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CLINICAL DATA ANALYSIS OF CDKS EXPRESSION AND PROGNOSIS IN BREAST CANCER

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Abstract – *Objective:* *We investigated the expression of CDKs and prognosis in breast cancer.*

Materials and Methods: *The Oncomine database examined CDK gene expressions in breast cancer. The prescient worth of CDKs in bosom malignant growth patients was analyzed utilizing the Kaplan-Meier Plotter. The expression changes of CDKs in tumor staging were analyzed in the GEPIA database. The role of CDKs in DNA replication and the cell cycle was analyzed utilizing the KEGG data set. Using the CBioPortal database, the association between CDKs gene expression and CDKs in breast cancer was investigated. The Encori database was used to study miRNAs that target CDKs.*

Results: *Oncomine data showed that the expressions of CDK1, CDK5 and CDK20 in breast cancer patients were upregulated, while mRNA expression levels in CDK2 and CDK6 decreased, and CDK3, CDK4 and CDK7~19 were not expression data. Results from the GEPIA database revealed that the expression levels of CDK1, CDK2, CDK4, CDK5, CDK7, CDK8, and CDK20 were greater in breast cancer tissues than in normal tissues, and that CDK1 and CDK5 were significantly different, and the expression levels of CDK3 and CDK1 in the former were lower than those in the latter, while those in the latter did not change. Kaplan-Meier Plotter data showed that CDK1, CDK3, CDK4 and CDK20 were associated with a dismal prognosis in individuals with breast cancer, while mRNA level in CDK8 was associated with progression after survival.*

Conclusions: *CDK1, CDK2c, CDK4, CDK5, CDK7, CDK8 and CDK20 can be used as molecular markers for breast cancer patients, or as potential targets for breast cancer therapy by targeting CDKs.*

KEYWORDS: *Breast cancer, CDKs, Clinical data, Kaplan-Meier Plotter analysis, Tumor markers.*

INTRODUCTION

About 20 serine/threonine kinases make up the Cyclin-dependent kinases (CDKs) family, which controls a variety of cellular biological processes, for instance CDKs control tumor cells by regulating cell cycle, gene transcription and RNA splicing proliferation and growth[1]. Research has indicated that the dysregulation of CDKs is involved in tumorigenesis and development, and targeting CDKs has become a promising tumor therapy strategy[2]. According to its roles, the CDK family is split into two subgroups. One class of CDKs controls the changeover between several cell cycle phases (including CDK1, also known as CDC2, CDK2, CDK3, CDK4 and CDK6), of which CDK3 can regulate the cell cycle G0 phase exit and en-

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ter G1 period[3]. Another class of CDKs is associated with gene transcription (including CDK7, CDK8, CDK9, CDK11, CDK12, CDK13, CDK19 and CDK20 also known as CCRKs)[4]. By phosphorylating RNA polymerase II's carbide terminal domain (CTD), CDKs participate in gene transcription (RNAP II)[2]. Given their ability to control cancer cell survival and growth processes, CDKs are regarded as promising therapeutic targets[4].

Breast cancer has supplanted lung cancer as the most common disease worldwide and the primary cause of cancer deaths in women, according to the most recent data on the global burden of cancer issued by the International Agency for Research on Cancer (IARC) of the World Health Organization in 2020[5]. Like other countries, breast cancer is currently the most common tumor in Chinese women. 9.6% of all breast cancer deaths and 12.2% of all newly diagnosed cases worldwide occur in China[6].

Considering that CDKs have a significant role in the occurrence and progression of cancers, especially breast cancer, we systematically explored the function of CDKs in clinical samples of breast cancer using bioinformatics. Based on online databases (Oncomine, GEPIA, KEGG, cBioPortal and ENCORI, etc.), the expression level, clinicopathological features, survival, and prognosis of CDKs in breast cancer patients were analyzed. In addition, miRNAs regulating CDKs were also analyzed. This research is beneficial for developing breast cancer treatments that target CDKs and for better understanding the role and function of CDKs in breast cancer.

