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Research article

Computational assessment of MCM2 transcriptional expression and identification of the prognostic biomarker for human breast cancer

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ABSTRACT

Minichromosome maintenance protein 2 (MCM2) is a highly conserved protein from the MCM protein family that plays an important role in eukaryotic DNA replication as well as in cell cycle progression. In addition, it maintains the ploidy level consistency in eukaryotic cells, hence, mutations or alteration of this protein could result in the disintegration of the fine-tuned molecular machinery that can lead to uncontrolled cell proliferation. Moreover, MCM2 has been found to be an important marker for progression and prognosis in different cancers. Therefore, we aimed to analyze the MCM2 expression and the associated outcome in breast cancer (BC) patients based on the publicly available online databases. In this study, server-based gene expression analyses indicate the upregulation of MCM2 ($p < 10^{-6}$; fold change>2.0) in various BC subtypes as compared to the respective normal tissues. Besides, the evaluation of histological sections from healthy and cancer tissues showed strong staining signals indicating higher expression of MCM2 protein. The overexpression of MCM2 was significantly correlated to promoter methylation and was related to patients' clinical features. Further, mutation analysis suggested missense as the predominant type of mutation (71.4%) with 18 copy-number alterations and 0.2% mutation frequency in the *MCM2* gene. This study revealed a significant correlation (Cox $p \leq 0.05$) between the higher MCM2 expression and lower patient survival. Finally, we identified the co-expressed genes with gene ontological features and signaling pathways associated in BC development. We believe that this study will provide a basis for MCM2 to be a significant biomarker for human BC prognosis.

1. Introduction

Cancer is a group of diseases that can develop anywhere in a body through abnormal cell proliferation. It is the second leading cause of death worldwide accounting for 1 out of every 6 deaths, hence, becomes one of the important concerns for human survival [1]. In 2018, about 18.1 million cancer cases were reported with an estimated 9.6 million deaths worldwide. Among them, about 2.1 million (11.6%) cases were BC in female patients with 0.63 million (6.6%) deaths [2]. At the early stages, BC shows no or a few symptoms. Due to the lack of awareness and difficulties in detection at an early stage, the death rate in BC patients is increasing gradually. Therefore, early diagnosis can potentially reduce the BC-associated death rate by providing time for the proper treatment plan. Furthermore, genetic or epigenetic alterations of genes can change their expression patterns which may facilitate the development of cancers. Differentially expressed genes (DEGs) can be the possible markers for the therapeutic target as it reflects cancer characteristics related to the patient's prognosis.

The cell cycle is the process of genome duplication and cell division which leads to the proliferation of cells [3]. It has four phases such as G1

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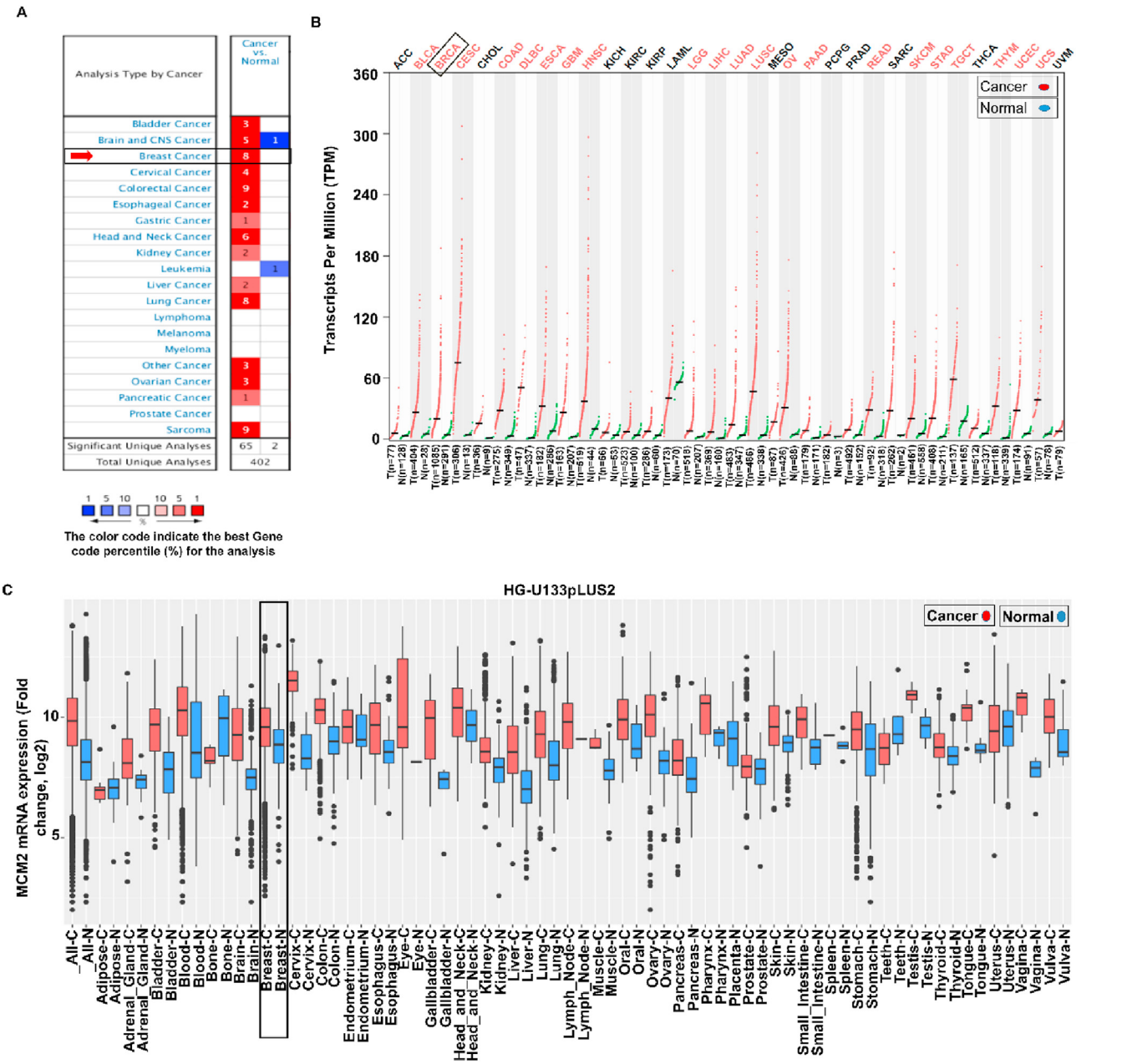

Figure 1. Tissue-wide gene expression pattern of MCM2 in multiple human cancer, (A) comparison between cancer versus normal tissues in which high and low expression of mRNA has indicated by red and blue colors, respectively, (B) The dot plot shows the gene expression profile of the MCM2 gene in 33 types of human cancers including tumors and paired normal tissue samples. Herein, red and green dashed lines represent the average expression value in all tumor and normal tissues, respectively, and (C) box-plot showing the MCM2 mRNA expression in tumors and respective normal tissues based on the GENT2 database where boxes represent by the median, dots indicate outliers, red-boxes define the tumor tissues, and blue-boxes is normal tissues.

(Gap 1), S (DNA synthesis), G2 (Gap 2), and M (mitosis and cytokinesis); and contains multiple checkpoints throughout the process to ensure the proper segregation and replication of chromosomes into daughter cells [4]. Each stage in the cell cycle undergoes a strict regulation by different factors [5]. For instance, proteins under the MCM family ensure that the chromosomal replication occurs only once per cell cycle [6]. MCM proteins 2–7 interact with origin recognition complex (ORC) and bind to replication origin, cell division cycle 6 (Cdc6), chromatin licensing, and DNA replication factor 1 in the early G1 phase resulting in the formation of the pre-replication complex which is essential for DNA replication, initiation, and elongation [7]. MCM proteins have also been found to play an interior role in genome stability [8]. Alteration in any of these factors can cause serious problems in cell cycles leading to uncontrolled cell growth followed by the development of neoplasm [4]. MCM proteins have been reported to be significant in cancer initiation and development. The gene expression pattern of these proteins has been found to be associated with a wide range of epithelial malignancies and indicates that the up-regulation of MCM proteins may occur at either genomic or transcriptional levels [9].

