Expression Profile and Prognostic Values of Mini-Chromosome Maintenance Families (MCMs) in Breast Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Mini-chromosome maintenance families (MCMs) were considered the key factors for DNA replication initiation. Emerging evidences indicate that MCM2-7 (MCMs) are highly expressed in tissues from various malignant tumors. However, little is known about the clinical values of MCMs in breast cancer.

Material/Methods: In our study, a comprehensive bioinformatics analysis was performed to investigate expression patterns, potential functions, and prognostic values of MCMs in breast cancer, through ONCOMINE, bc-GenExMiner v4.1, Kaplan-Meier Plotter, cBioPortal and GeneMANIA databases.

Results: We found that mRNA levels of MCMs were significantly elevated in breast cancer, especially in fast-growing and spreading tumor subtypes. These over-expressed MCMs predicted worse prognosis for breast cancer patients with shorter relapse-free survival (RFS) and overall survival. Among these six factors, high expression of MCM2/4/5/7 significantly reduced the RFS for patients with Luminal-A or B breast cancer and elevated MCM6/7 indicated shorter RFS for patients with basal-like or HER2-positive breast cancer. We also found that genomic alteration of MCMs was frequently found in breast cancer and the most common alteration was mRNA up-regulation and amplification. Furthermore, MCMs were highly correlated with CDC45, CDC7, TIMELESS, ORC6, MCM10, ORC5, ORC4 and ORC3, mainly functioning to control the DNA replication initiation and genome stability.

Conclusions: These results suggest that MCMs are attractive prognostic biomarkers for breast cancer. Our study also provides useful clinical information about the potential of MCMs as therapeutic targets.

MeSH Keywords: Breast Neoplasms • Databases, Genetic • Ki-67 Antigen • Minichromosome Maintenance Proteins

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Background

Breast cancer is the most common cancer in women and every year, nearly 1.7 million new cases are diagnosed worldwide, or 30% of all newly diagnosed cancers in women [1,2]. Although the pathogenesis of breast cancer has been extensively studied, patients who are at high risk of recurrence still suffer from low survival. Therefore, specific and sensitive markers for prognosis of breast cancer are very crucial to identify cancer patients with high risk of metastasis or recurrence, and for appropriate selection and administration of therapies for individual patients. However, conventional predictors such as tumor diameter, lymph node metastasis, histological grade, and hormone receptor and HER2 status as well as Ki-67 proliferation index seem to be insufficient for assessing risk of metastasis and prognosis in patients with breast cancer [3]. Hence, novel predictive and prognostic biomarkers are needed to identify patients who have worse prognosis and need to be treated more aggressively.

Proliferative capacity is one of the most essential features of malignant tumor [4], and proliferation of tumor cells can impact aggressiveness, clinical behavior, and prognosis of breast cancer [5]. Thus, accurate measurement of tumor proliferative capacity may not only provide useful information about patient prognosis but also help to select a more appropriate therapeutic regimen [6]. Many genes associated with DNA or cell replication are highly expressed in breast cancer tissues compared with that in normal tissues, which makes them useful biomarkers for prognosis or diagnosis of breast cancer.

The mini-chromosome maintenance family (MCM) has been considered as the key factor for DNA replication initiation [7]. The MCM family mainly consists of six highly conserved proteins, MCM2-7. Despite sharing the same MCM name, other genes including *MCM1*, *MCM8*, and *MCM10*, are not similar to MCM2-7 [8]. Previous studies have demonstrated that MCM2-7 interacts collectively to form the core of the DNA helicase and plays a critical role in unwinding and melting the double helix during DNA synthesis [9]. The MCM2-7 complex is considered the key convergence point that connects initiation of genome replication with cell growth-signaling pathways [10]. Accumulating evidences indicates that MCM2-7 in tissues from various clinical malignant tumors are more highly expressed than that in normal tissues [9,11]. Other molecular studies also have demonstrated that overexpression of MCM2-7 may not only be a biomarker of proliferative cancer cells but may also indicate the potential for recurrence of cancer and identify cells as being precancerous [8,12,13]. Evidence from the abovementioned studies supports the hypothesis that MCMs (MCM2-7) may be potentially important biomarkers for routine clinical use in breast cancer detection and prognosis.

In contrast with traditional biological assays, microarray technology allows simultaneous measurement of tens of thousands of DNA fragments for copy number variation analysis or of mRNA transcripts for gene expression [14]. As a result, microarray analysis has become one of the most important components of biomedical and biological research. Recently, the prognostic significance of several biomarkers such as PAKs, COL10A1 and BIRC5 in breast cancer has been evaluated using an online database [15-17]. In the current study, we systemically analyzed mRNA transcription levels of MCMs and their prognostic values in patients with breast cancer. Such an analysis serves to deepen our understanding of the complexity and heterogeneity of the molecular biology of breast cancer and demonstrates its clinical significance in diagnosis, prognosis, and even potentially in guiding decisions about therapy for breast cancer.

Material and Methods

ONCOMINE and GEPIA database analysis

ONCOMINE is an online cancer microarray database and integrated data-mining platform (*www.oncomine.org*) [18]. In this

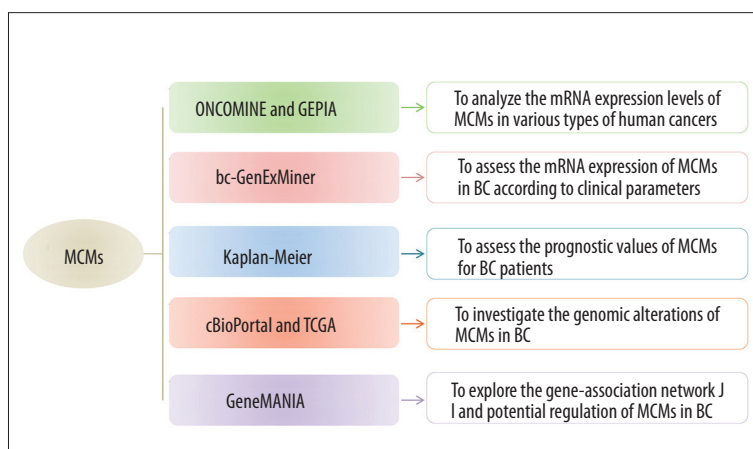**Figure 1.** The databases used in this study.

Figure 1 content:
- MCMs
  - ONCOMINE and GEPIA → To analyze the mRNA expression levels of MCMs in various types of human cancers
  - bc-GenExMiner → To assess the mRNA expression of MCMs in BC according to clinical parameters
  - Kaplan-Meier → To assess the prognostic values of MCMs for BC patients
  - cBioPortal and TCGA → To investigate the genomic alterations of MCMs in BC
  - GeneMANIA → To explore the gene-association network l and potential regulation of MCMs in BC

A

Analysis type by cancer	Cancer vs. normal MCM2		Cancer vs. normal MCM3		Cancer vs. normal MCM4		Cancer vs. normal MCMS		Cancer vs. normal MCM6		Cancer vs. normal MCM7	
Bladder Cancer	3		2		3		2		1		3	
Brain and CNS Cancer	4	1	4		1	1	6	1	2	1	2	
Breast Cancer	7		1		10	1	2	1	2		1	
Cervical Cancer	3		2		3		3		4		4	
Colorectal Cancer	7		4		4		2		2		7	
Esophageal Cancer	2		2		2		1		2		3	
Gastric Cancer	1		1		3		2				2	
Head and Neck Cancer	7		1		4		1		3		5	
Kidney Cancer	2					2	3				2	
Leukemia		1	1			2	1		1	1		3
Liver Cancer	2		2		3		2		3		2	
Lung Cancer	8		3		8		4		8		3	
Lymphoma							2				1	
Melanoma			2		3		1		1			
Myeloma			1				1		1			
Other Cancer	4		2		3	1	8		6		2	1
Ovarian Cancer	3				3		1		3		3	
Pancreatic Cancer	1				1				2			
Prostate Cancer					2							
Sarcoma	10		7		11		5		10		4	
Significant Unique Analyses	63	2	35		63	7	46	2	46	2	45	4
Total Unique Analyses	402		446		453		436		436		424	

1	5	10		10	5	1

%

B

Figure 2. Expression of MCM genes across different types of cancers (A) Transcription levels of MCMs in twenty human cancers compared with normal tissue were obtained from ONCOMINE, with the gene rank ≤ top 5%, fold change ≥2 and threshold of P≤0.001. Significant mRNA underexpression or overexpression of MCMs was shown as blue or red cell. Cancer types are organized by their tissue of origin, and the degree of color was determined by the gene rank percentile of the highest-ranking analyses. (B) MCMs expression levels in breast cancer compared with normal tissues in the GEPIA database.