MATERIALS AND METHODS

Database analysis for Oncomine

Information from this database can be used to identify target gene expressions in various malignancies. The levels of CDKs mRNA expression in tumor and healthy tissues were examined. The *p*-value threshold was 0.05, the multiple changes was 2, and the genes were in the top 5%.

GEPIA data

TCGA and GTEx databases are the foundation of GEPIA, which may be used to examine RNA expression in various cancer and healthy tissue samples. GEPIA was used for correlation analysis of CDKs in breast cancer.

Kaplan and Meier Plotter

The impact of target genes on patients' chances of survival for different cancer types could be predicted using this method. Using a Kaplan-Meier Plotter, the predictive value of CDKs and their regulatory mechanisms in breast cancer were studied.

CBioPortal database

The invasive breast cancer database was selected (including 1084 samples). CDKs cancer genome map were based on cBioPortal analysis and construction. For analysis, diploid samples of the genome map with mutations, possible changes in the number of DNA copies, and Z-scores (microarray) for mRNA expression were chosen.

KEGG database

KEGG analyzes visible genes on CDKs cell cycle maps with multiple sub databases, including genomes, biochemical reactions, biochemical substances, diseases and drugs, and common PATHWAY information.

ENCORI database

ENCORI (**http://starbase.sysu.edu.cn/panCancer.php**) to find targeted CDKs microRNAs and regulate CDKs miRNA expression levels were determined.

RESULTS

CDKs transcription levels in people with breast cancer

We compared and examined the transcription levels of CDKs in breast cancer clinical samples with normal breast samples using the Oncomine database. In breast cancer patients, the degrees of mRNA articulation of CDK1, CDK5 and CDK20 were significantly upregulated, while the degrees of mRNA articulation of CDK2, CDK6 and CDK11B were decreased, and the articulation levels of other CDKs were not observed (Figure 1). The transcription levels of CDK1, CDK5 and CDK20 were significantly different between breast cancer tumor tissue with healthy tissue (Figure 1 and Table 1).

	CDK1		CDK2		CDK3		CDK4		CDK5		CDK6		CDK7		CDK8		CDK9		CDK10		CDK11A		CDK11B		CDK12		CDK13		CDK14		CDK15		CDK16		CDK17		CDK18		CDK19		CDK20	
Analysis Type by Cancer	**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**		**Cancer vs. Normal**	
Bladder Cancer	5								2			1	1						2		2							1			3			1	3					
Brain and CNS Cancer	7	1	3				8			5	2					1		1	1									2					1	1		1				1
Breast Cancer	18	1		1					10			5												1															1	
Cervical Cancer	3		2				1																										1				1			
Colorectal Cancer	12		7			1	14		2		9		3	1	11									1		1											2			
Esophageal Cancer	3		1				1					1		3														1							1			3		
Gastric Cancer	3		1				1																		1															
Head and Neck Cancer	4						5		1		4																	2									1			
Kidney Cancer	2				1							1						1				1				1					2		2	1	5	2	3			
Leukemia		8	1	1		1	4	2		1	8	6					1	1						1			2	5	4						1		1		
Liver Cancer	4						2			1			1																											
Lung Cancer	11						1		4												1											2					1			
Lymphoma	5						6		4																	3		1		1	1				3			5		
Melanoma	1		1				1		1			1																			1								
Myeloma					1				1								1								1														
Other Cancer	4	1	5				7		3		2			1								1						1						1	2			5		
Ovarian Cancer	4		1						1																						1			1				1		
Pancreatic Cancer	2										2	1	1																						1	1				1
Prostate Cancer																			1						1											1				
Sarcoma	10		5				2																	1			6								1				
Significant Unique Analyses	97	11	26	2	67	9	53	2	29	7	27	16	6	5	11	1	2	3	4		3	1		2	2	2	4	5	14	9		1	10		4	8	11	7	8	15	1	2
Total Unique Anlyses	455		431		453		463		458		469		456		348		457		388		362		380		419		459		406		267		423		430		448		407		411	

Fig. 1. Transcriptional levels of CDKs in various cancers. Number of datasets with higher expression levels of CDKs in various types of carcinoma samples compared to normal samples. Oncomine (http://www.oncomine.org).