MCM2, also known as DNA replication licensing factor, is one of the members of the MCM protein family. It is an important element of the pre-replication complex (pre-RC) that regulates the helicase activity resulting in the formation of the replication forks. The helicase activity of the MCM2 protein plays a critical role in the initiation of DNA replication and unwinding of the DNA strands [10]. The overexpression of MCM2 protein correlates with cell proliferation and carcinogenesis [11]. Therefore, it can be utilized as the diagnostic and prognostic tool for human malignancy in the clinical setting [1]. The overexpression of

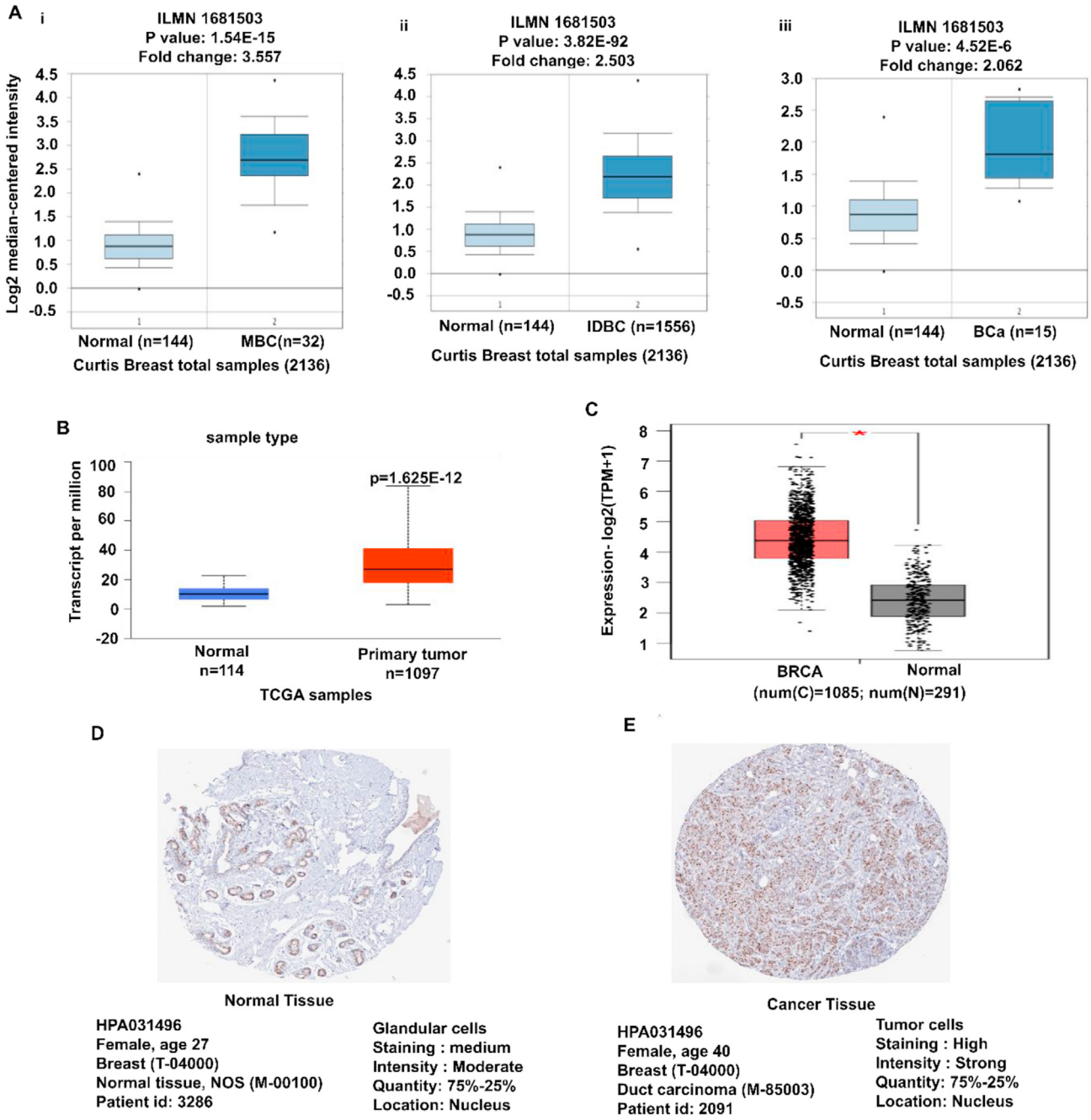

Figure 2. MCM2 expression analysis in various BC subtypes in which (A) box-plots showing comparative expression between normal (left) and cancer tissue (right) - MBC (i), IDBC (ii), and BCa (iii), (B–C) box-plots showing the expression of MCM2 mRNA in breast cancer and normal tissues using the UALCAN and GEPIA2 servers, respectively, and (D–E) the immunohistochemistry images of MCM2 in BC tissues and normal tissues retrieved from the HPA database. Abbreviation: MBC = Medullary breast carcinoma, IDBC = Invasive Ductal Breast Carcinoma, BCa = Breast carcinoma, n = number of samples, HPA = Human Protein Atlas.

MCM2 mRNA (messenger RNA) indicated that it can be a prospective biomarker for the diagnosis of human BC, colorectal cancer [12], anal neoplasia [13], esophageal [14], and bladder cancers [15]. In prostate, lung, ovary, and renal cancers, overexpression of MCM2 protein is related to regional recurrence, shorter survival of patients, and distant metastases [16, 17, 18]. Previously, two independent prognostic markers MCM5 and MCM6 have shown to overexpress in ovarian cancer and melanoma, respectively [17,19]. Likewise, MCM7 expression was demonstrated to be a remarkable biomarker for cervical cancer as well as a prognostic factor in colorectal, lung, and ovarian cancer [20,21].

Human BC is a heterogeneous disease with different neoplasms originating from the epithelial cells. The molecular underpinning of the BC cells led to the identification of prognostic tools to estimate therapeutic reaction and predict gene expression signatures which can lead to the long-term survival of the patients [22]. Traditional prognostic and predictive features including the status of lymph node, size of the tumor, histological grade, and types of the hormonal receptors (i.e., estrogen and progesterone receptors) are not sufficient for prognosticating and early-stage determination of the disease [23]. Hence, there is a need for potential biomarkers to detect primary and operable BC at early stages, which can reduce the burden of overtreatments [22]. In human BC, the MCM2 is thought to have a strong prognostic value since overexpression of MCM2 is correlated with patient survival, regional recurrence, and distant metastases [23]. Thus, the relevance of the distinct up-regulation

Table 1. The expression of MCM2 in the various subtype of BC from the Oncomine database.

Datasets	Parameters	Samples	Cox *p*-value	Gene rank	Fold change
Curtis Breast (n = 2136)					
	Normal	144			
	Medullary breast carcinoma	32	1.54E-15	112	3.557
	Invasive Ductal Breast Carcinoma	1556	3.82E-92	214	2.503
	Breast carcinoma	14	4.52E-6	371	2.062
TCGA Breast (n = 553)					
	Normal	61			
	Mixed Lobule and Ductal Breast Carcinoma	7	1.18E-6	183	2.200
	Invasive Lobular Breast Carcinoma	36	1.06E-12	506	2120
	Invasive Ductal Breast Carcinoma	389	5.24E-31	554	2.877
	Invasive Breast Carcinoma	76	1.30E-17	795	2.184
Richardson Breast (n = 47)					
	Normal	7			
	Ductal Breast Carcinoma	40	2.30E-5	1223	4.065