Table 1. The mRNA transcription level of MCMs in different types of breast carcinoma (BC) compared with normal breast tissues (*ONCOMINE* database).

Gene	Types of BC *vs*. breast	Fold change	*p*-Value	*t*-Test	Gene Rank	Ref
MCM2	Medullary BC	3.557	1.54E-15	13.256	112 (in top 1%)	Curtis [25]
	Invasive ductal BC	2.503	3.82E-92	34.511	214 (in top 2%)	Curtis [25]
	BC	2.062	4.52E-6	6.693	371 (in top 2%)	Curtis [25]
	Mixed lobular and ductal BC	2.200	1.18E-6	8.165	183 (in top 1%)	TCGA*
	Invasive lobular BC	2.120	1.06E-12	8.432	506 (in top 3%)	TCGA*
MCM3	Medullary BC	2.101	1.62E-11	9.579	524 (in top 3%)	Curtis [25]
MCM4	Ductal BC	3.081	3.36E-14	13.705	4 (in top 1%)	Perou [24]
	Invasive ductal BC	3.192	3.89E-48	22.602	59 (in top 1%)	TCGA*
	Invasive BC	2.814	9.54E-26	12.992	206 (in top 2%)	TCGA*
	Invasive lobular BC	2.048	1.02E-13	9.018	375 (in top 2%)	TCGA*
	Medullary BC	3.126	1.96E-13	11.226	263 (in top 2%)	Curtis [25]
	Invasive BC stroma#	−10.866	1.27E-26	−19.402	522 (in top 3%)	Finak [27]
MCM5	Medullary BC	2.622	2.34E-17	15.507	48 (in top 1%)	Curtis [25]
	Intraductal cribriform breast adenocarcinoma	2.082	2.53E-4	5.919	847 (in top 5%)	TCGA*
	Invasive BC stroma#	−4.433	4.62E-23	−20.696	836 (in top 5%)	Finak [27]
MCM6	Ductal BC	3.149	8.27E-9	8.698	216 (in top 2%)	Richardson [26]
	Medullary BC	2.551	1.26E-12	10.679	362 (in top 2%)	Curtis [25]
MCM7	Medullary BC	2.196	2.81E-11	9.248	558 (in top 3%)	Curtis [25]

* TCGA – The Cancer Genome Atlas – Invasive Breast Carcinoma Gene Expression Data. # The transcription level of MCMs was down-regulated in tumor stroma and normal breast tissues, but up-regulated in breast carcinoma.

study, ONCOMINE 4.5 was used to analyze mRNA expression levels of MCMs in various types of breast cancers (Figure 1). Transcription levels of MCMs in cancer specimens were compared with that in normal tissue counterpart. Search criteria were for mRNA datasets and normal tissue *vs.* cancer tissue analysis only, and the cut-off of fold change, P value, and gene rank were defined as 2, 0.001 and the top 5%, respectively. In addition, we used the GEPIA database (*http://gepia. cancer-pku.cn*) [19] to analyze the hub genes expression in breast cancer (Figure 1).

Breast cancer gene-expression miner v4.1

bc-GenExMiner v4.1 is a statistical tool that can be used to mine 36 published annotated genomic databases of information on 4823 patients with breast cancer [20,21]. Three types of analyses are available: "expression", "prognostic," and "correlation" analyses, all of which have different subtypes. In this study, the expression module was used to assess expression of MCMs in breast cancer according to clinical parameters (Figure 1), such as nodal status, hormonal receptors, Scarff Bloom & Richardson grade status, and molecular subtypes.

Kaplan-Meier plotter database analysis

Kaplan-Meier (K-M) plotter, an open database (*www.kmplot. com*) including data on expression of 54 000 genes and survival information on 6234 patients with breast cancer [22], was used to assess the prognostic value of transcription levels of MCMs in breast carcinoma (Figure 1). To evaluate the prognostic significance of the target genes, samples from patients with breast carcinoma were divided into two groups (low *vs.* high expression) based on median gene expression. Then, we analyzed the overall survival (OS) and relapse-free survival (RFS) in patients with breast cancer with different intrinsic subtypes using K-M survival curves. The hazard ratio (HR) with 95% confidence intervals (CI) and log rank P value were calculated and displayed on the plot, and the number-at-risk was also shown below the main plot.

cBioPortal database analysis and TCGA data

The cBioPortal database provides integrative analysis of large-scale cancer genomics and clinical profiles datasets from 212 cancer studies (*www.cbioportal.org*) [23,24]. TCGA (the Cancer Genome Atlas) includes both pathological and sequencing data

Table 2. Correlation of MCMs mRNA expression with clinicopathological features for patients with breast carcinoma.

Variables	MCM2		MCM3		MCM4		MCM5		MCM6		MCM7	
	No.	mRNA/ p-value	No.	mRNA/ p-value	No.	mRNA/ p-value	No.	mRNA/ p-value	No.	mRNA/ p-value	No.	mRNA/ p-value
Age												
≤51	1392	0.0149	1392	<0.0001	1353	0.6471	1361	0.0757	1392	0.0226	1492	<0.0001
>51	2210	↓	2210	↓	2055	–	2142	–	2209	↓	2263	↓
Nodal status												
–	2493	0.4368	2493	0.0062	2380	0.0959	2447	0.4971	2493	0.7000	2447	0.1717
+	1562	–	1562	↓	1494	–	1509	–	1561	–	1761	–
ER (IHC)												
–	1446	<0.0001	1559	<0.0001	1519	<0.0001	1525	<0.0001	1559	<0.0001	1583	<0.0001
+	3766	↓	3988	↓	3836	↓	3923	↓	3987	↓	4104	↓
PR (IHC)												
–	804	<0.0001	946	<0.0001	936	<0.0001	946	<0.0001	946	<0.0001	1076	<0.0001
+	1249	↓	1439	↓	1390	↓	1439	↓	1439	↓	1545	↓
HER2 (IHC)												
–	1409	0.0436	1409	0.1203	1357	0.0419	1409	0.2175	1409	0.1689	1596	0.2159
+	201	↑	201	–	198	↑	201	–	201	–	217	–
Triple-negative status												
Not	1060	<0.0001	1144	<0.0001	1094	<0.0001	1144	<0.0001	1144	<0.0001	1144	<0.0001
TNBC	3942	↑	4200	↑	4072	↑	4200	↑	4200	↑	4200	↑

ER (IHC), PR (IHC) and HER2 (IHC) represent oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 by immunohistochemistry, respectively. TNBC represents basal-like subtype (PAM50) status and/or triple negative breast cancer status by IHC.

on 30 different cancers [25]. In this study, the invasive breast carcinoma dataset (TCGA, Cell 2015) containing 818 samples was selected for further analysis of MCMs (Figure 1) [26]. Search parameters included putative copy-number alterations from GISTIC, mutations, mRNA expression z-scores (RNA Seq V2 RSEM, ±2), and protein expression z-scores (RPPA, ±2) with default settings.

GeneMANIA database analysis and the target gene function prediction

GeneMANIA database (*http://genemania.org*) was used to make gene-association networks for MCMs to predict the most related genes in our gene set (Figure 1) [27]. The gene interaction network was constructed by predicted interaction, shared protein domains, physical interaction, co-expression, and pathway. The query genes were in stripped circles and the related genes

were colored based on their involvement in specific processes, such as "G1/S transition of mitotic cell cycle", "DNA replication", "nuclear chromosome part", "MCM complex", "DNA helicase activity", and "DNA replication initiation".