TABLE 1. Transcriptional expression of CDKs in different types of breast cancer (Oncomine).

	Type of Breast Cancer vs. Normal Breast Tissue	Fold Change	p-value	t-test	Source and/or Reference
CDK1	Mucinous breast carcinoma	2.476	7.32E-18	12.474	Curtis Breast Statistics
	Invasive ductal breast carcinoma	3.275	4.98E-135	45.054	Curtis Breast Statistics
	Medullary breast carcinoma	4.030	3.78E-15	13.152	Curtis Breast Statistics
	Breast carcinoma	2.958	3.55E-6	7.036	Curtis Breast Statistics
	Invasive ductal and invasive lobular breast carcinoma	2.931	1.42E-31	16.310	Curtis Breast Statistics
	Tubular breast carcinoma	2.523	1.03E-23	13.953	Curtis Breast Statistics
	Invasive breast carcinoma	3.396	1.90E-8	8.405	Curtis Breast Statistics
	Invasive lobular breast carcinoma	2.325	2.36E-48	18.721	Curtis Breast Statistics
	Mucinous breast carcinoma	8.868	8.74E-5	10.841	TCGA Breast Statistics
	Male breast carcinoma	6.998	1.09E-5	13.620	TCGA Breast Statistics
	Invasive ductal breast carcinoma	5.857	1.51E-47	25.541	TCGA Breast Statistics
	Invasive lobular breast carcinoma	4.761	8.14E-17	10.614	TCGA Breast Statistics
	Invasive breast carcinoma	5.176	6.31E-35	16.809	TCGA Breast Statistics
	Intraductal cribriform breast adenocarcinoma	2.050	8.25E-9	8.46	TCGA Breast Statistics
CDK5	Lobular breast carcinoma	2.449	3.03E-10	11.111	Zhao Breast Statistics
	Invasive ductal breast carcinoma	2.435	2.37E-11	9.468	Zhao Breast Statistics
	Invasive breast carcinoma	2.094	1.70E-9	22.064	Gluck Breast Statistics
	Invasive ductal breast carcinoma	2.166	1.85E-40	21.508	TCGA Breast Statistics
	Invasive ductal breast carcinoma	2.156	1.73E-102	41.097	Curtis Breast Statistics
	Tubular breast carcinoma	2.007	1.27E-35	18.930	Curtis Breast Statistics
	Invasive ductal and invasive lobular breast carcinoma	2.108	9.17E-49	21.978	Curtis Breast Statistics
	Mucinous breast carcinoma	2.246	1.66E-25	17.521	Curtis Breast Statistics
	Medullary breast carcinoma	2.107	1.30E-15	12.930	Curtis Breast Statistics
	Breast carcinoma	2.023	1.22E-10	13.638	Curtis Breast Statistics
CDK20	Intraductal cribriform breast adenocarcinoma	2.225	3.23E-19	17.058	TCGA Breast Statistics

Transcriptional level analysis of CDKs in breast cancer

We compared the expression of CDKs gene in clinical breast cancer samples with healthy breast tissue. The results showed that the expression levels of CDK1, CDK2, CDK4, CDK5, CDK7C and CDK8 in breast cancer tissues were higher than those in healthy tissues, and the expression levels of CDK1 and CDK5 were significantly different (p <0.05) (Figure 2AB). Compared with healthy breast tissues, expressions of CDK3 and CDK9 were decreased in breast cancer tissues, while expressions of CDK6 were not significantly changed (Figure 2AB).

CDKs are expressed in different stages of breast cancer

GEPIA database was used to analyze the expression of CDKs in different stages of breast cancer clinical samples. The results showed that CDK1 had significant differences in different stages of breast cancer (p < 0.05), while other subtypes of CDKs had no significant differences in breast cancer stages (Figure 3).

Clinical breast cancer patients' chance of survival and the predictive significance of CDKs

Kaplan-Meier database was used to detect the relationship between CDKs mRNA levels with survival of clinical breast cancer patients. The results showed that CDK1, CDK4 and CDK20 had significant differences with no recurrence rate (RFS) and overall survival (OS) (p <0.05). CDK3 with RFS were significantly different (p <0.05). CDK8 mRNA levels were correlated with the survival rate after progression and were significantly different from that of PPS (p <0.05) (Figure 4).

