI)

network with a confidence score of 0.4. DAVID (https://david.ncifcrf.gov/) was used for gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of MCMs. The related information of their involvement in biological processes (BPs), molecular functions (MFs), cell components (CCs) and different pathways was identified based on p-values < 0.05.

3. Results

3.1 Transcriptional levels of MCMs in BC

The Oncomine database contains and compares available microarray data from multiple cancer types, including BC, with those from normal samples. The expression of MCMs in cancer cells is shown in Fig. 1. For most cancers, members of MCM family were upregulated; for BC, exceptions existed for *MCM1*, for which expression level was downregulated, and for *MCM9*, for where available data were still lacking. MCMs transcriptional levels in BC are summarized in **Supplementary Table 1**. Upregulation of *MCM4* transcriptional level was present in 10 datasets [27–31], followed by *MCM2* in 8 datasets [28,29,31] and *MCM10* in 7 datasets [28,29,31]. However, owing to the relatively small sample size, whether the expression of *MCM5*, *MCM6* and *MCM8* was elevated in BC remains controversial.

3.2 Relationship between MCM mRNA and protein expression levels in BC

Further investigation of the transcriptional levels of the MCMs in BC and in different subtypes using GEPIA online tool and UALCAN database showed that *MCM2*, *MCM4* and *MCM10* were significantly upregulated in the tumor tissues compared with expression in normal tissues, but there was no significant difference in the expression of the other MCMs between BC and normal tissues (Fig. 2A); when divided into different subtypes of breast invasive carcinoma, the results showed that almost all MCM family members were significantly upregulated in these subtypes compared with levels in normal sample, except *MCM1* and *MCM9*, which were downregulated; of these, the expression levels of *MCM1* in triple negetive BC and *MCM9* in luminal and triple nagetive BC were not significantly different compared with those in normal tissues (Fig. 2B). IHC (Immunohistonchemistry, IHC) staining images for MCM proteins in BC and normal sample obtained from the HPA database demonstrated that MCM2, MCM4 and MCM10 protein levels were more highly expressed in BC tissue than in their counterparts, whereas others were not significantly different in BC tissue (Fig. 3).

3.3 Association between mRNA expression of MCMs and BC patient prognosis

To further investigate the critical role of MCMs in predicting the prognosis of BC patients, we analyzed the association between MCMs transcription levels and BC patients survival using Kaplan-Meier plotter analysis. The BC patients were divided into high and low expression groups according to the median value. High expression levels of MCM family members were significantly correlated with OS, RFS and DMFS: *MCM2*, *MCM4, MCM5, MCM6, MCM7* and *MCM10* correlated with worse OS, RFS, and DMFS, *MCM8* correlated with the poor DMFS, *MCM9* correlated with better OS and RFS, and *MCM1* correlated with better RFS (Fig. 4, **Supplementary Fig. 1**). In addition, we found that the expression levels of *MCM2, MCM3, MCM7* and *MCM10* varied significantly in different tumor stages of BC, indicating that they are associated with clinical stages (Fig. 5).

3.4 Alteration to MCMs in BC and correlations among them

We next analyzed MCM alterations using the cBioPortal, which is an online database for Cancer Genomics, providing images and analyses of large-scale cancer genomics datasets [32]. As shown in Fig. 6, the most common genetic change among 1101 BC patients was high mRNA expression (Fig. 6A); MCMs were altered in 494 BC patients (45%) and multiple alterations were detected in 129 (11.8%) (Fig. 6B). Among them, *MCM4, MCM10* and *MCM2* alterations were associated with 23%, 16%, and 11% of BC cases, respectively surpassing the alterations to other MCMs. The relationship between MCM mRNA levels was analyzed using the GEPIA online tool coupled with Pearson's correction (Fig. 6C). In BC, a close correlation between the mRNA expression of *MCM2*-*MCM7*, but not between *MCM1* and the other nine MCMs was found, and the following MCMs showed significant and positive correlations: *MCM8* with *MCM2*, *MCM3*, *MCM5*, *MCM6* and *MCM10*; *MCM9* with *MCM4*; *MCM10* with *MCM2-7* and *MCM8*. Among them, *MCM2* and *MCM6* exhibited the highest positive correlation with a Spearman's correlation coefficient of 0.77.