Statistical analysis

Student's *t* test was used to evaluate mRNA levels of MCMs in normal tissues and breast cancer. The Welch's test was performed along with Dunnett-Tukey-Kramer's tests for pairwise comparison when appropriate in bc-GenExMiner database. Log-rank test was carried out to compare patient survival with different MCMs expression in Kaplan-Meier plotter database. P<0.05 was considered to be statistically significant.
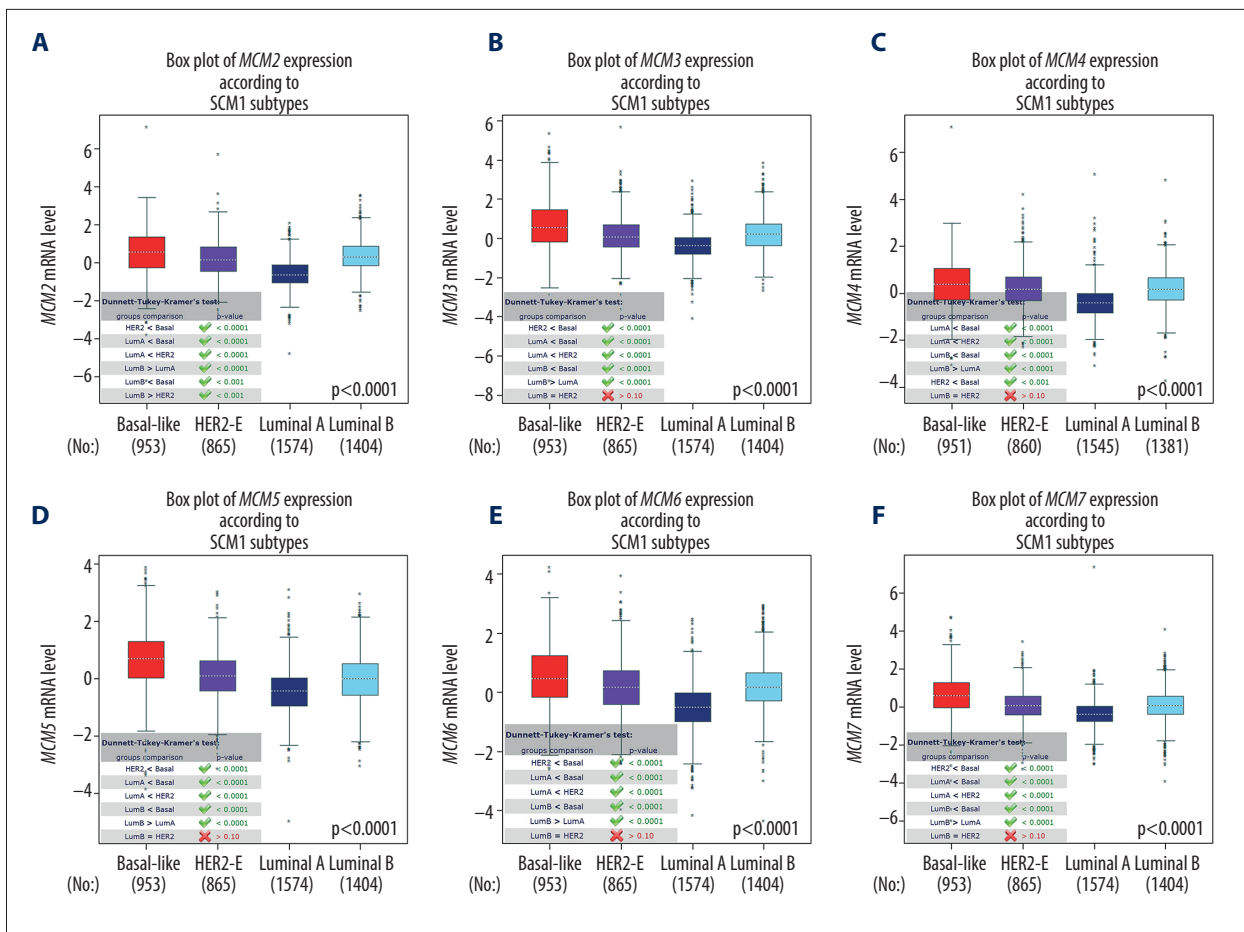
Figure 3. (A–F) mRNA expression levels of MCMs in four molecular subtypes of breast cancer. The Welch's test was performed along with Dunnett-Tukey-Kramer's tests for pairwise comparison when a global significant difference existed (P<0.05).

Results

MCMs were overexpressed in patients with breast cancer

Transcription levels of MCM2-7 in 20 types of human cancer compared to normal tissues were identified using ONCOMINE database. Figure 2A shows the percentile of highest-ranking analyses was depicted for each cancer tissue type and each MCM gene. Oncomine analysis revealed that MCMs were dramatically overexpressed in a wide variety of datasets in different cancer types (Figure 2A). For patients with breast cancer, the mRNA expression of MCM2 was significantly elevated in seven datasets. Overexpression of MCM2 was found in different types of breast carcinoma tissues, including invasive ductal, medullary, invasive lobular, and mixed lobular and ductal breast cancer (Fold changes were 2.503, 3.557, 2.120, and 2.200, respectively) (Table 1). Similarly, upregulation of MCM4 was also reported in breast cancer samples in multiple datasets (Figure 2A), with a fold change of 3.081 in ductal breast cancer [28], 3.192 in invasive ductal breast cancer, 2.048 in invasive lobular breast cancer, and 3.126 in medullary breast

cancer [29]. Both MCM3 and MCM7 were only upregulated in medullary breast cancer in a dataset from the study by Curtis with fold change equaling 2.101 and 2.196, respectively [29]. Consistent with the findings above, GEPIA analysis also revealed that MCMs were more highly expressed in breast cancer tissues than in normal tissues (Figure 2B).

As shown in Table 1, the transcription level of MCM6 was higher in ductal (fold change=3.149) and medullary breast cancer (fold change=2.551) than that in normal breast tissues [29,30]. Another MCM factor with increased mRNA expression in patients with breast cancer was MCM5. The fold change was 2.622 in medullary breast cancer and 2.082 in intraductal cribriform breast adenocarcinoma compared with normal breast tissues. Of note, MCM4 and MCM5 were observed dramatically downregulated in tumor stroma relatively to control type-matched tissue, with the fold change as low as −10.866 and −4.433, respectively [31].
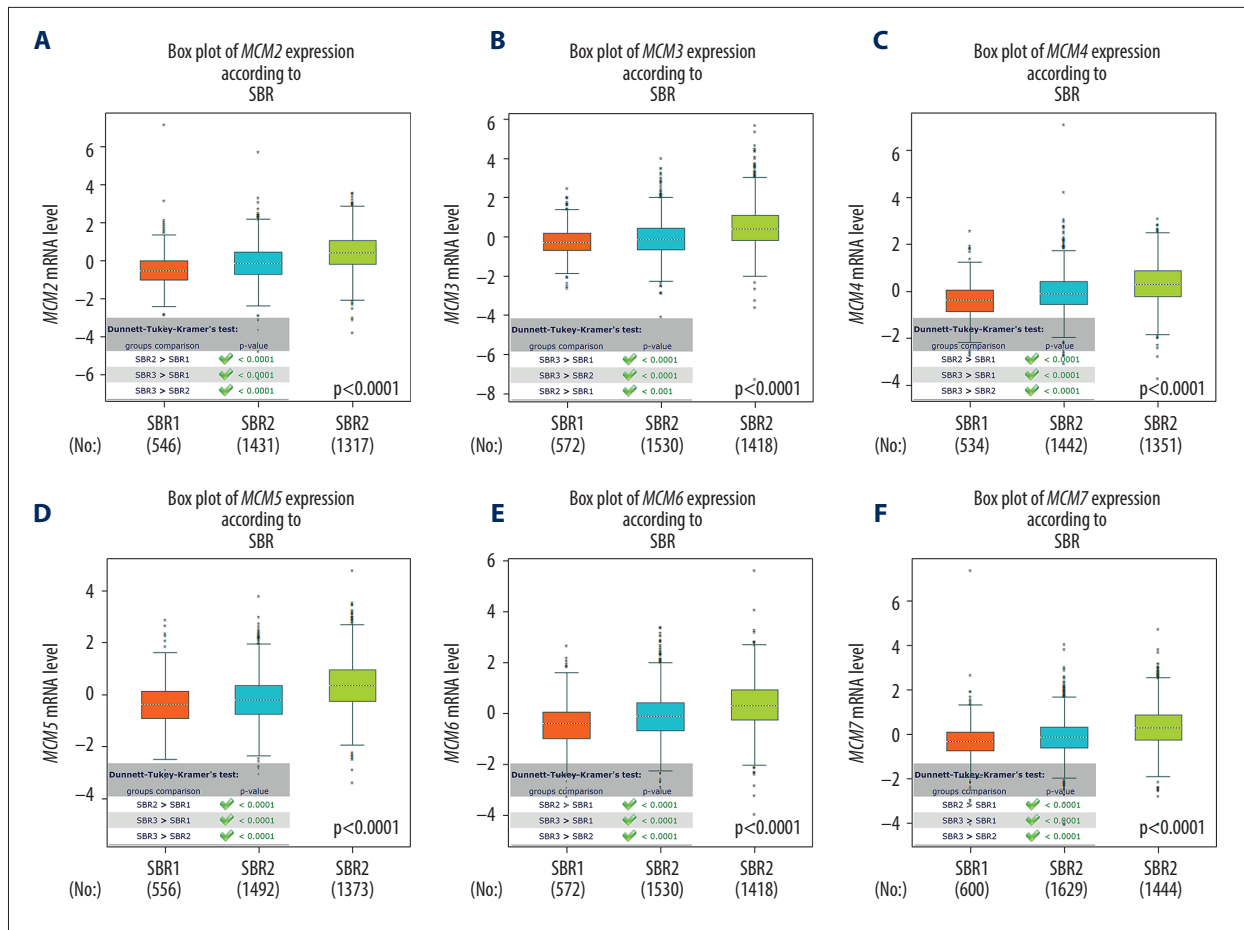
Figure 4. (**A–F**) mRNA expression levels of MCMs in different Scarff Bloom & Richardson grade status (SBR).