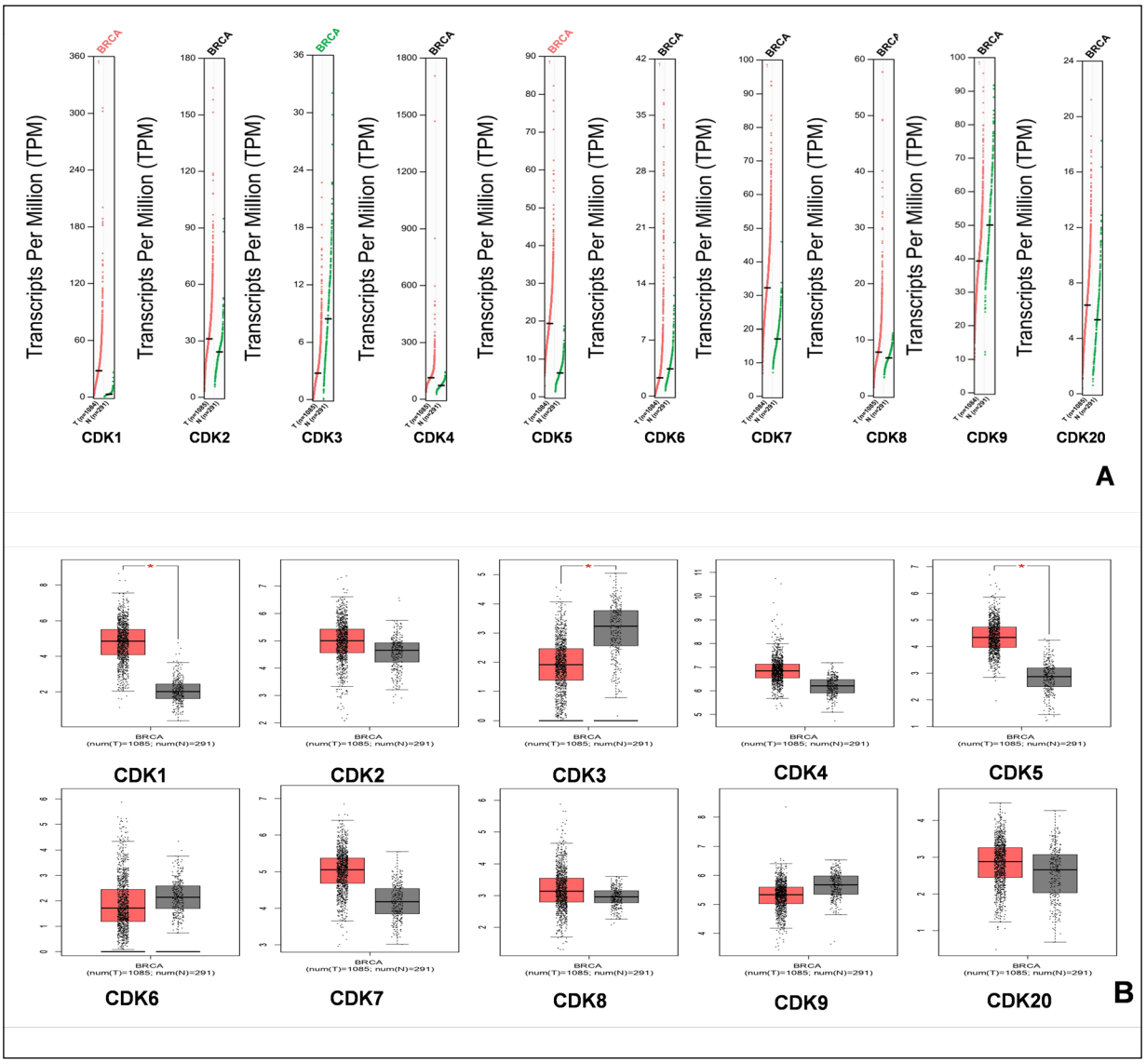


Fig. 2. Expression of CDKs in Breast Cancer (A. scatter diagram; B. box plot). GEPIA database (http://gepia.cancer-pku.cn/).