3.5 MCM-regulated biomolecular network analysis of BC genomics

To explore how MCMs exert their regulatory effects on the occurrence and development of BC, potential MCM-regulated genes were obtained from the STRING online tool. MCM-related gene network showed that MCMs had a close correlation with 50 genes, which were cell cycle-related or involved in DNA damage/repair, including POLA1, ORC6, RPA3, CDC7 and RFC2 (Fig. 6D). Further functions related to pathways of MCMs and their frequently altered neighbor genes were predicted by analyzing GO and KEGG processes in the DAVID. GO enrichment analysis is based on three aspects to predict the functional roles of target host genes. These results indicated that the MCMs in the BPs of GO enrichment were markedly related to DNA replication, telomere maintenance, telomere organization, and cell cycle transition (Fig. 7A). For CCs of GO en-

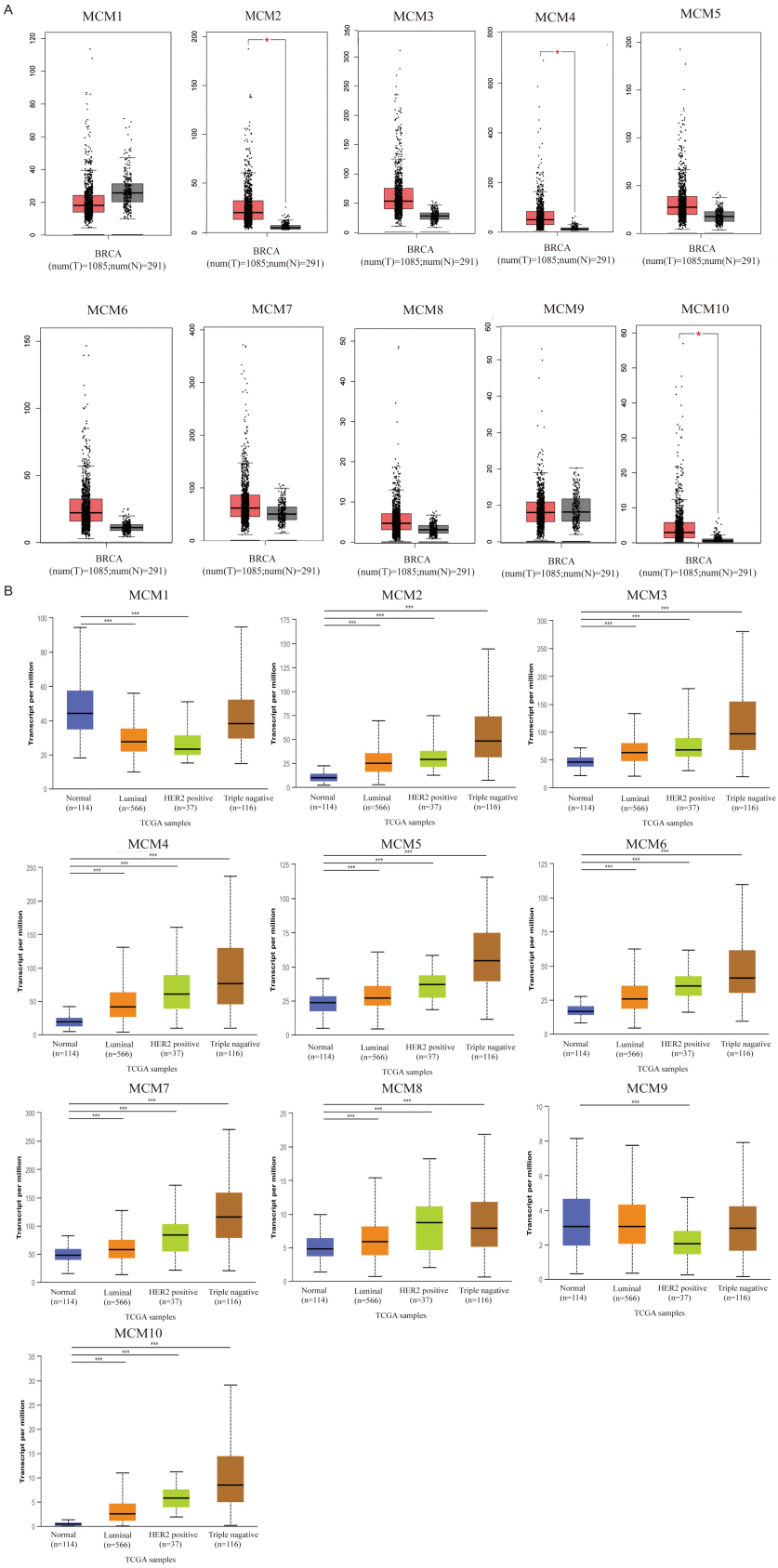

Fig. 2. The mRNA Expression of MCMs in BC (GEPIA, UALCAN). (A) The mRNA expression of *MCM1-MCM10* in BC. (B) The mRNA expression of *MCM1-MCM10* in major subclasses of BC. *$p < 0.05$; ***$p < 0.001$; MCM, minichromosome maintenance gene; T, tumor; N, normal; num, number.