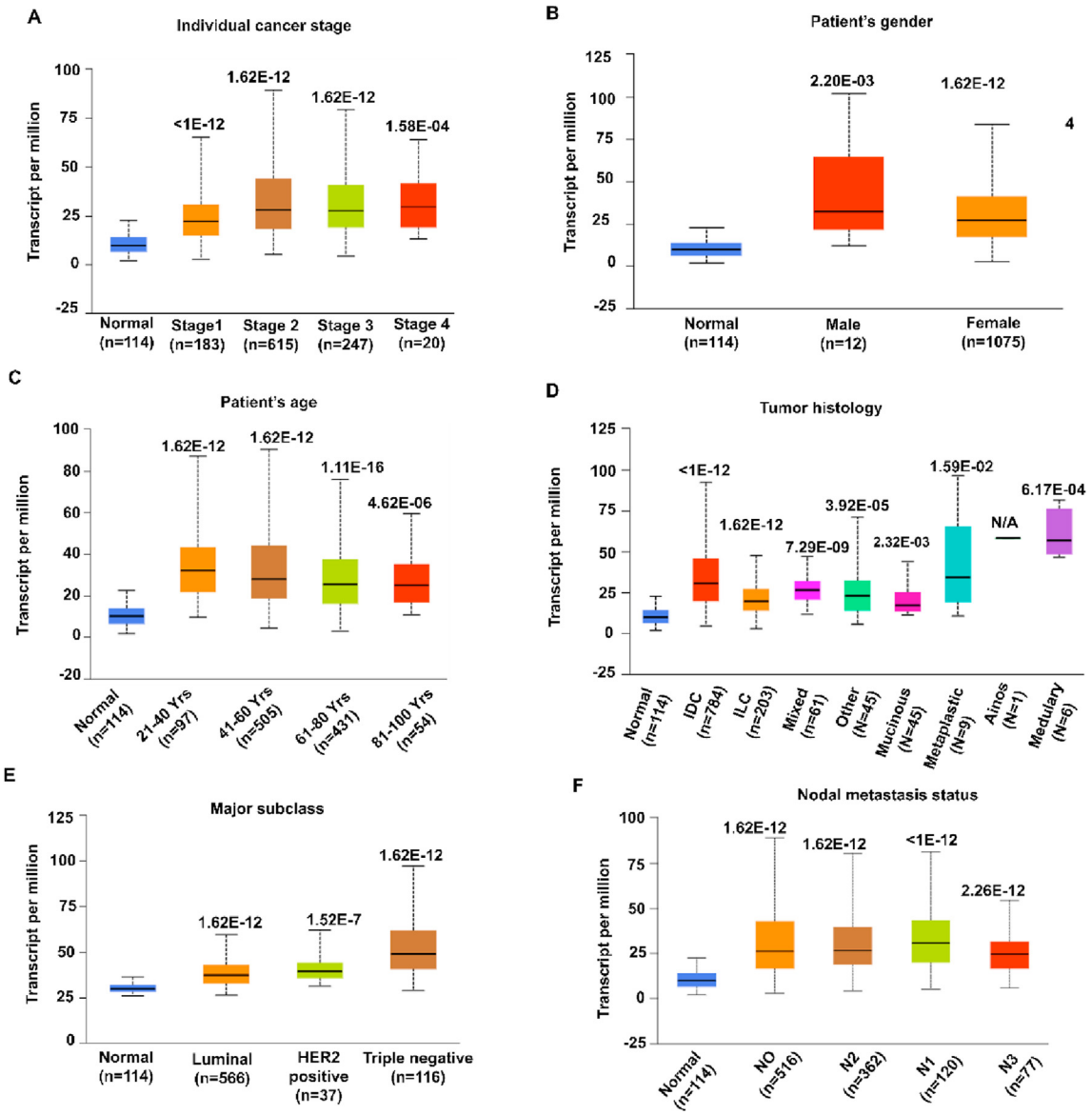

Figure 3. The expression analysis of MCM2 with clinical characteristics of BC patients. The MCM2 mRNA expression in BRCA showing (A) individual cancer stage, (B) patient's gender, (C) age group, (D) tumor histology, (E) major subclasses, and (F) nodal metastasis. These graphs were generated by comparing significant changes between normal variables and other variables. Abbreviation: HER2, human epidermal growth factor receptor 2; IDC, infiltrating ductal carcinoma; Mixed, mixed histology; Medullary, medullary carcinoma; INOS, infiltrating carcinoma NOS; ILC, infiltrating lobular carcinoma; Mucinous, mucinous carcinoma; Metaplastic, metaplastic carcinoma; N0, no regional lymph node metastasis; N1, metastases in 1–3 axillary lymph nodes; N2, metastases in 4–9 axillary lymph nodes; N3, metastases in 10 or more axillary lymph nodes.

Table 2. The relationship between MCM2 and clinicopathological features of breast cancer.

Type of samples	Expression of mRNA*	Number of samples	Cox *P*-value
Normal	↓	114	
Tumor	↑	1097	1.62E-12
Individual cancer stages			
Normal	↓	114	
Stage 1	↑	183	<1E-12
Stage 2	↑	615	1.62E-12
Stage 3	↑	247	1.62E-12
Stage 4	↑	20	1.54E-04
Patient's race			
Normal	↓	114	
Caucasian	↑	748	1.62E-12
African American	↑	179	<1E-12
Asian	↑	61	6.65E-10
Patient's gender			
Normal	↓	114	
Male	↑	12	2.20E-03
Female	↑	1075	1.60E-12
Patient's age			
Normal	↓	114	
21-40 Yrs	↑	97	1.62E-12
41-60 Yrs	↑	505	1.62E-12
61-80 Yrs	↑	431	1.11E-16
81-100 Yrs	↑	54	4.62E-06
Major subclasses			
Normal	↓	114	
Luminal	↑	566	1.62E-12
HER2 positive	↑	37	1.52E-07
Triple-negative	↑	116	1.62E-12
Major subclasses (incl. TNBC type)			
Normal	↓	114	
Luminal	↑	566	1.62E-12
HER2pos	↑	37	1.52E-07
TNBC-BL1	↑	13	2.74E-07
TNBC-BL2	↑	1113	1.98E-04
TNBC-IM	↑	2011	1.25E-06
TNBC-LAR	↑	820	1.11E-01
TNBC-MSL	↑	8	4.70E-03
TNBC-M	↑	29	2.77E-06
TNBC-UNS	↑	27	3.90E-06
Menopause Status			
Normal	↓	114	
Pre-menopause	↑	230	1.62E-12
Perimenopause	↑	37	5.18E-07
Post-menopause	↑	700	1.62E-12
Tumor histology			
Normal	↓	114	
IDC	↑	784	<1E-12
ILC	↑	203	1.62E-12
Mixed	↑	29	7.28E-09
Other	↑	45	3.92E-05
Mucinous	↑	17	2.32E-03
Metaplastic	↑	9	1.58E-02
INOS	↑	1	N/A
Medullary	↑	6	6.16E-04
Nodal Metastasis status			
Normal	↓	114	
NO	↑	516	1.62E-12
N1	↑	362	1.62E-12
N2	↑	120	<1E-12
N3	↑	77	2.26E-12

*Down-arrow (↓) indicates the underexpression while up-arrow (↑) indicates the overexpression.