These results suggest that breast cancer patients with high expressions of CDK1, CDK3, CDK4, CDK8, and CDK20 have a poorer prognosis.

Gene recombination, gene correlation and co-expression gene network of CDKs in breast cancer

Analysis of CDKs gene mutations or recombination using the CBioPortal database showed that 127 (13%) of 1084 breast cancer samples had CDK mutations (Figure 5 A). Correlation analysis of CDKs mRNA expression in breast cancer showed that CDK1 is positively correlated with CDK2, CDK4, CDK6, CDK8, and negatively correlated with CDK9, CDK20 ($R > 0$, $p < 0.05$). CDK2 is positively correlated with CDK4, CDK6, CDK8, and negatively correlated with CDK5, CDK9, CDK20 ($R > 0$, $p < 0.05$). CDK3 is positively correlated with CDK4, CDK5, CDK9, CDK20, and negatively correlated with CDK7, CDK8 ($R > 0$, $p < 0.05$). CDK4 is positively correlated with CDK5, CDK7 ($R > 0$, $p < 0.05$). CDK5 is positively correlated with CDK7, CDK9, CDK20, and negatively correlated with CDK6, CDK8 ($R > 0$, $p < 0.05$). CDK6 is positively correlated with CDK8, and negatively correlated with CDK7, CDK9, CDK20 ($R > 0$, $p < 0.05$). CDK7 is positively correlated with CDK20, and negatively correlated with CDK8 ($R > 0$, $p < 0.05$). CDK8 is negatively correlated with CDK9, CDK20 were negatively correlated. CDK9 was positively correlated with CDK20 ($R > 0$, $p < 0.05$) (Figure 5 B).