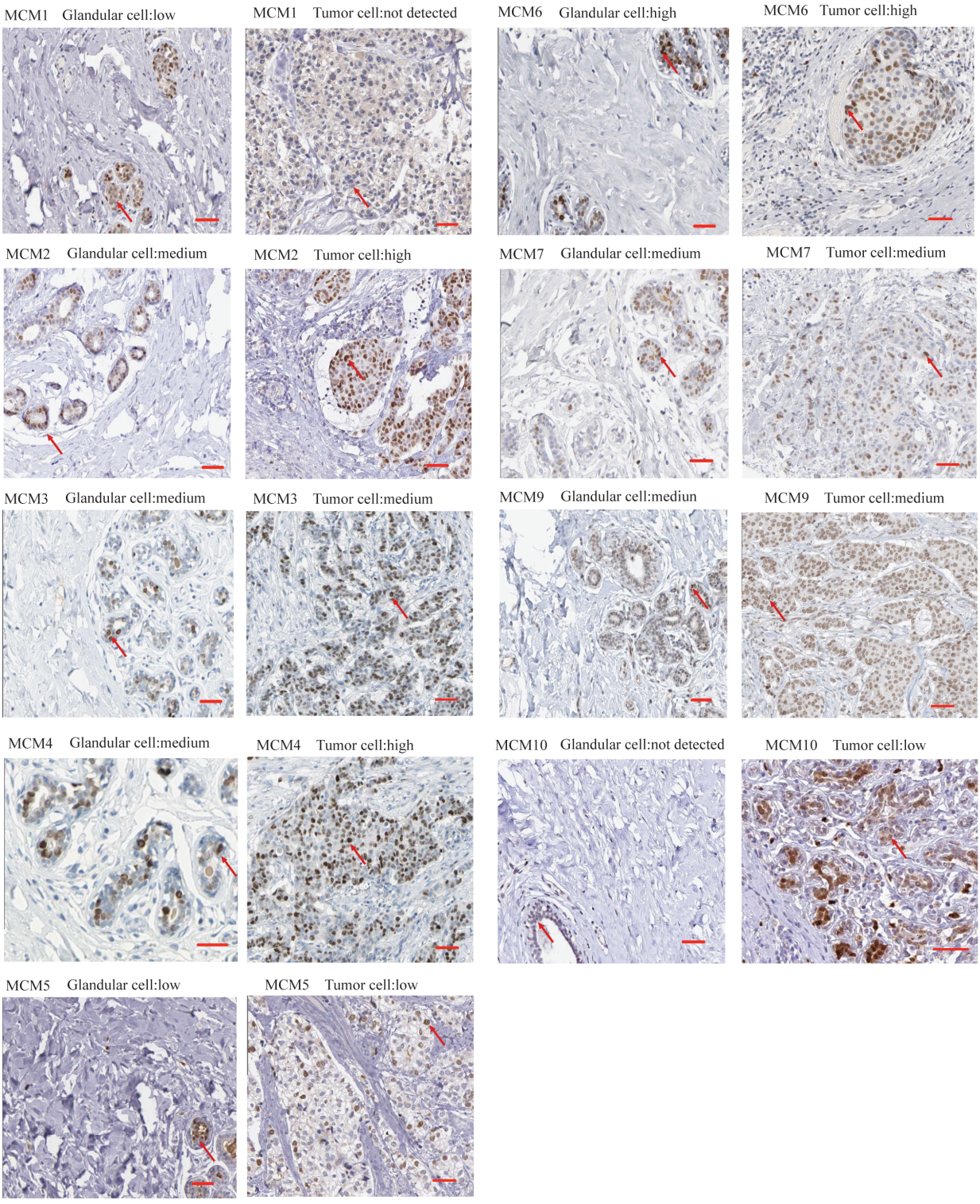

Fig. 3. Immunohistochemistry Staining Images of MCMs Proteins in BC (HPA). The red arrows refer to glandular cell in normal tissue (left) and tumor cell (right) in tumor tissue. Scale bar = 50 μm. The total staining score of tissues is from not detected to high. MCM, minichromosome maintenance gene.

richment, the MCMs were particularly enriched in nuclear chromosome parts, chromosomal regions, replication forks and protein-DNA complexes (Fig. 7B). In addition to MF, MCMs were remarkably associated with chromatin binding, damaged DNA binding, single-stranded DNA binding and telomeric DNA binding (Fig. 7C). Moreover, the results of KEGG analysis demonstrated that MCMs were enriched in the cell cycle, DNA replication, nucleotide excision repair, mismatch repair, and homologous recombination (Fig. 7D).