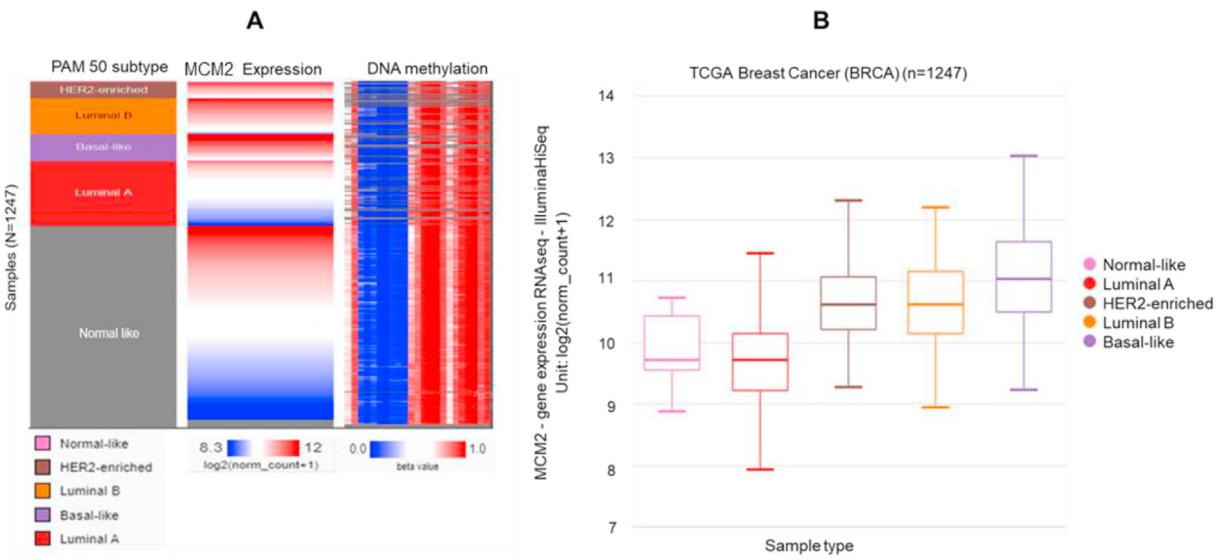

Figure 4. Promoter methylation of the *MCM2* gene in BC tissue. (A) Heat map of MCM2 expression and DNA methylation status; (B) MCM2 expression in different breast cancer DNA methylation clusters. The results were generated using the UCSC Xena server based on TCGA dataset. Red color indicates higher expression while blue color indicates lower expression.

Table 3. A list of MCM2 mutational positions and types in BC from the TCGA dataset.

Sample ID	Cancer type	Protein change	Mutation type	Number of samples
TCGA-A2-A0T5-01	Breast Invasive Ductal Carcinoma	H287P	Missense	1067
TCGA-BH-A2L8-01	Breast Invasive Lobular Carcinoma	E254	Nonsense	279
TCGA-BH-A0HF-01	Breast Invasive Ductal Carcinoma	E184K	Missense	551
TCGA-BH-A2L8-01	Breast Invasive Lobular Carcinoma	E254	Nonsense	423
TCGA-A2-A0T5-01	Breast Invasive Ductal Carcinoma	H287P	Missense	1076
TCGA-A2-A04Q-01	Breast Invasive Ductal Carcinoma	M706I	Missense	19
TCGA-A8-A08R-01	Invasive Breast Carcinoma	H287P	Missense	128

feature of MCM2 during human BC led us to undertake a comprehensive analysis to further explore its expression pattern in human BC.

2. Materials and methods

2.1. Expression analysis of MCM2 in various cancers and normal tissues

The expression patterns of *MCM2* transcript (mRNA) in various cancers and respective normal tissues were analyzed using the Oncomine (https://www.oncomine.org) [24,25], GEPIA2 (http://gepia2.cancer-pku.cn) [26,27], and GENT2 (http://gent2.appex.kr) databases [28,29]. The Oncomine database contains microarray data from 86,733 tumors and 12,764 normal tissues in which eight datasets were used for comparative analysis. The threshold value was set to fold change>2, p-value $< 10^{-4}$, mRNA as data type, and 10% of gene ranking. The GEPIA2 database includes the expression data of 9,667 tumors and 602 healthy tissues from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) portal. On the other hand, the GENT2 contains normal and tumor tissue data from GPL570 platform (HG-U133_Plus_2). For GEPIA2 and GENT2 databases, all the MCM2 queries regarding breast cancer were performed with default settings.

2.2. Expression analysis of MCM2 in normal and cancerous human breast tissues

The mRNA expression levels of *MCM2* gene in BC and normal tissue counterparts were evaluated with the Oncomine [24,25], UALCAN (http://ualcan.path.uab.edu/) [29,30], GEPIA2, and HPA (https://www.proteinatlas.org/) databases [31]. The GEPIA2 database contains data from 1,085 tumors and 291 normal tissues related to BC where the type of cancers can be predicted by the query sample based on the intensity of gene expression. The UALCAN is a web-resource to perform in-depth analysis of the cancer transcriptome such as TCGA expression data [29,30]. The UALCAN webserver contains data from 1097 cancers and 114 normal tissues where users can determine the potential biomarkers and validate the potential gene of interest. Using this server, we compared the relative patterns of *MCM2* expression in cancers from the TCGA database. The analysis of MCM2 mRNA expression was performed with default settings and the cox p-value less than 0.05 was considered as statistically significant. Furthermore, the immunohistochemistry of MCM2 protein in BC and normal tissues was visualized using the HPA031496 dataset deposited in the HPA server [31].

2.3. Association of MCM2 expression in clinical features and promoter methylation

The mRNA expression of the MCM2 gene in BC patients was analyzed based on their clinicopathological characteristics in TCGA datasets with the UALCAN server [29,30]. The analysis was performed by comparing breast cancer tissues with healthy tissues where all the parameters were kept defaults. We used the UCSC Xena server (https://xenabrowser.net/) to visualize and analyze the functional genomics data, for example, DNA methylation. In doing so, we collected DNA methylation data of 1247 BC samples from TCGA datasets [32]. Herein, we considered cox p-value less than 0.05 as statistically significant.

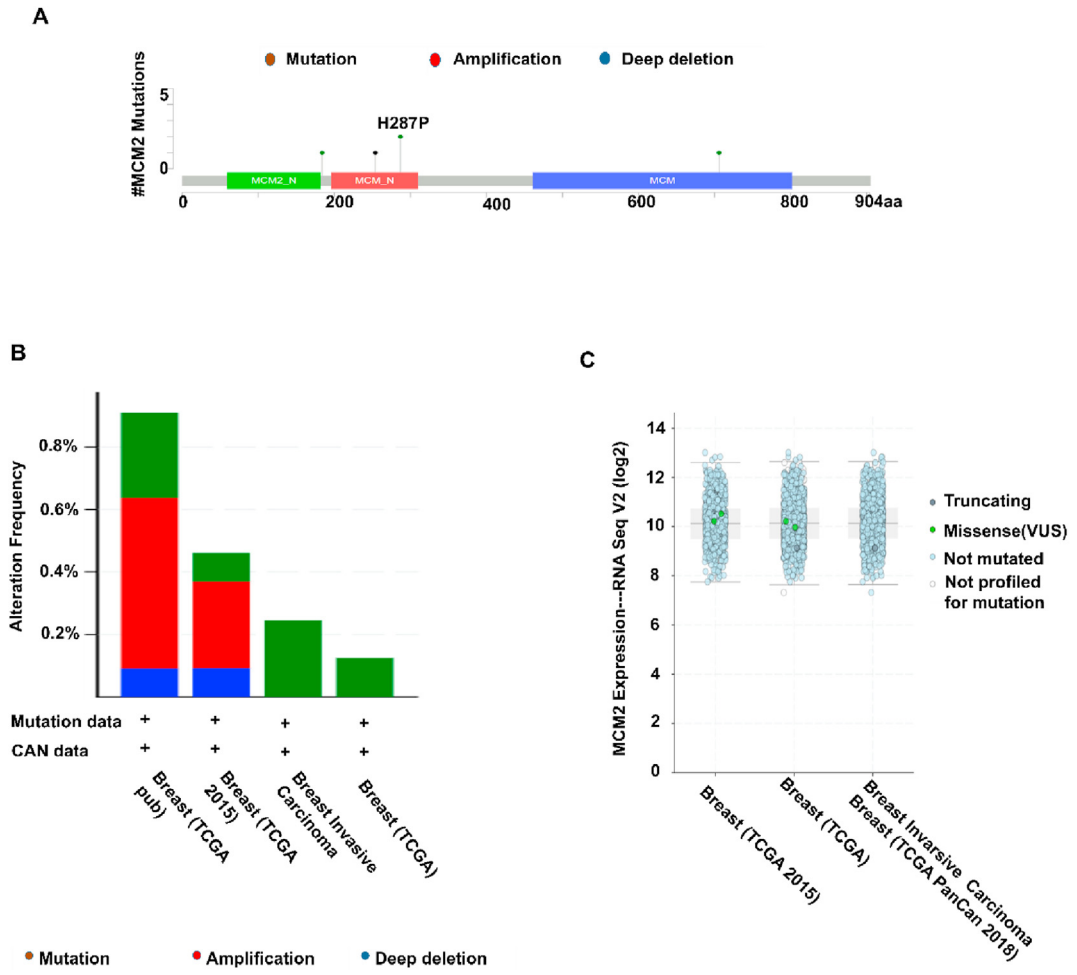

Figure 5. Genetic alteration and mutations of MCM2 in BC tissue. Herein (A) lollipop plot shows the type of alteration in seven mutation spots within the MCM2 peptide sequence (0–904 AA), (B) bar diagram shows the mutation frequencies and genome alteration in the *MCM2* gene, and (C) the correlation between the expression and copy number alteration of MCM2 in TCGA dataset. Abbreviation: BC, breast cancer; TCGA, the cancer genome atlas; and CNA, copy number alteration.