Transcription levels of MCMs were highly correlated with clinicopathological features in patients with breast cancer

Based on different clinicopathological parameters, mRNA expression levels of MCMs between groups of patients were compared with bc-GenExMiner v4.1. As shown in Table 2, for age criterion, patients ≤51 yrs had higher levels of expression of MCM2/3/6/7 than those >51 yrs. As for nodal status, there was no significant difference between the two groups, except for downregulation of MCM3 in patients with negative nodes. Progesterone receptor (PR) and estrogen receptor (ER) status were observed to be negatively related with MCM2/3/4/5/6/7 expression. Conversely, mRNA levels of MCM2/3/4/5/6/7 were significantly increased in patients with triple negative breast cancer (TNBC). Furthermore, MCM2 and MCM4 were also overexpressed in patients with HER2-positive breast cancer.

As shown in Figure 3, MCM2/3/4/5/6/7 mRNA expression levels in basal-like breast cancer were the highest among the four molecular subtypes. The lowest mRNA levels of MCM2/3/4/5/6/7 were found in patients with Luminal-A breast cancer. However, no significant difference in MCM transcript levels was achieved between HER2-positive and Luminal-B subtypes of breast cancer, except for MCM2, which was upregulated in Luminal-B breast cancer. With regard to Scarff Bloom & Richardson grade status (SBR), higher mRNA levels of MCM2/3/4/5/6/7 were associated with more advanced SBR grade and all groups in the comparison (SBR3 >SBR2 >SBR1) met the cutoff, P<0.05 (Figure 4).

Increased mRNA expression of MCMs predicted poorer OS and RFS in patients with breast cancer

We then assessed prognostic values of MCMs for patients with breast cancer. Kaplan-Meier plotter survival analyses and log-rank test analyses revealed that high levels of expression of MCM2/3/4/5/6/7 were significantly associated with poor OS, with HRs of 1.76, 1.29, 1.71, 1.27, 1.52, and 1.63, respectively (Figure 5). Also, RFS analysis revealed a strong correlation with all six MCM factors. As depicted in Figure 6, overexpression of MCM2/3/4/5/6/7 resulted in shorter RFS for patients with all breast cancer subtypes.
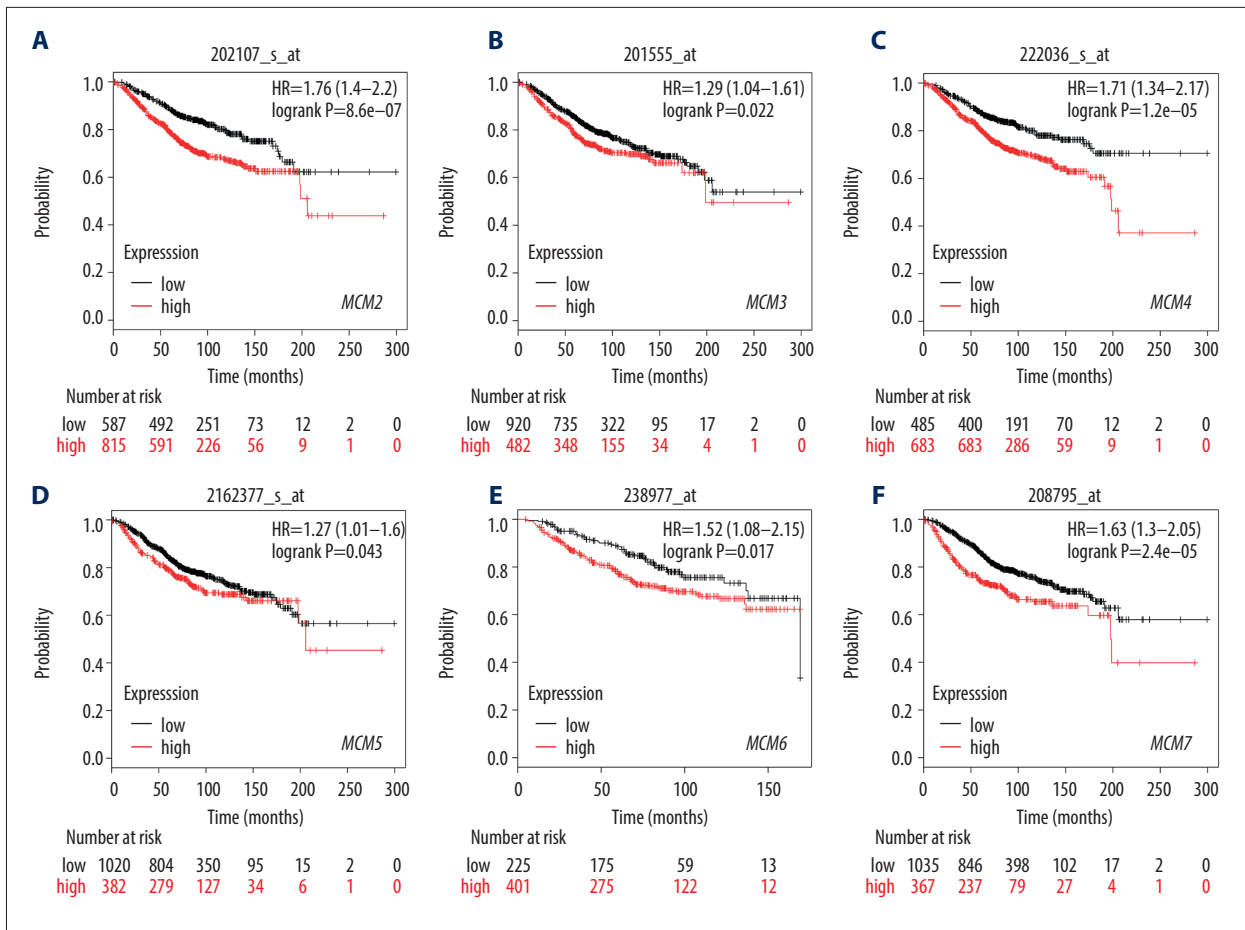
Figure 5. (**A–F**) Prognostic values of mRNA levels of MCMs in patients under all breast cancer subtypes (OS in Kaplan-Meier plotter).

Prognostic value of MCMs in different molecular subtypes of breast cancer

We further investigated the relationship between MCMs and RFS in different molecular subtypes of breast cancer. In Luminal-A subtype, high expression of MCM2/4/5/7 significantly reduced RFS in patients with breast cancer (Figure 7). A similar expression pattern was shown in patients with Luminal-B subtype breast cancer, and overexpression of MCM2/4/5/7 was also correlated with decreased RFS and worse prognosis (Figure 8). For patients with HER2-positive breast cancer, elevated MCM6/7 expression predicted shorter RFS (Figure 9). However, in the subtype with basal-like breast cancer, only patients with high expression of MCM6 presented with shorter RFS (Figure 10). Interestingly, in this study, we found that high levels of expression of MCM3 were associated with longer RFS in patients with HER2-positive breast cancer (HR=0.56 [0.38–0.83]), and high mRNA levels of MCM3/4 showed favorable RFS in patients with basal-like breast cancer (HR=0.7 [0.55–0.91] and 0.75 [0.57–0.99], respectively) (Figures 9, 10).

Genomic alterations in MCMs in breast cancer and potential gene-gene interaction network for MCMs

We also investigated genomic alterations in MCMs in breast cancer by examining mutational data publicly available in the cBioPortal database. The frequency of alteration in each kind of the selected genes is shown in Figure 11A. Overall, alterations in MCMs occurred in 279 of 817 sequenced invasive breast carcinoma samples (TCGA, Cell 2015), with an incidence as high as 34%. More than 45% of them (127/279) had two or more alterations. This analysis also showed that MCM4 has the highest incidence of alteration among the six MCMs (24%). The most common alteration in MCMs was mRNA upregulation and amplification.