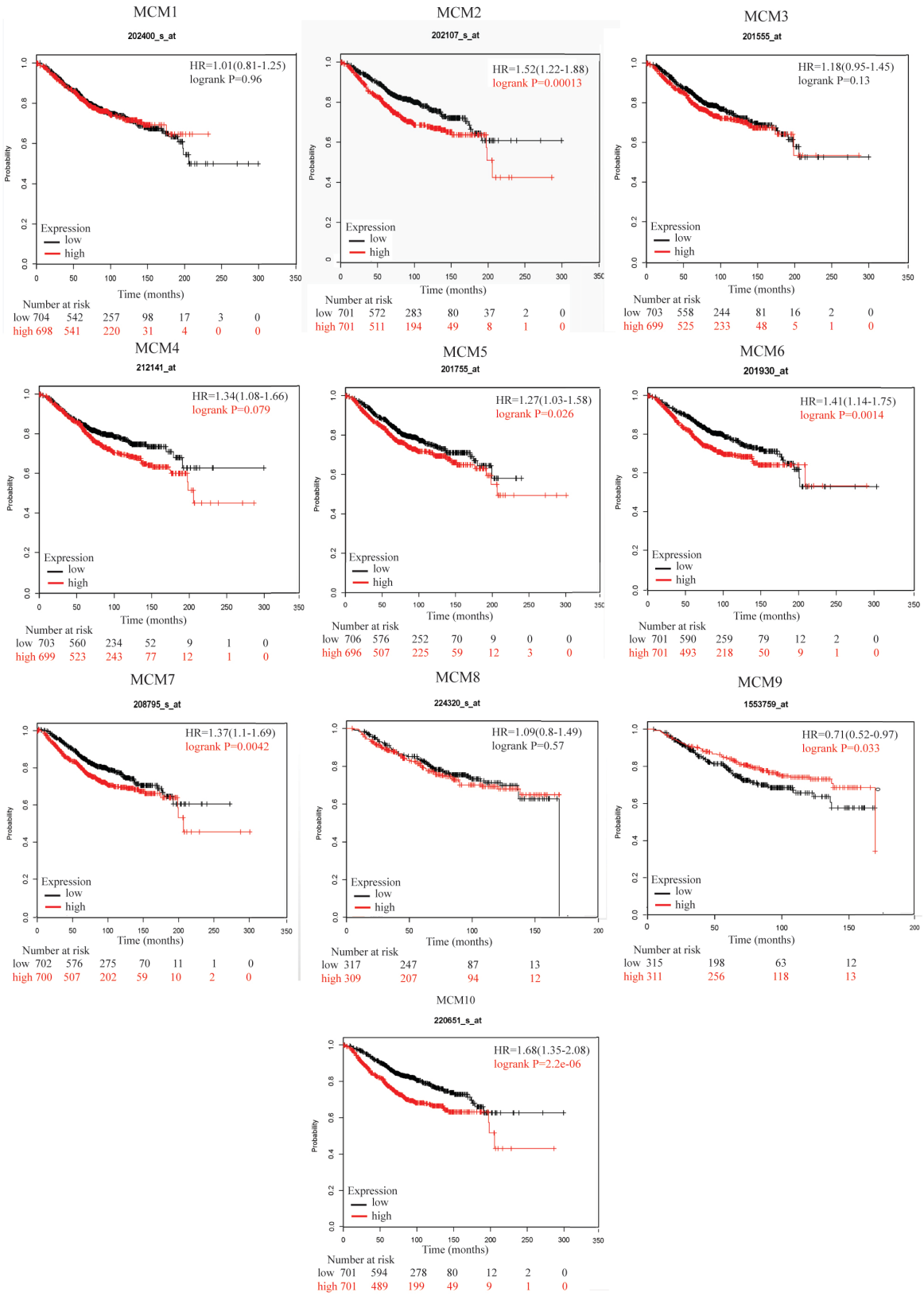

Fig. 4. The Prognostic Value of mRNA Level of MCM Factors in BC Patients (Kaplan-Meier Plotter). OS of *MCM1-MCM10* plotted for all patients (OS: n = 1402). HR with 95% CIs are displayed. *p*-values were calculated by log-rank test. Logrank *p* less than 0.05 is displayed in red font, indicating that the difference is significant. OS, overall survival; HR, hazard ratio; CIs, confidence intervals; MCM, minichromosome maintenance gene.