2.4. Determination of mutations and copy number alterations of the MCM2 gene

The prevalence of mutations and copy number alterations of the *MCM2* gene in human BC were examined using the cBioPortal server (https://www.cbioportal.org/) [33]. The cBioPortal is an interactive online platform designed for exploring, visualizing, and analyzing multidimensional cancer genomics datasets. Total 3834 samples were retrieved from the four TCGA database studies (Cell 2015, Firehose legacy, Nature 2016, and PanCancer Atlas), which were then analyzed by using the cBioPortal server. Mutation and somatic copy number alterations have become one of the major challenges for understanding the cancer genomics. In this study, the GISTIC algorithm (Genomic Identification of Significant Targets in Cancer) with default parameters was used to identify mutations and aberrant regions in our desired gene which may contribute to cancer pathogenesis.

2.5. Analyzing the relationship between MCM2 expression and survival of BC patients

The effect of MCM2 on BC patient's survival was analyzed with PrognoScan server (http://dna00.bio.kyutech.ac.jp/PrognoScan/) and the survival plots were generated using KM-Plotter [34,35]. The PrognoScan is a widely used server for survival analysis based on the genomics datasets from multiple cancers. It uses quickly confirmed disease prophecies including overall survival (OS), relapse-free survival (RFS), distant metastasis-free survival (DMFS), and post-progression survival (PPS) [36,37]. The cox *p*-value of less than 0.05 was considered as statistically significant.

2.6. Profiling of genes Co-expressed with MCM2 in human BC tissues

The co-expression profile of the *MCM2* gene in BC was assessed and the corresponding heatmap was retrieved from the Oncomine server. In addition, co-expression profiling revealed that the *MCM4* gene is highly correlated with the *MCM2* gene in BC patients. To confirm their co-expression levels in BC, a heatmap relating to two genes was anticipated with the UCSC Xena web server based on the TCGA dataset [32]. The predicted co-expressed BC genes from TCGA were also analyzed with UCSC Xena server. Furthermore, a positive correlation between the levels of MCM2 and MCM4 transcripts in BC patients were confirmed by the GEPIA2 server.

2.7. Analysis of gene ontology and signaling pathways related to BC development

The gene ontology (GO) and signaling pathways of co-expressed genes and respective bar plots were retrieved from Enrichr server

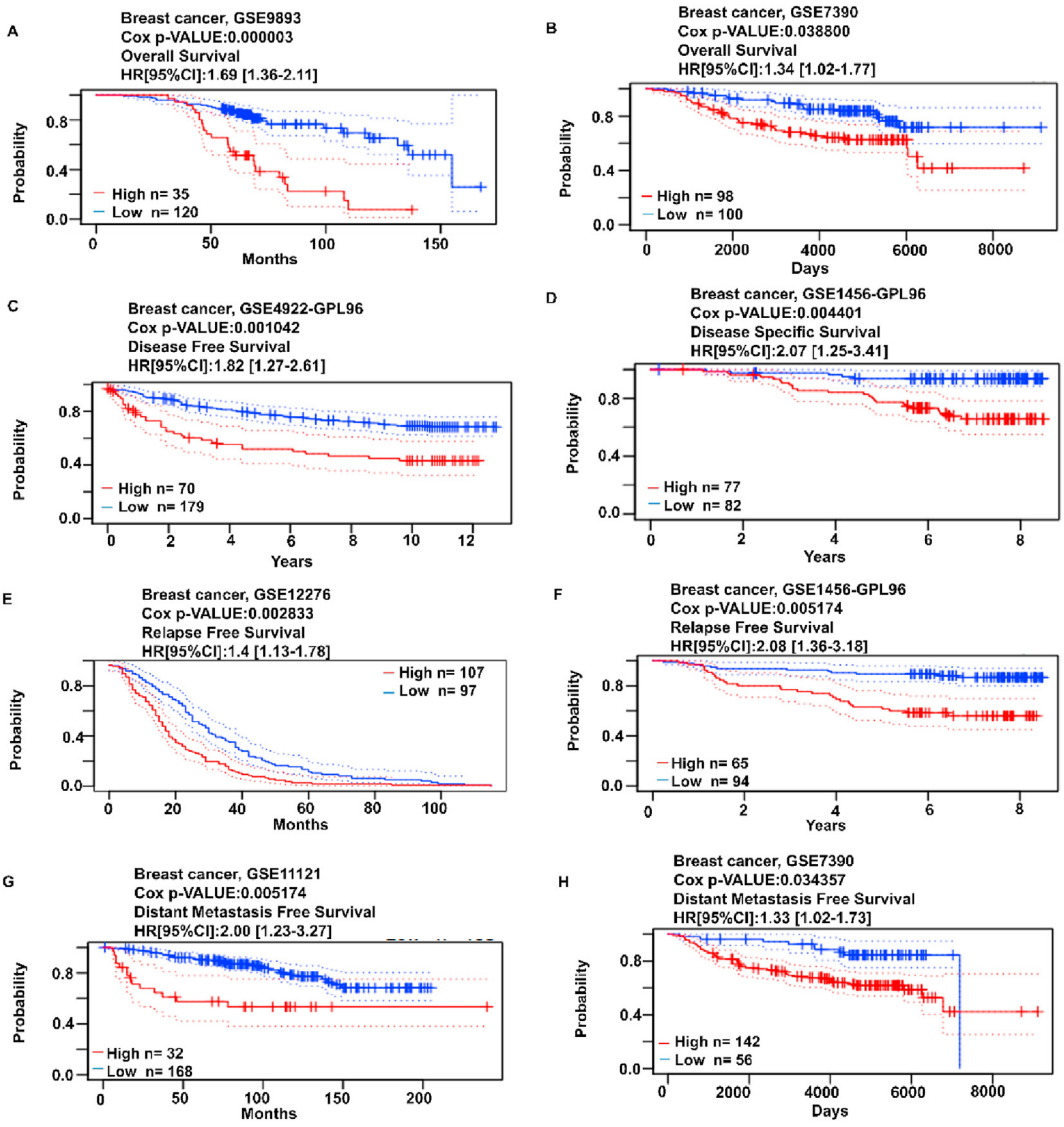

Figure 6. The relationship between MCM2 gene expression and survival of BC patients. The survival curves demonstrate patients' survival with the high (red) and low (blue) expression of MCM2 in Kaplan-Meier plots where (A–B) showing overall survival, (C) diseases free survival, (E–F) relapse-free survival, and (G–H) distant metastasis-free survival. The analysis was focused on the MCM2 expression in BC patients. Abbreviation: HR, hazard ratio; CI, confidence interval; and BC, breast cancer.

(http://amp.pharm.mssm.edu/Enrichr/) [38]. The Enrichr is a widely used comprehensive enrichment analysis server that works by comparing multiple genomics datasets. Based on the GO terms, the input genes were categorized into biological processes, molecular functions, and cellular components. Likewise, signaling pathways were determined using Bioplanet 2019, Reactome 2016, and KEGG 2019 databases via Enrichr server [38]. For both cases, we considered the cox *p*-value less than 0.05 as statistically significant.