To further explore the gene-gene interaction network and potential regulation of MCMs in breast carcinoma, we then performed data mining and constructed an interaction network using GeneMANIA online software. MCMs were found to be correlated with other genes in terms of the following additional traits: predicted interaction (36.10%), physical interaction (35.98%), shared protein domains (22.90%),
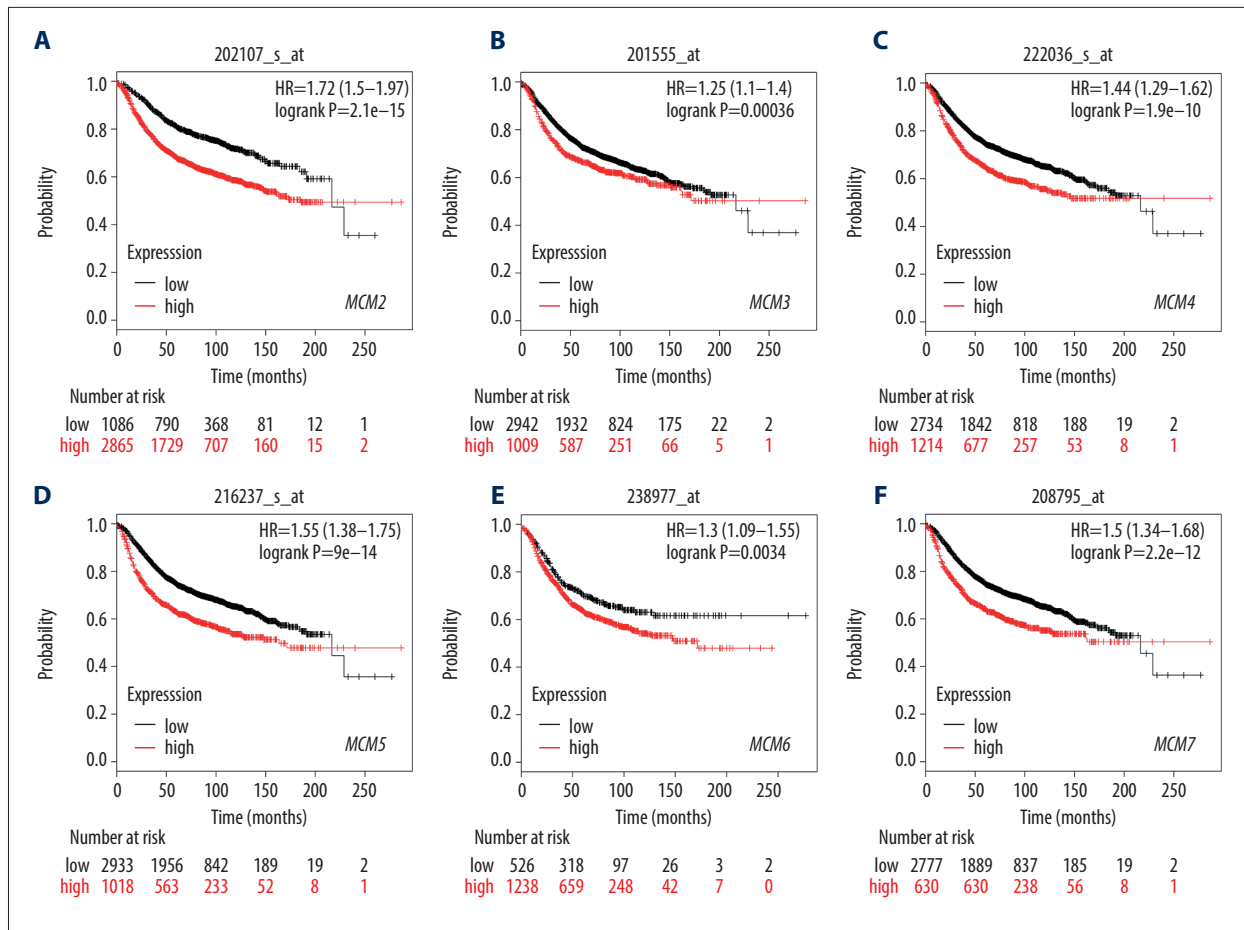
Figure 6. (**A–F**) Prognostic values of mRNA levels of MCMs in patients under all breast cancer subtypes (RFS in Kaplan-Meier plotter).

co-expression (4.70%), and pathway (0.32%) (Figure 11B–11G). The network consists of 26 genes, including six identified genes and 20 additional genes that were pulled by GeneMANIA. Overall, the analysis showed that these six MCM factors interacted closely with each other. As illustrated in Figure 11C, 11E, and 11F, physical interactions, co-expression, and certain pathway are apparent between MCMs and known genes, such as CDC45, CDC7, TIMELESS, ORC6, MCM10, ORC5, ORC4, and ORC3. Among the targeted genes, the protein products of MCMs have common structural domains with MCMDC2, MCM9 and MCM8 (Figure 11D). In addition, the network in Figure 11B also predicted other functional relationships between MCMs and CDC45, CDC7, TIMELESS, ORC5, GINS4, and ORC4.

Discussion

Overexpression of and mutations in proliferation-related genes have been proved to be strongly correlated with tumor formation. Identification of novel biomarkers for cell proliferation is urgent because of their role in diagnosis, prognosis, and treatment of breast cancer, particularly for specific subtypes. MCMs

were first identified in genetic screenings for *Saccharomyces cerevisiae* mutants that were defective in the maintenance of circular minichromosomes [32]. Previous reports have shown that MCMs are crucial for initiation of genome replication in all eukaryotic cells and play a key role in restricting replication during the cell cycle [33,34]. Compared with the other proliferative factors, MCMs may be more sensitive biomarkers because they are stably expressed throughout the cell cycle. Resting tumor cells that are still replication-competent can also be identified by MCMs, which make MCMs the ideal markers for proliferation in contrast to Ki-67 and PCNA [35]. Emerging evidence suggests that MCMs may predict tumor recurrence and progression and may serve as potential targets for tumor therapy [9,33].

As a member of the MCM family, the role of MCM2 during cell cycle and DNA proliferation has been firmly established. In the absence of MCM2, proliferation of eukaryotic cells cannot be completed [36]. An increasing number of studies have demonstrated that MCM2 is a promising cell proliferation biomarker for a variety of cancers, such as breast, stomach, and thyroid cancers [6,7]. Furthermore, in Yousef's study, MCM2
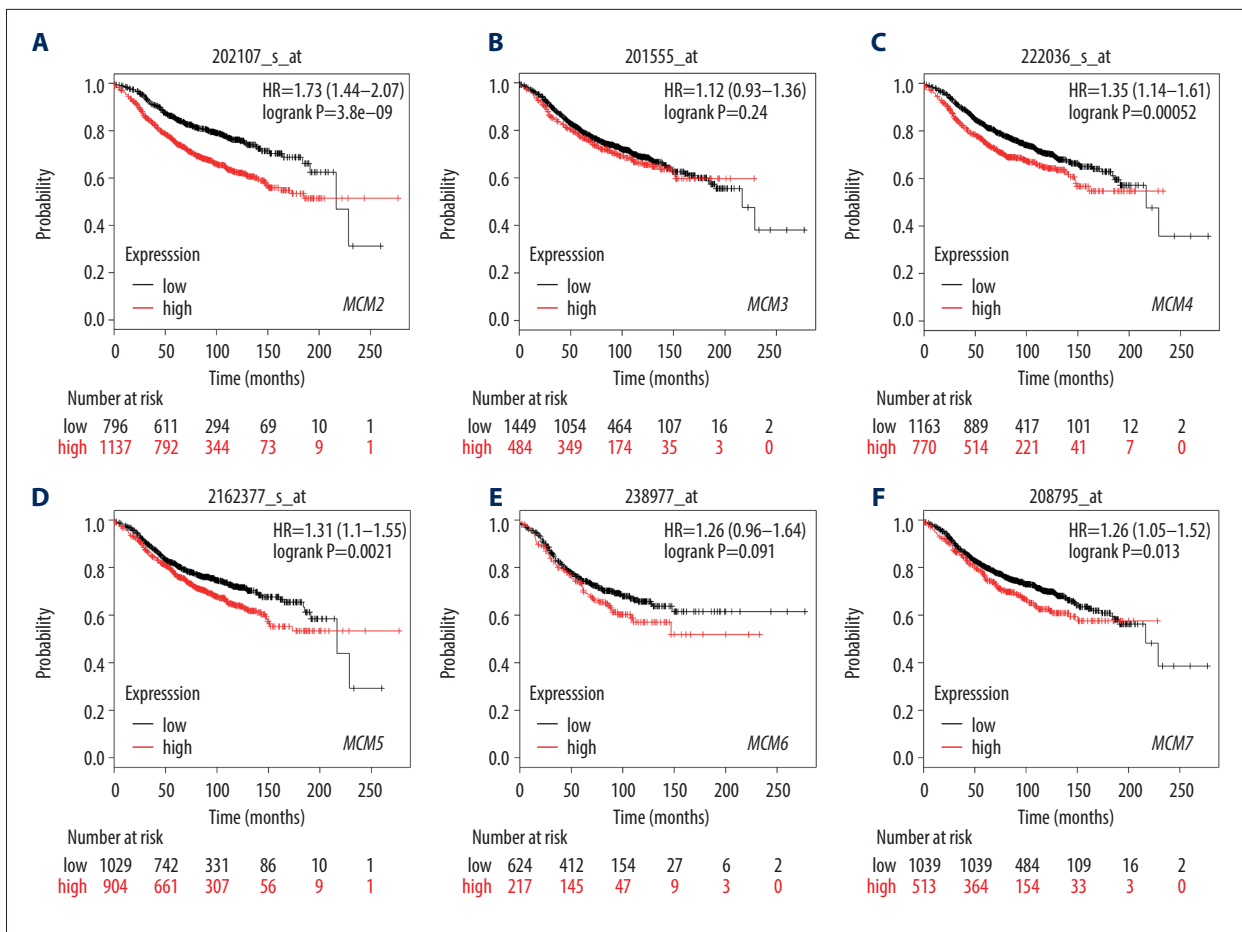
Figure 7. (**A–F**) Prognostic values of mRNA levels of MCMs in patients with Luminal-A breast cancer (RFS in Kaplan-Meier plotter).