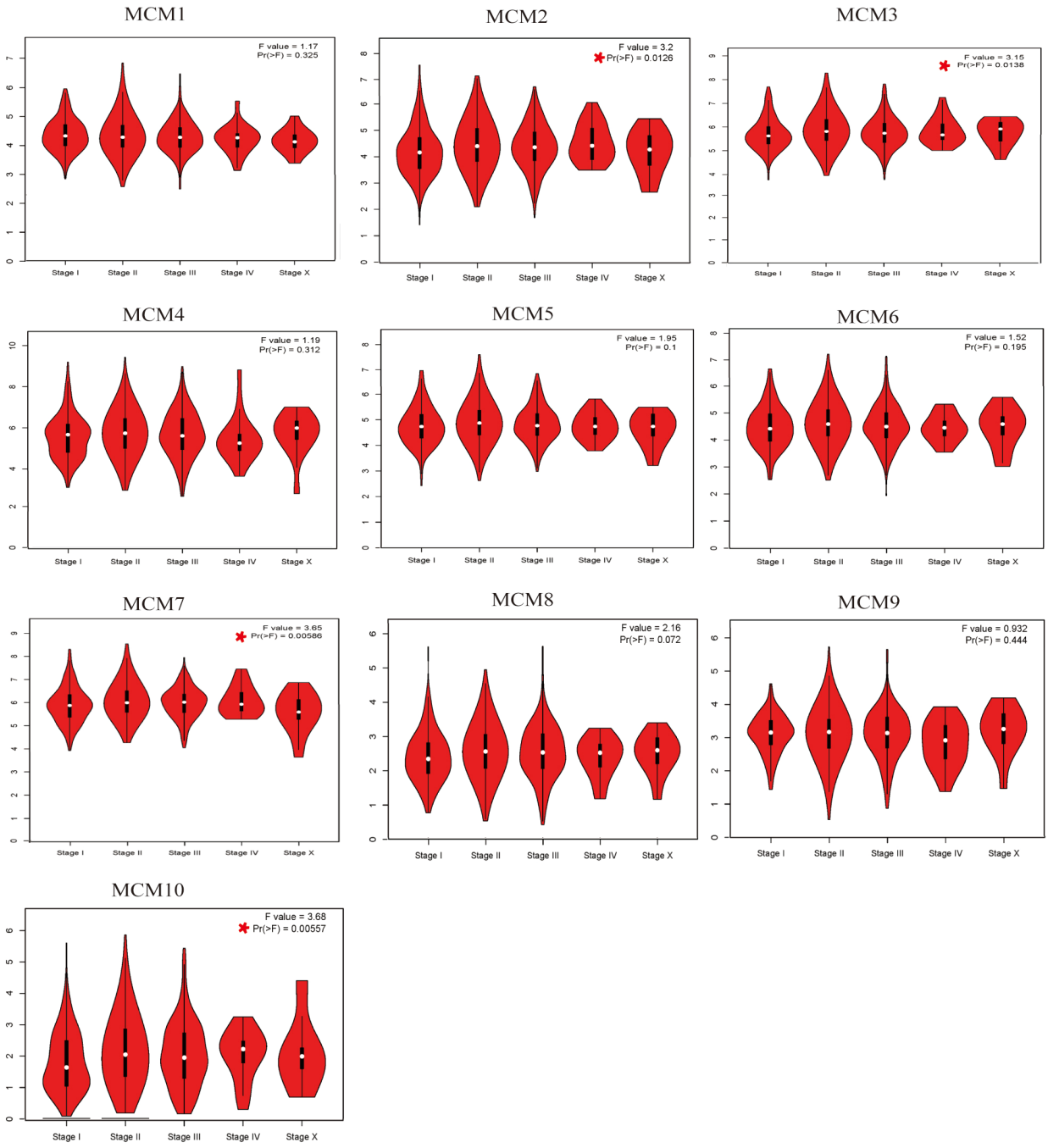

Fig. 5. Correlation between MCM Expression and Tumor Stage in BC Patients (GEPIA). In the violin charts, the white dots, black bars and black lines represent the median, 95% CIs and interquartile range respectively; the distribution density is represented by the width of the red shape. CIs, confidence intervals; MCM, minichromosome maintenance gene. F-value, the statistical value of F test (one-way ANOVA); Pr (>F), *p*-value. *Pr (>F) $\leq$0.05 indicates MCM expression is significantly different among the tumor stages I to IV.