3. Results

3.1. The mRNA expression of MCM2 in different types of cancer

By utilizing the three-independent web-based platforms, we demonstrated the differential patterns of *MCM2* transcript in different cancer tissues and their respective healthy counterparts. Among the 402 unique datasets in the Oncomine server, only 67 studies ranked in the top 10% showed statistically significant results. Interestingly, the upregulation of MCM2 was evident in different cancers including breast, bladder, brain, cervical, colorectal, esophageal, gastric, head and neck, kidney, liver, lung, other, ovarian, sarcoma, and pancreatic cancers (Figure 1A). Observation of the data also showed that *MCM2* is highly expressed in BC patients while downregulated in leukemia only (Figure 1A). The expression of *MCM2* in 33 different human cancers and their corresponding normal tissues were shown in Figure 1B. Further analysis of the data was performed using the Affymetrix HG-U133pLUS2 platform from the GENT2 database that reveals the overexpression of *MCM2* in various cancers as depicted in Figure 1C. The results suggest strong and significant evidences relating the higher expression of MCM2 in BC tissues as compared to the normal tissues (Figure 1).

3.2. Expression of MCM2 transcript in human BC tissues

The Oncomine platform was used to assess the MCM2 gene expression for each subtype of BC compared to the normal tissues. The result revealed the overexpression of MCM2 in various BCs, such as medullary breast carcinoma, invasive ductal breast carcinoma, breast carcinoma, mixed lobular and ductal breast carcinoma, invasive lobular breast carcinoma, invasive breast carcinoma, and ductal breast carcinoma (Figure 2Ai-iii and Table 1). Further evaluation of TCGA datasets with

Table 4. The association of MCM2 expression and survival in human BC patients.

Dataset	Endpoint	Probe ID	N	Cox *P*-value	HR*
GSE12276	Relapse Free Survival	202107_s_at	204	0.002833	1.42
GSE9195	Distant Metastasis Free Survival	202107_s_at	77	0.001732	3.99
GSE9195	Relapse Free Survival	202107_s_at	77	0.003532	3.04
GSE11121	Distant Metastasis Free Survival	202107_s_at	200	0.005174	2
GSE1378	Relapse Free Survival	8241	60	0.006214	1.15
GSE9893	Overall Survival	3845	155	0.000003	1.69
GSE2034	Distant Metastasis Free Survival	202107_s_at	286	0.029374	1.43
GSE1456-GPL96	Relapse Free Survival	202107_s_at	159	0.000724	2.08
GSE1456-GPL96	Disease Specific Survival	202107_s_at	159	0.004401	2.07
GSE1456-GPL96	Overall Survival	202107_s_at	159	0.02056	1.7
E-TABM-158	Disease Specific Survival	202107_s_at	117	0.013239	0.59
GSE3494-GPL96	Disease Specific Survival	202107_s_at	236	0.011551	1.81
GSE4922-GPL96	Disease Free Survival	202107_s_at	249	0.001042	1.82
GSE7390	Distant Metastasis Free Survival	202107_s_at	198	0.034357	1.33
GSE7390	Overall Survival	202107_s_at	198	0.0388	1.34
GSE7390	Relapse Free Survival	202107_s_at	198	0.033839	1.27

*HR denotes the hazard ratio and N is the number of samples.

UALCAN and GEPIA2 servers also exhibited the upregulation of MCM2 in BC (Figure 2B and 2C). Moreover, comparative immunohistochemistry analysis was performed between normal and cancer tissues using the HPA database. Among the 31 BC patients, sample 4 showed strong staining signals, while normal glandular cells showed moderate or weak staining signals indicating low *MCM2* expression (Figure 2D and 2E). The results suggest that MCM2 is highly expressed in BC tissue compare to normal tissues (Figure 2 and Table 1).

3.3. Association of MCM2 expression with clinical characteristics of BC patients

The relation between MCM2 expression and the clinical features of BC patients and healthy individuals (control) was performed using TCGA data via UALCAN database. The results exhibit enhanced expression of MCM2 irrespective of individual cancer stages, patient's race, gender, age, major subclass with and without different TNBC-type, menopause status, tumor histology, and nodal metastasis depicted in Figure 3 and listed in Table 2. The analysis asserted increased expression of MCM2 at later stages as compared to the early stages (Figure 3A). Besides, the expression level was enhanced in male patients compared to female patients. Also, Figure 3C shows higher expression at the early age group (21–40 years old). Upregulation of MCM2 expression can also be seen in terms of other clinicopathological parameters including patient's race (Table 2), tumor histology (Figure 3D), major subclasses (Figure 3E), major subclasses with TNBC-type (Table 2), nodal metastasis status (Figure 3F), and menopause status (Table 2). These results indicate that the expression of MCM2 and clinical characteristics is significantly higher in BC compared to healthy individuals.

3.4. Promoter methylation of BC from TCGA dataset

Promoter methylation is one of the essential epigenetic regulatory factors that plays a significant role in gene silencing, tissue differentiation, cellular development, and genetic imprinting. Aberrant hypermethylation of high-density CpG regions, known as CpG Islands (CGIs), or genome-wide hypo-methylation have been found to be associated with cancers. Therefore, we examined the correlation between MCM2 expression and methylation in PAM50 BC subtypes. Comparing the MCM2 expression heatmap and DNA methylation status revealed that MCM2 expression might be negatively related with some CpG sites (blank frame) (Figure 4A). By comparing MCM2 expression in different DNA methylation clusters, we also confirmed that MCM2 expression gradually increased with decreasing DNA methylation in BC (Figure 4B).

3.5. Mutations, copy number alterations, and expression of mutant MCM2 transcript

To determine the alterations of the *MCM2* gene in BC, a total of 3834 samples from 4 BC studies were analyzed by utilizing the cBioPortal database (Table 3). The MCM2 were altered in 18 (<0.1%) of quarried samples having a somatic mutation frequency of 0.2%. A total of 7 mutations were detected in patients with multiple samples. Besides, there were 5 missenses and 2 non-sense mutations located within 1–904 residues of MCM2 and MCM domain. Furthermore, the highest mutation was reported as a missense among 1067 samples in breast invasive ductal carcinoma and fell in a hotspot of H287P (Figure 5A). Alteration frequency was found highest (0.91% of 1099 cases) for breast TCGA among the four categories of the studies (Figure 5B). Mutated MCM2 mRNA expression was profiled in the seven cases of analysis showing the highest five missense and 2 non-sense types of mutations in BC (Figure 5C). Cumulatively, these findings suggested that the up expression of *MCM2* in BC might not be associated with mutations or copy number alterations even though these alterations were markedly found in MCM2 protein (Figure 5).

3.6. MCM2 expression and clinical prognosis of BC patients

The relationship between the level of MCM2 expression and patient's survival in BC was analyzed with the PrognoScan database (significant at Cox *P*-value < 0.05). The analysis showed a negative correlation in all cases of overall survival, disease-free survival, disease-specific survival, relapse-free survival, and distant metastasis-free survival with a hazard ratio (HR) larger than 1 (Figure 6 and Table 4). For dataset GSE9893 and GSE7390, patients with low MCM2 expression ($n = 120$ and 100) had significantly higher overall survival whereas higher expression ($n = 35$ and 98) of MCM2 lead to lower overall survival as depicted in Figure 6A-B. Similarly, dataset GSE4922-GPL96 and GSE1456-GPL96 showed lower disease-free survival and disease-specific survival, respectively, with high *MCM2* expression and *vice-versa* (Figure 6C-D). Furthermore, dataset GSE12276, GSE1456-GPL96, GSE11121, and GSE7390 indicate negative correlation since lower MCM2 expression (97, 94, 168, and 56) had significantly higher relapse-free survival and distant metastasis-free survival unlike its higher expression (107, 65, 32, and 142) as shown