appeared to be an attractive alternative to Ki-67, which could help to measure cell proliferation in breast cancer and sort out Luminal-A or B subtypes of breast cancer, which have clinical features and treatment that likely are divergent [6]. Except for Ki-67, the change in cells from proliferation to differentiation or quiescent state is also accompanied by rapid disappearance of MCM3 [37]. Data also show that the decline in proliferative activity parallels decreased expression of Ki-67, whereas MCM3 starts to decrease at a later stage [37]. In contrast to Ki-67, MCM3 is significantly increased in severe dysplasia samples compared with mild dysplasia samples [38]. Therefore, for patients with oral dysplastic lesions, MCM3 may be a more sensitive proliferative biomarker than Ki-67. Similarly, Rezazadeh's study also shows that MCM3 is a better cytologic biomarker than Ki-67 for patients with squamous cell carcinoma as MCM3 levels are significantly higher than Ki-67 in the group with cancer [39]. MCM2 and MCM3 are very important members of the MCM family. Translocation of the MCM complex from cytoplasm to nucleus and nuclear targeting of other family members are dependent upon MCM2 and MCM3 proteins.

Genetic instability plays a pivotal role in human cancer cell development [40]. Recognition of it will offer new insights into tumor pathogenesis. Mutations in MCM4 are associated with genome instability and DNA replication and repair will be perturbed in the presence of mutant MCM4 [40]. A high percentage of MCM4 expression (>70%), which is significantly higher than Ki-67, has been demonstrated to be significantly correlated with shorter survival and lymph node metastasis in esophageal adenocarcinoma [41]. MCM5, another MCM complex component, has also been demonstrated to be linked with increased proliferative rate in cells in breast cancer, gastric adenocarcinoma, and bladder cancer [42-44]. In bladder cancer cell lines, cell proliferation and invasion can be increased by estrogen receptor beta with upregulation of MCM5 expression [44]. The core of the MCM complex consists of MCM6 and MCM7 subunits. Previous studies have demonstrated that MCM6 is overexpressed in various types of cancers and plays a key role in cell proliferation [45]. The high level of expression of MCM6 in serum is a promising independent diagnostic marker for hepatocellular carcinoma, especially in aflphafetoprotein-negative patients [46]. Also, MCM6 is a potential marker for predicting shorter survival in patients with glioma [47]. However,
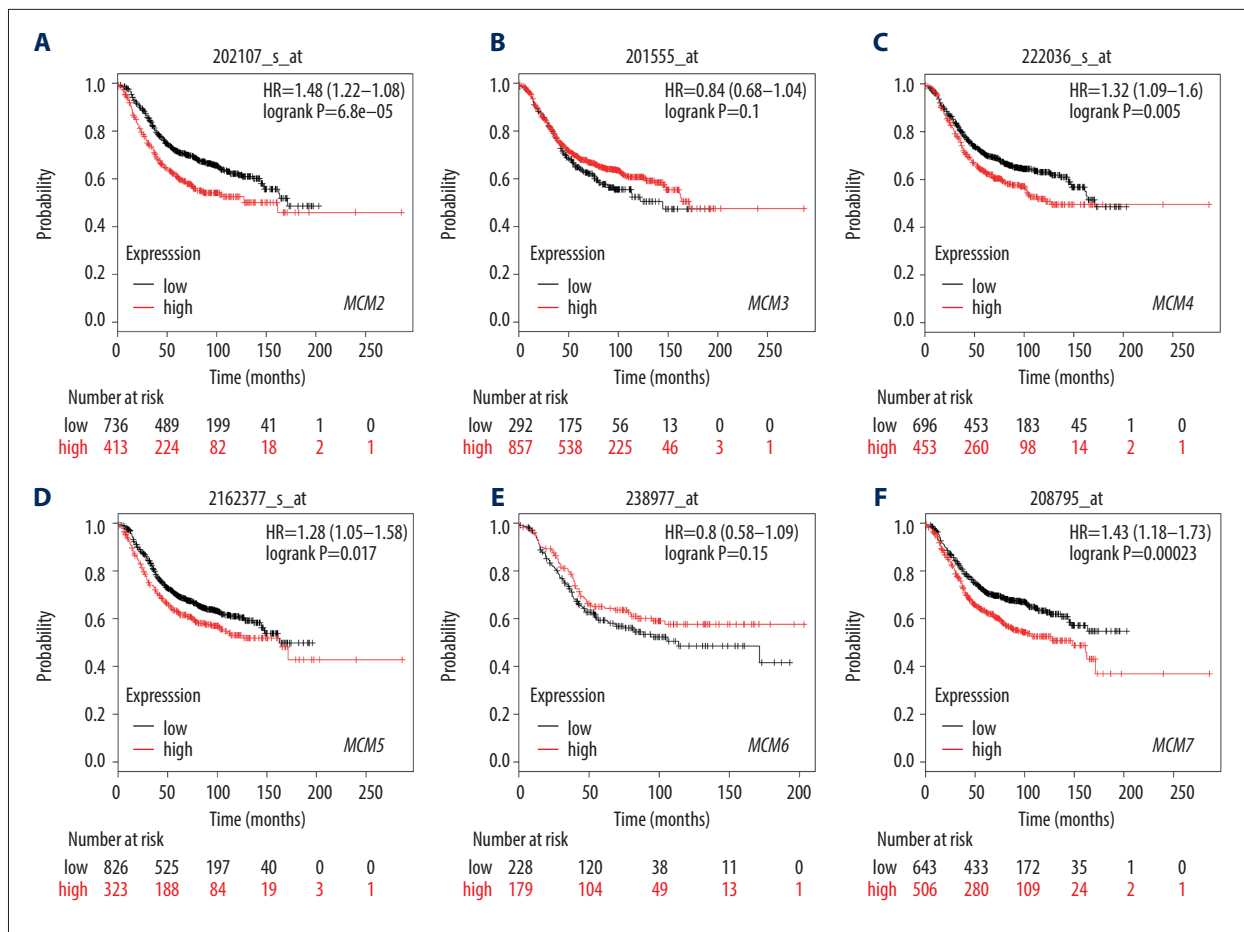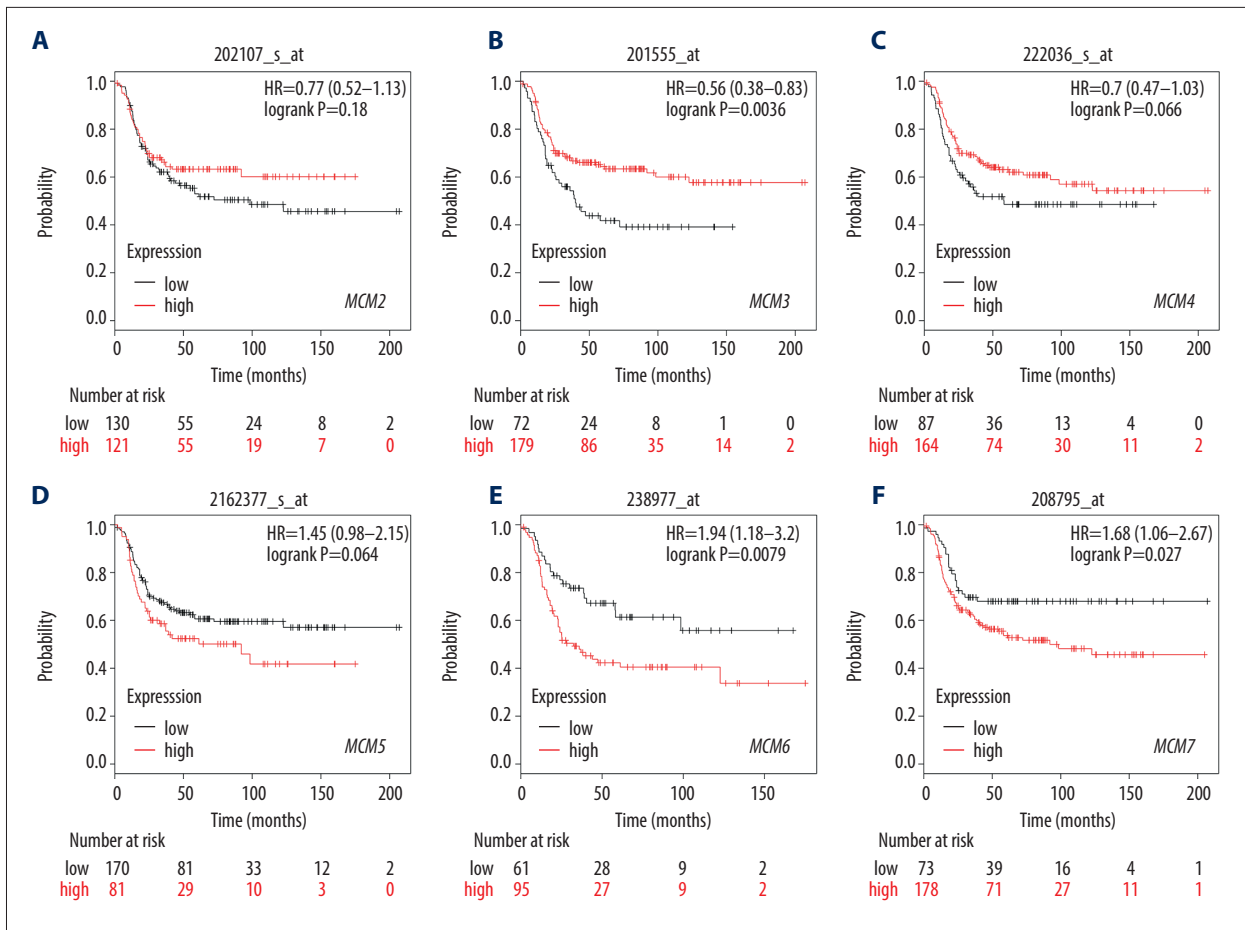
Figure 8. (**A–F**) Prognostic values of mRNA levels of MCMs in patients with Luminal-B breast cancer (RFS in Kaplan-Meier plotter).