4. Discussion

The proliferation of neoplastic cells is usually accompanied by dysregulated DNA replication [33]. As an essential molecule in DNA replication, MCM protein were found to be a determinant of the initiation and progression of malignancy [34]. MCM mutations appear more frequently in neoplastic cells than in normal cells [35], which leads to chromosome loss, DNA damage and increased recombination [36,37]. The role of MCM factor dysregulation in the tumorigenesis and prognosis of cancers has been partially confirmed, and further bioinformatics analysis of BC had not yet been performed.

MCM1, as a member of the MADS box transcription factor family, affects processes such as cell cycle, growth,

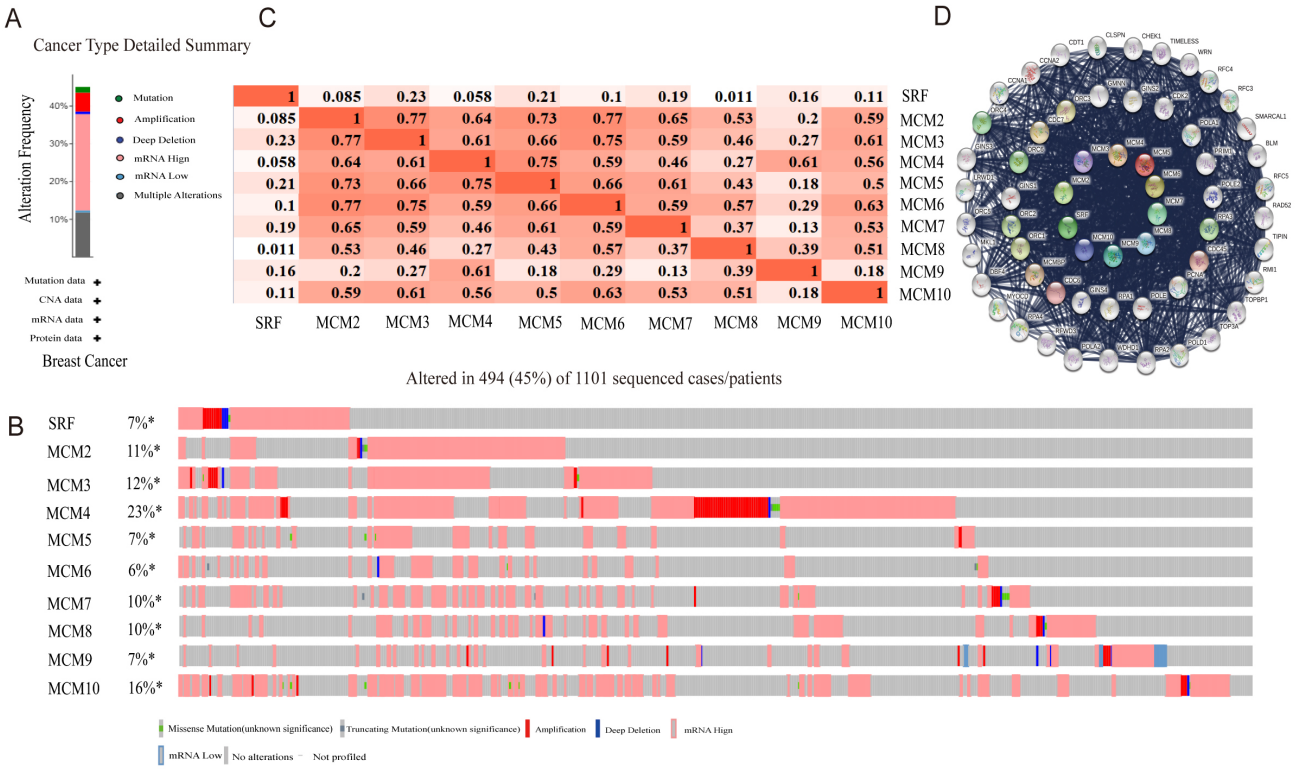

Fig. 6. MCM mRNA Expression and Mutation Analysis in BC (cBioPortal, GEPIA, STRING). (A) The summary of alteration frequency of MCMs in BC. (B) Genetic alterations of each MCM family member in BC patients. (C) Correlation among mRNA expression of each MCM family member; Spearman's correlation coefficients are presented. (D) The network for MCMs and the 50 most frequently altered neighbor genes. MCM, minichromosome maintenance gene.

differentiation and apoptosis by regulating the activation of neighbor genes [38]. The current study revealed that the downregulation of *MCM1* was significantly associated with HER2 positive BC and luminal BC, and with poor RFS.