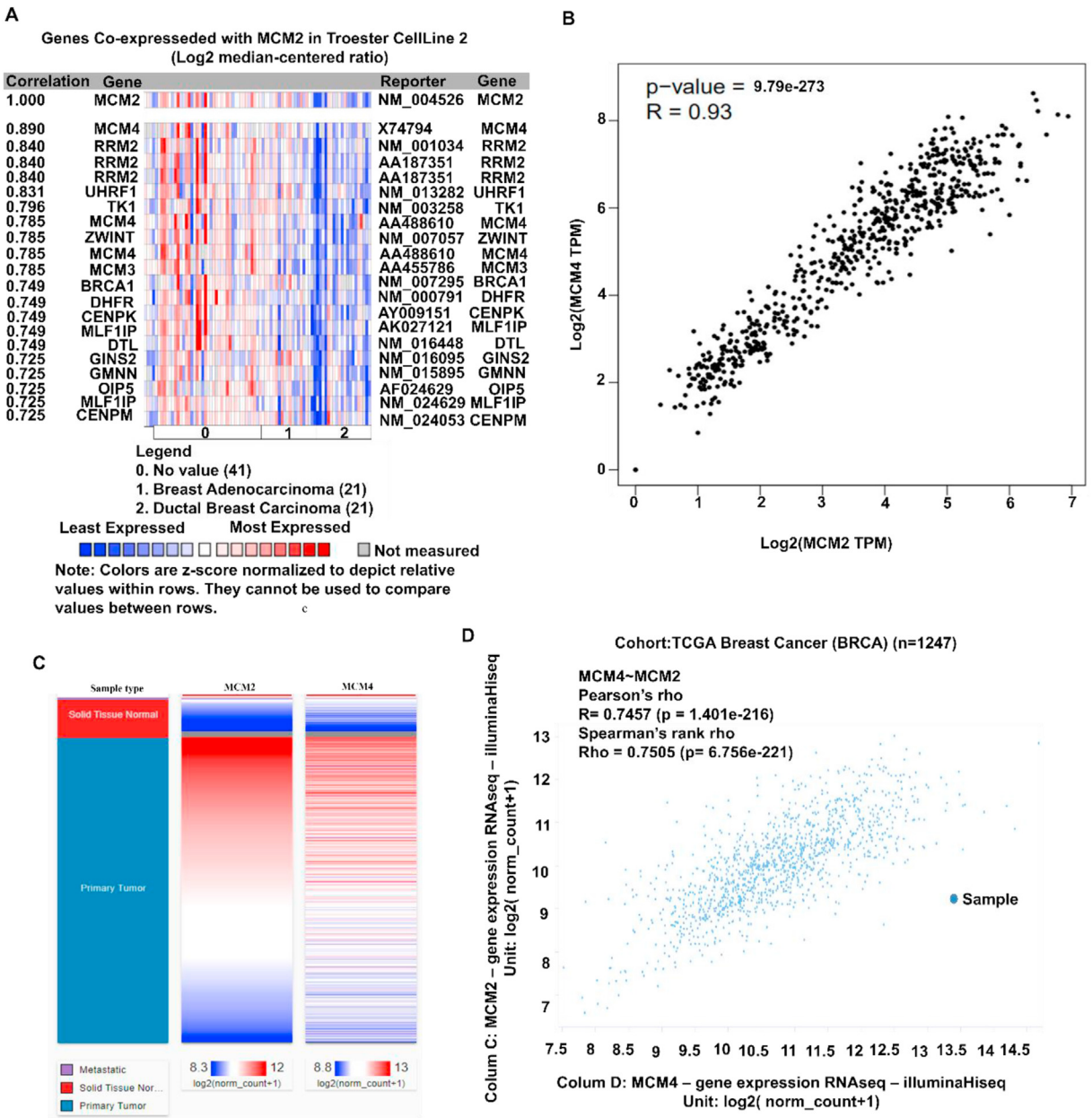

Figure 7. Co-expression profile of the *MCM2* and co-expressed genes in human BC. The figure shows (A) co-expression profile of MCM2 derived from the Oncomine database, (B) correlation analysis between MCM2 and MCM4 obtained by GEPIA2 server, (C) heatmap of mRNA expression for MCM2 and MCM4 genes across BC in the TCGA database, and (D) co-expression analysis between MCM2 and MCM4 genes in BC using UCSC Xena server.

in Figure 6E-H. Therefore, these results confirmed that the increased expression of *MCM2* could confer a poor prognosis in BC patients.

3.7. Analysis of gene signatures linked to MCM2 and human BC

We explored the co-expression profile of MCM2 with 21 genes across 83 BC samples through the Oncomine database (Figure 7A). The result showing the expression of MCM4 (mini-chromosome maintenance 4) was mostly co-expressed (R = 0.890) among the total 21 genes. The positive correlation between MCM2 and MCM4 (R = 0.93) with the spearman coefficient was confirmed using the GEPIA2 server (Figure 7B). Further, the confirmation of positive correlation between MCM2 and MCM4 was done using Pearson (r = 0.7457) and Spearman (r = 0.7505) correlation analyses for BC patients using TCGA data through UCSCXena server (Figure 7C-D). These analyses suggest that *MCM2* might be positively associated with the *MCM4*-mediated signaling pathway in BC progression.

3.8. Gene ontologies and signaling pathways related to MCM2 and BC progression

Based on the MCM2 and correlated genes, we identified signaling pathways and gene ontological features that lead to the progression of BC in humans. For pathway determination, we considered results from three databases depicted in Figure 8A-C. For KEGG human 2019 database, we found different significant pathways including cell cycle, DNA replication, spliceosome, mismatch repair, progesterone-mediated, p53 signaling pathway, base excision repair, protein processing, ABC transporters, etc. (Figure 8A). Likewise, analysis of Reactome 2016 showed pathways related to cell cycle, TP53 regulates transcription of cell cycle