little is known about its prognostic significance in breast cancer. Similarly, as a proliferative factor, MCM7 is correlated with tumorigenesis in a variety of malignant tumors and associated with poor prognosis [48,49]. MCM7 has been reported to be a crucial target of some oncogenic and tumor suppressor signaling pathways, which implies that multiple layers of regulation may be involved in MCM7's biological function and its oncogenic properties [50]. One of the mechanisms is that MCM7-mediated DNA replication is potentiated by epidermal growth factor receptor through Lyn phosphorylation in human breast cancers [48]. Although these findings suggest that high levels of MCM expression tend to indicate increased biological malignant aggressiveness, other studies in various cancers, including ER-positive breast cancer, have indicated the potential role of MCM overexpression in predicting better prognosis [51]. Therefore, the prognostic significance of MCMs in breast carcinoma remains to be elucidated.

Our study showed that these six DNA replication licensing factors, MCM2-7, had distinct mRNA expression signatures in breast carcinoma tissues compared with normal tissues. MCM2-7 were significantly overexpressed in invasive lobular,

medullary, invasive ductal, and mixed ductal and lobular breast cancer. The observation is noteworthy that MCM4/5 mRNA levels were dramatically downregulated in invasive breast carcinoma stromal tissue. Our analysis also indicated that mRNA levels of MCMs were significantly upregulated in younger patients with higher SBR grade status and lower expression of ER and PR. Meanwhile, the highest levels of MCM expression were found in patients with basal-like breast cancer or TNBC. These results predicted that upregulated MCMs were associated with fast-growing and spreading tumors.

In general, survival analyses revealed that higher expression of MCM2/3/4/5/6/7 was highly correlated with poorer RFS and OS in patients with all subtypes of breast cancer. Among them, higher mRNA levels of MCM2 and MCM5 mainly indicated shorter RFS for patients with Luminal-A or B breast cancer. Overexpression of MCM3 was correlated with favorable outcome for patients with basal-like or HER2-positive breast cancer. MCM4 in tumor tissue predicted shorter RFS in patients with Luminal-A or B breast cancer but longer RFS in patients with TNBC. In addition, MCM6 status was negatively correlated with RFS in patients with basal-like or HER2-positive breast
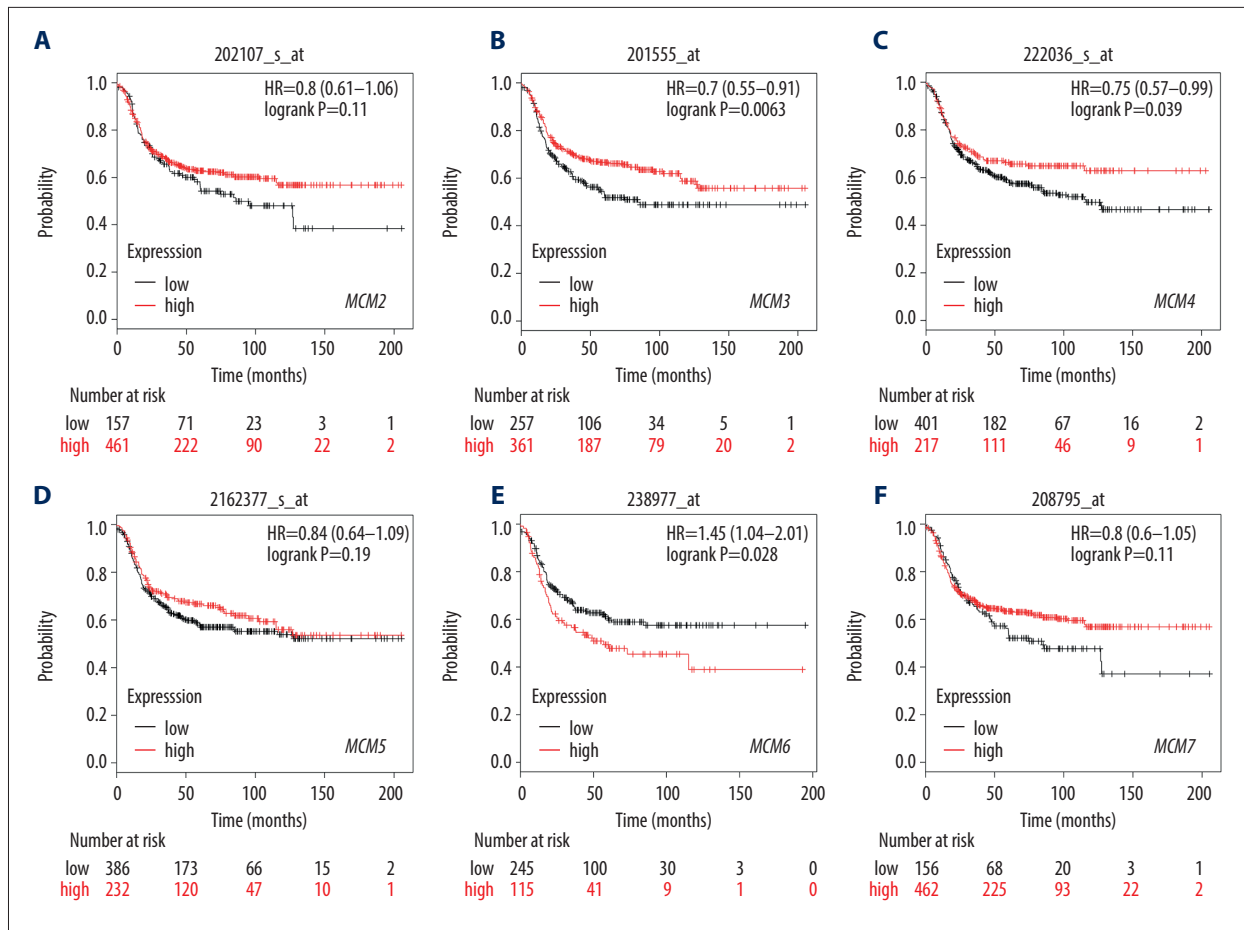
Figure 9. (A–F) Prognostic values of mRNA levels of MCMs in patients with HER2-positive breast cancer (RFS in Kaplan-Meier plotter).