The MCM 2–7 complex is a major factor in DNA replication initiation and elongation [39]. Our results showed that the transcriptional levels of all members of MCM complex in BC and its subclasses were upregulated and that they were closely correlated with each other, which could indicate their regulation based on similar transcription factors or signaling pathways. Combining the analysis of multiple databases, the mRNA expression levels of *MCM2, MCM4* and *MCM10* were significantly upregulated, whereas others were only slightly increased in BC tissue. Upregulated mRNA expression of *MCM2, MCM4* and *MCM10* was in accordance with protein expression levels and was associated with poor OS, RFS and DMFS. The genetic alteration rates of *MCM4, MCM10* and *MCM2* were highest, which might be the reason for their mRNA upregulation. As a member of the MCM protein complex family, the mRNA expression of *MCM2* was firmly established as instrumental in DNA replication and cell proliferation [40,41]; therefore, the precise control of *MCM2* expression is crucial for the maintenance of genomic stability [42]. It has been reported that the knockdown of *MCM2* prevents tumor cells from proliferating [43], whereas high expression of *MCM2* is positively associated with tumor size [44]. The present study also found that *MCM2* was correlated with tumor stage, which is in agreement with the findings of Wojnar *et al.* [24]. The *MCM4* gene mutation destabilizes the MCM 2–7 complex, causing DNA replication impairment and chromosome instability, which indicates its causative role in BC progression [45,46]. A previous study showed that *MCM4,* for which a mutation was determined to be a causative factor in BC progression, could be a potential novel prognostic and predictive indicator [19,46]. Although not included in MCM 2–7 complex, *MCM10* has a critical role in the initiation of DNA replication and tumor progression through interactions with the MCM 2–7 complex [47,48]. *MCM10* was reported to induce BC metastasis via the Wnt/β-catenin pathway and can be defined as a potential diagnostic biomarker and a promising target for BC [25]. However, Kwok *et al.* [19] demonstrated that the converging overexpression of more than four MCMs at the mRNA level is associated with a significantly higher risk of poor prognosis.

As to other members of the MCM 2–7 complex, the expression levels of *MCM3*, *MCM5*, *MCM6* and *MCM7* differed significantly only between normal tissue and each major subclass of invasive BC. MCM3 protein was reported