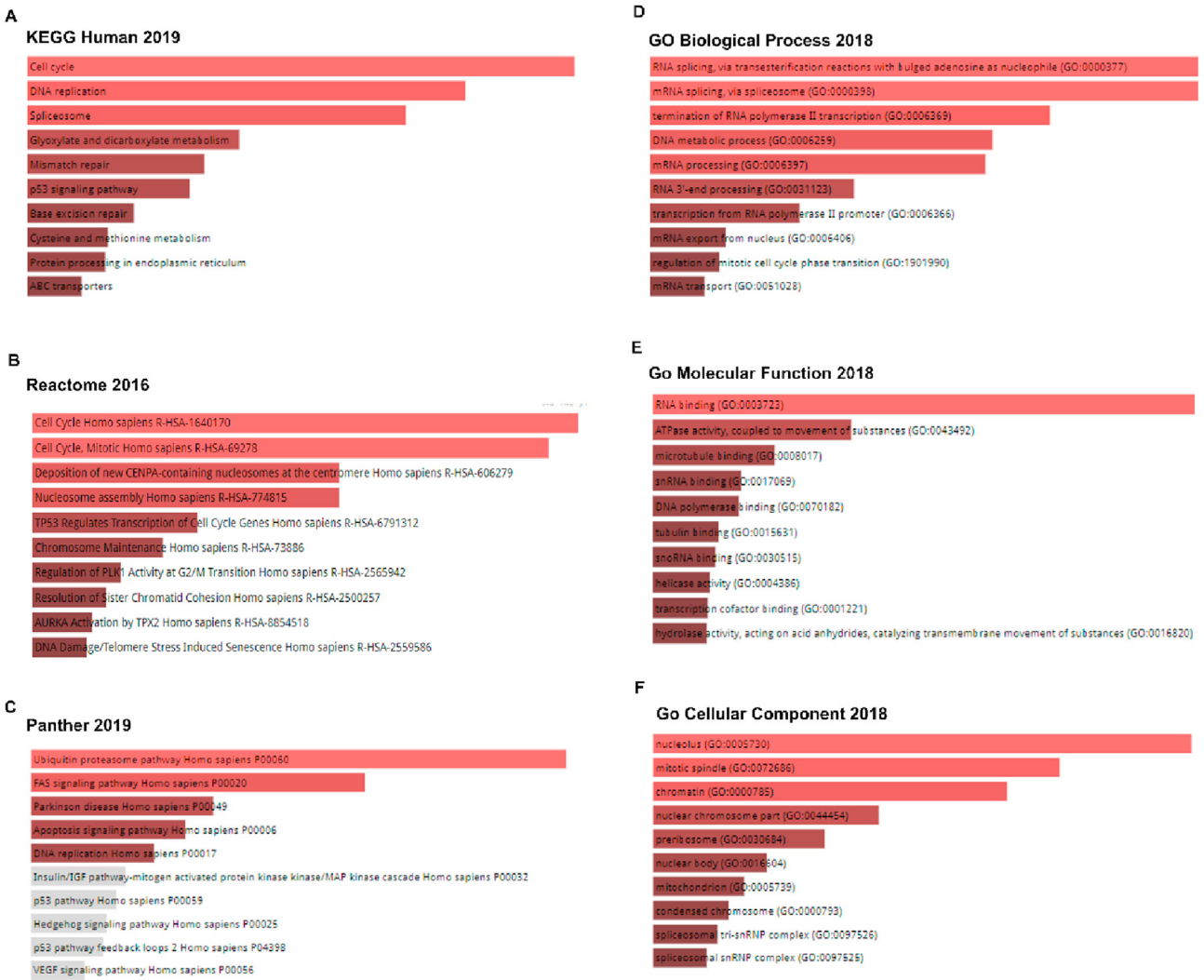

Figure 8. Pathways and gene ontologies related to MCM2 and BC expression. Pathways are obtained from (A) KEGG human 2019, (B) Reactome 2016, (C) Panther 2019, (D) GO biological process 2018, (E) GO molecular function 2018, and (F) GO cellular component 2018. The length and the color gradient of the bar represent the level of significance (the brighter the color, the more significant the term is).

genes, resolution of sister chromatid cohesion, mitotic prometaphase, Rho-GTPase signaling, Rho-GTPases activate formin, deposition of new CENPA-containing nucleosomes at the centromere, nucleosome assembly, and separation of sister chromatids, etc. (Figure 8B). Finally, Panther 2019 suggested pathways such as beta1, beta2, and beta3adrenergic receptor signaling pathway, TGF-beta signaling pathway, ATP synthesis, nicotine pharmacodynamics pathway-amyloid secretase pathway, p53 pathway and its feedback loops 2, heme biosynthesis, etc. (Figure 8C). These pathways might be related to tumor development and involved in breast neoplasia. Furthermore, we computed the GO terms for MCM2 and positively correlated genes. The suggested GO features mainly include RNA splicing, via transesterification reactions with bulged adenosine as a nucleophile, transcription from RNA polymerase II promoter activity (Figure 8D), RNA binding, ATPase activity, coupled to the movement of substances (Figure 8E), nucleus and mitotic spindle activity (Figure 8F). Therefore, these pathways may also be related to cancer development and involved in BC tumorigenesis (Figure 8).

4. Discussion

Human BC is a heterogeneous disease and has different subtypes with a variety of biological behaviors and risk profiles that make the clinical management challenging [39]. Despite the declining mortality rate, BC is still one of the prominent causes of cancer deaths [40]. Therefore, the accurate prediction of the BC outcome is crucial to both patients and physicians as well as to researchers. Because, prognosis helps patients to know about their illness and to set up future courses [41,42]. For example, early prognosis by an Australian study ensured the opportunity of anticancer treatment for 85% of cancer patients in which 75% were incurable [41]. Besides, essential BC treatment relies on the precise prediction of the outcome [43]. Therefore, it is urgent to enroll a strong prognostic index to facilitate accurate predictions regarding the survival and/or response to the treatment of BC patients [44,45]. In this study, we evaluated the significance of MCM2 as a prognostic marker in BC prediction using bioinformatics [46].

Based on the multiple databases, we found that the expression level of MCM2 is positively correlated with the progression of BC. The analysis of MCM2 expression in BC exhibits a negative correlation with all cases of overall survival, diseases free survival, relapse-free survival, distant metastasis-free survival with the overall HR $>$ 1. We have seen the noxious impact of high MCM2 levels on the survival rate than those with low MCM2 levels. A previous study suggested that the increased expression of *MCM2* could confer a poor prognosis in BC patients [10]. The expression patterns of MCM2 in cancer tissues were significantly associated with different clinical characteristics of BC patients including, tumor histology, patient's race, gender, age, TNBC status, etc. Therefore,

these results require further investigation since a high expression level of MCM2 may confer a risk of subsequent malignant transformation. The methodological aspects of modern immunohistochemistry that combines computer-aided systems and digital imaging will provide greater realization in immunohistochemical scoring [47,48]. The immunohistochemical data of MCM2 demonstrates strong nuclear immunoreactivity of MCM2 in each BC cell. Strong and highly intense staining of cancer cells compared to that of normal glandular cells ascertain the higher levels of MCM2 expression in BC tissues. Previous MCM2 expression was detected at higher levels than that of Ki-67 in normal breast tissues and breast cancers [49].

Most importantly, there are four facts, i.e., somatically acquired genetic, epigenetic, transcriptomic, and proteomic alterations which forms a series of histopathological process, thereby, causing cancer progression [47]. Any alteration in the genomic region either loss or gain can lead to either suppressive or oncogenic effects [50]. To explore the copy number alterations, mutations, and mutant mRNA expressions of MCM2, the cBioPortal webserver was utilized. Among the queried sample, 18 (<0.1%) found altered with the somatic mutation frequency of 0.2%. In total, 7 mutations were reported having the highest mutation to the H287P hotspot. Next, we compared *MCM2* expression and DNA methylation status that reveals no significant association. The comparison between different DNA methylation clusters shows that *MCM2* expression increases with the decrease in DNA methylation. It has also been shown that *MCM2* DNA methylation status is decreased in human cancer [51].

Furthermore, co-expression and correlation analysis, we observed that 21 genes showed a positive correlation with the *MCM2* gene and MCM4 was positively correlated with MCM2 expression (R = 0.89). Co-alteration of *MCM2* and *MCM4* was also confirmed by other analyses by other platforms as well. MCM4 regulates the initiation of DNA replication and may play an essential role in the proliferation of some NSCLC cells. Taken together with higher expression in NSCLCs and its correlation with clinicopathologic characteristics such as non-adenocarcinoma [52].

Finally, we analyzed the possible MCM2 related pathways in BC using the correlated genes. In the KEGG pathway analysis, the correlated genes were mostly related to the cell cycle. This is plausible since any disturbance in the cell cycle can lead to cancer progression by facilitating proliferation, genomic, and chromosomal instability [3]. In addition to the cell cycle, the correlated genes are found to be involved in DNA replication [53]. In GO analysis, the most enriched ontology terms were RNA splicing via trans-esterification reactions with bulged adenosine as the nucleophile that involves a bulge site during self-splicing [54]. Another mostly correlated molecular function was RNA binding in the cellular components. Collectively, the pathways and GO enrichment analysis imparts the importance of MCM2 and its correlated genes in different oncogenic processes.

5. Conclusion

In this study, we aimed to identify molecular signatures that play key roles in the development and progression of BC. In cancer, prognostic factors are important for efficient treatment, leaving patients with minimum risk profiles, and preventing side effects of overtreatment. To determine the candidacy of MCM2 as a potential prognostic marker in BC development, we analyzed the mRNA expression, DNA methylation, mutations and CNAs, correlated genes, and the prognostic features. Importantly, our evaluation exhibits marked upregulation and a positive correlation of MCM2 to the BC development. Furthermore, it revealed the possible signaling pathways and gene ontological features related to MCM2 and its expression in BC progression. These pathways could be the potential checkpoints to inhibit or reduce the development of cancer. In conclusion, MCM2 could be an effective biomarker and a potential therapeutic target to control BC in humans.

Declarations

Author contribution statement

A. Samad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

F. Haque: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Z. Nain: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Alam: Performed the experiments; Wrote the paper.

M. Al Noman: Performed the experiments; Contributed reagents, materials, analysis tools or data.

M. Molla and M. Khan: Contributed reagents, materials, analysis tools or data.

M. Hossen and M. Islam: Contributed reagents, materials, analysis tools or data; Wrote the paper.

F. Ahammad: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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