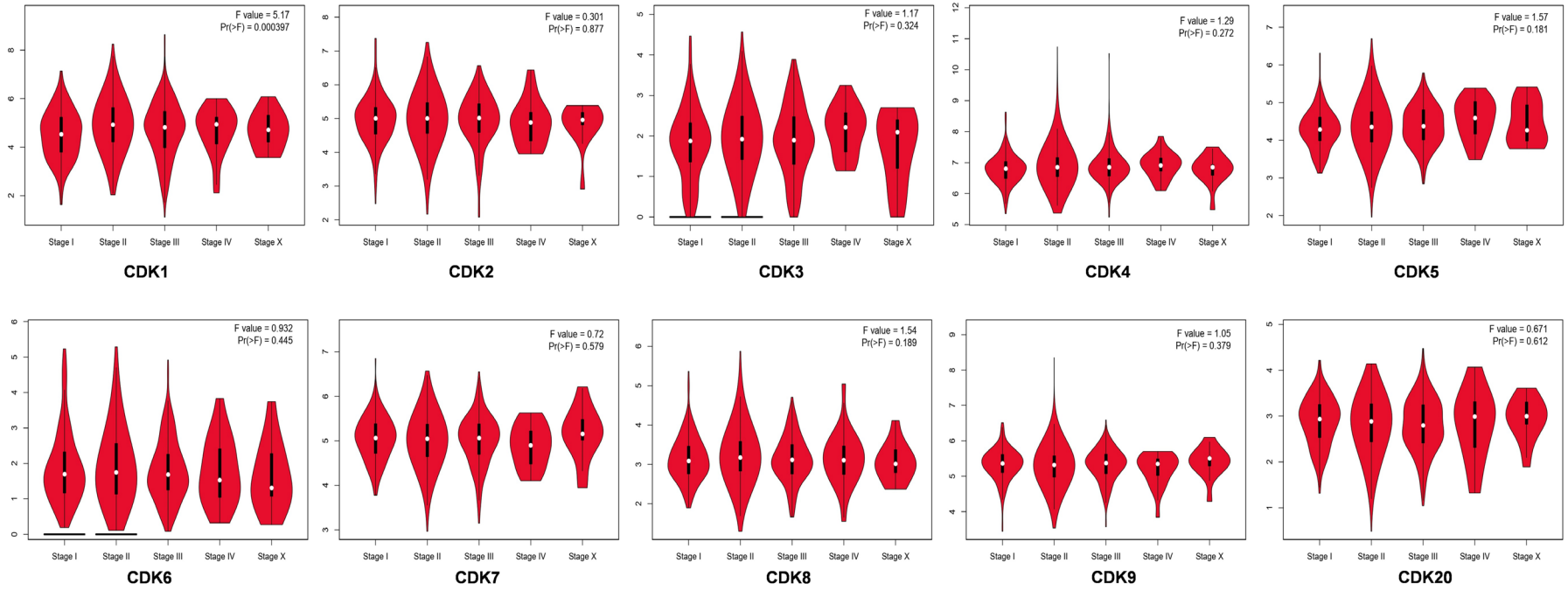


Fig. 3. Correlation between CDKs expression and tumor stage in breast cancer patients. GEPIA database (http://gepia.cancer-pku.cn/).

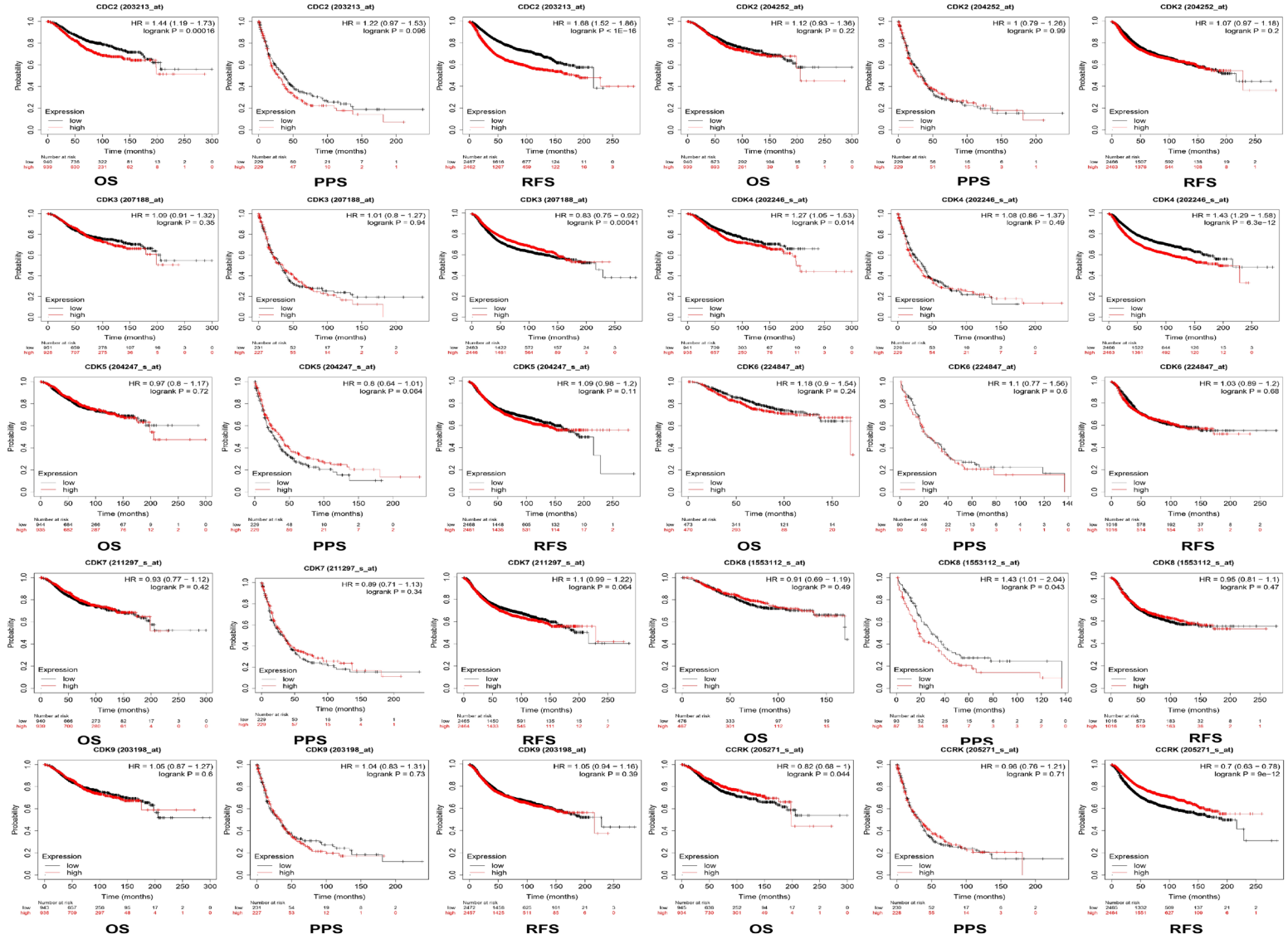
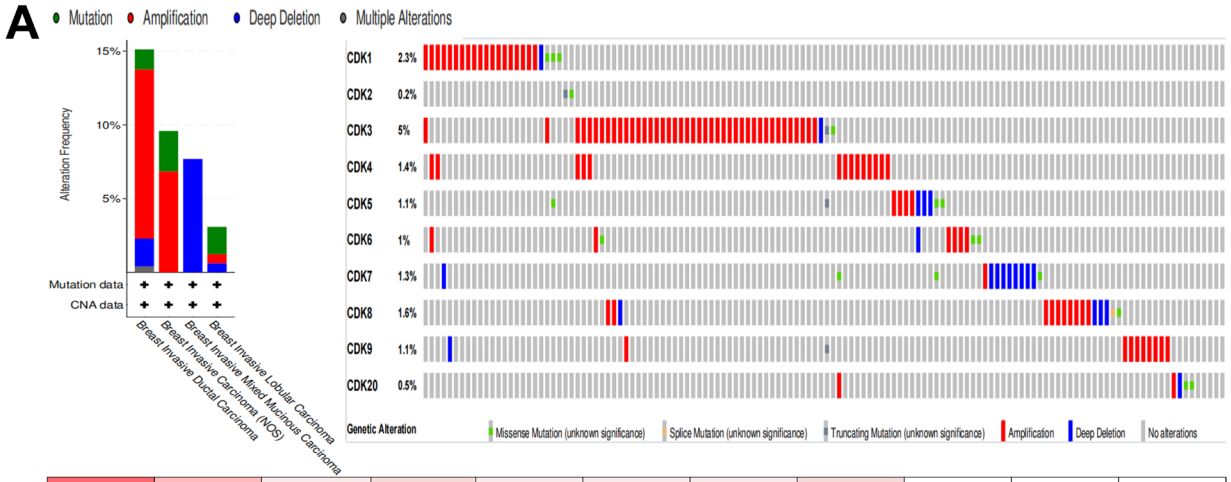