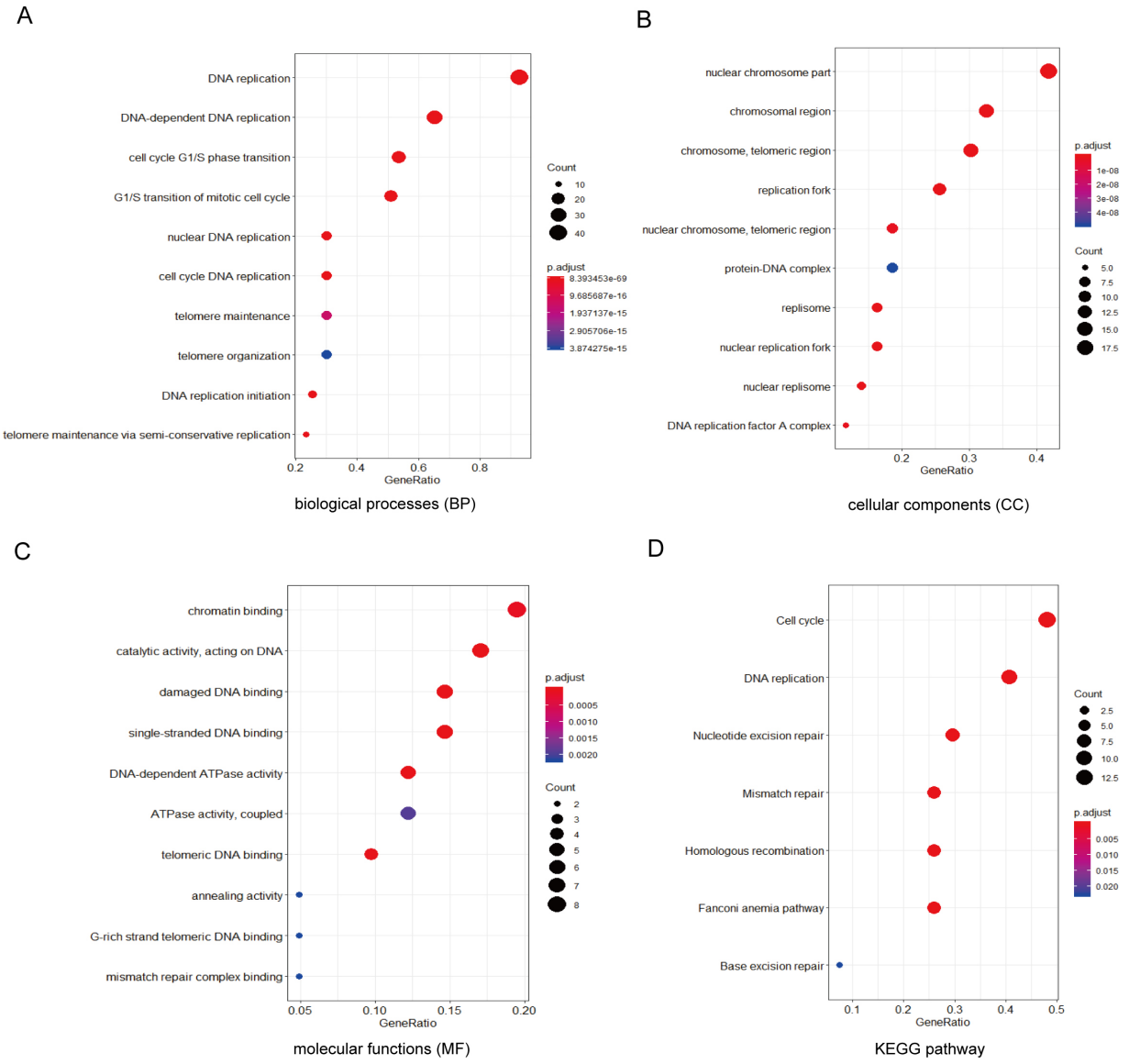

Fig. 7. The GO and KEGG Enrichment Analysis in BC (DAVID). GO enrichment analysis predicted the functional roles of target host genes based on three aspects by DAVID tools, including (A) BP (B) CC and (C) MF. (D) Alterations were predicted by the analysis of KEGG. The size of the dots represents the count of the enriched MCMs, and the dot color represents the adjusted *p*-value.

to be a contributing factor to cell proliferation, and high *MCM3* expression is association with poor prognosis in invasive ductal carcinoma patients [49]. However, no association between *MCM3* expression and BC patient outcomes was found in the present study. However, a significant correlation was observed between *MCM3* mRNA expression and tumor stage. *MCM5* is involved in the cell cycle regulation [50]. High expression level of *MCM5* was proved to be significantly associated with poor prognosis in BC patients in the current study. *MCM6* also appears to have a prognostic value. In our report, *MCM6* upregulation was found to be significantly associated with poor prognosis in BC patients, which is in agreement with the study by Kwok, H. F. *et al*. [19]. As a licensing factor crucial for DNA replication, when MCM7 protein is indirectly phosphorylated by activated epidermal growth factor receptor, its phosphorylation increases its association with other MCM proteins, thereby promoting DNA synthesis complex assembly, together with cell proliferation, which is correlated with poor BC patient survival [51]. In the current study, high expression level of *MCM7* was associated with tumor stage and adverse prognosis.

Of the remaining members of the MCM family, *MCM8* might be associated with chromosomal instability [52]. The current study showed that high *MCM8* expression is associated with poor prognosis. Little is known about the role of *MCM9* in BC. The prognostic value of *MCM9* for BC has not been investigated yet; however, its upregulated

was found to be associated with better OS and RFS in our study.

5. Conclusions

In conclusion, we analyzed the mRNA and protein expression, gene alterations, prognostic values, and functions of MCMs in BC. Our results indicated that the mRNA and protein expression of *MCM2, MCM4* and *MCM10* was upregulated in BC patients, which was associated with poor OS, RFS and DMFS. Furthermore, genetic alterations were comparatively more frequent for *MCM2*, *MCM4*, and *MCM10*, which themselves appeared to directly affect DNA replication impairment and chromosome instability in BC, or indirectly affect these processes—through mutations affecting adjacent cell cycle-related genes or those involved in DNA damage/repair. Our findings suggest that *MCM2*, *MCM4* and *MCM10* are potential prognostic markers and therapeutic targets for BC.

Author contributions

JX—Performing the research, visualization, writing-Original draft preparation. YZ—Designing the study, data analysis, writing-Original draft-review & editing. YG—Designing the study, conceptualization, Data curation.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/EJGO/43/2/10.31083/j.ejgo4302029.

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