cancer and MCM7 overexpression dramatically decreased the RFS probability of patients with HER2-positive, Luminal-A or Luminal-B breast cancer. Our study also revealed that MCM genomic alteration was frequently found in invasive breast carcinoma, which accounted for more than 34% of all samples. The most common alteration in MCMs was mRNA upregulation and amplification. Meanwhile, we tried to investigate the mechanisms of MCMs in regulating DNA replication origin. Results from GeneMANIA showed that MCMs were highly correlated with CDC45, CDC7, TIMELESS, ORC6, MCM10, ORC5, ORC4, and ORC3, mainly functioning to control DNA replication initiation and genome stability. Based on what we found above, MCM2-7 may be promising markers for diagnosis and prognosis of breast cancer.

As a replication protein, Ki-67 labeling index has been routinely evaluated in breast cancer. However, Ki-67 is not present in the early G1 and G0 phase of quiescent tumor cells and the level of Ki-67 may be influenced by nutrient deprivation and other external factors [43]. Besides, rather than being directly correlated with cell division, Ki-67 is considered as an "efficiency factor" in ribosome biosynthesis [52].

Therefore, the diagnostic and prognostic value of Ki-67 is limited. MCM factors, especially MCM2, MCM3 and MCM4, are more frequently expressed than standard proliferation marker Ki-67 in tumor tissues and stable throughout the cell cycle, hence, MCMs may serve as more sensitive proliferative biomarkers than Ki-67 for cytological evaluation of breast cancer [8,39,41]. In addition, it is suggested that elevated MCMs may help cancer cells resist chemotherapy and suppression of reserved MCM complexes can provide an effective sensitizing approach to increase the therapeutic index of chemotherapy drugs in cancer [53]. Therefore, elevated levels of MCMs may be potential therapeutic targets for breast carcinoma. In this study, the ONCOMINE and GEPIA databases were used to analyze expression of MCMs in breast cancer and a Kaplan-Meier plotter was used to assess prognostic values of MCMs in patients with breast cancer [18,19,22]. Furthermore, OSbrca is a novel online database for breast cancer prognostic biomarker investigation based on an independent cohort [54]. Further experiments and clinical evidence are needed to elucidate the prognostic and therapeutic value of MCMs in breast cancer.

Figure 10. (**A–F**) Prognostic values of mRNA levels of MCMs in patients with basal-like breast cancer (RFS in Kaplan-Meier plotter).

Conclusions

In our study, a comprehensive bioinformatics analysis was performed to investigate the expression patterns, potential functions, and prognostic values of MCMs in breast carcinoma. Our results showed that MCMs were overexpressed in breast carcinoma and the overexpression predicted shorter OS and RFS for patients. High expression of MCM2/4/5/7 significantly reduced RFS in patients with Luminal-A or B breast carcinoma and elevated MCM6/7 predicted shorter RFS for patients with basal-like or HER2-positive breast carcinoma. On the one hand, MCMs might act as attractive prognostic biomarkers for breast carcinoma. In addition, effective therapeutic targets are essential for treatment of breast carcinoma. Our results provide useful clinical information about the potential of MCMs as therapeutic targets.
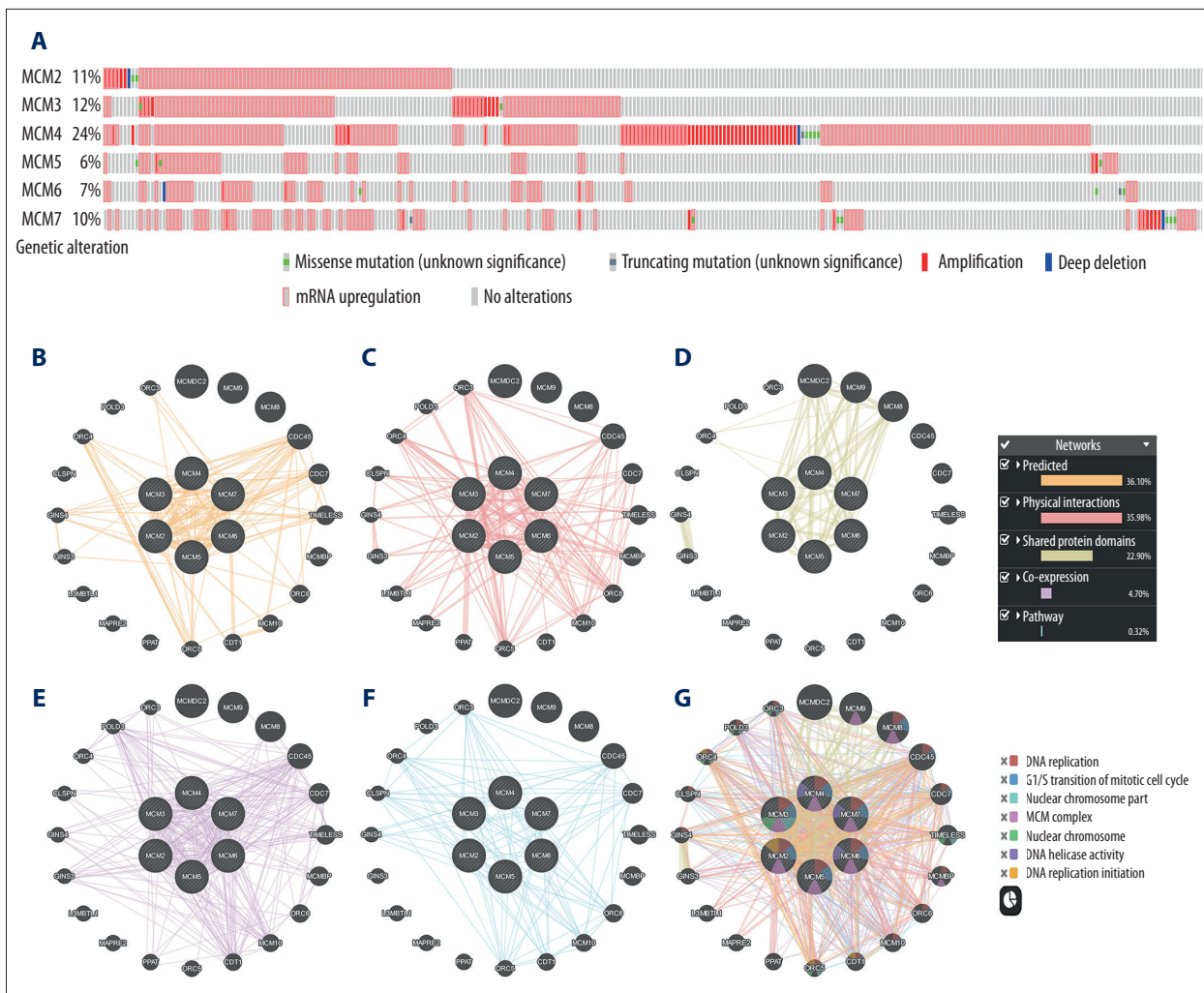
Figure 11. Genomic alterations of MCMs in breast cancer (cBioportal) and potential gene-gene interaction network for MCMs with paths colored according to their functions (GeneMANIA). (**A**) Distribution and proportion of MCMs alterations in breast cancer samples were displayed above. (**A**) Samples without alterations on the right side were cropped from the figure. **G:** The integrated interaction network of MCMs with other related genes was constructed by **B:** predicted interaction, **C:** physical interaction, **D:** shared protein domains, **E:** co-expression, and **F:** pathway.

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