Fig. 4. Prognostic value of the expression levels of MCMs in patients with BC. k-Mplotter database (http://kmplot.com/analysis/index.php?p=background).



B

1	0. 49	0. 06	0. 21	0. 02	0. 15	0. 00	0. 26	−0. 07	−0. 17	CDK1
0. 49	1	0. 02	0. 18	−0. 1	0. 1	−0. 03	0. 29	−0. 13	−0. 09	CDK2
0. 06	0. 02	1	0. 17	0. 23	0. 04	−0. 07	−0. 13	0. 22	0. 21	CDK3
0. 21	0. 18	0. 17	1	0. 1	0. 05	0. 07	0. 05	0. 04	−0. 06	CDK4
0. 02	−0. 1	0. 23	0. 10	1	−0. 15	0. 10	−0. 22	0. 24	0. 19	CDK5
0. 15	0. 1	0. 04	0. 05	−0. 15	1	−0. 15	0. 34	−0. 15	−0. 22	CDK6
0	−0. 03	−0. 07	0. 07	0. 1	−0. 15	1	−0. 15	0. 02	0. 17	CDK7
0. 26	0. 29	−0. 13	0. 05	−0. 22	0. 34	−0. 15	1	−0. 20	−0. 27	CDK8
−0. 07	−0. 13	0. 22	0. 04	0. 24	−0. 15	0. 02	−0. 20	1	0. 19	CDK9
−0. 17	−0. 09	0. 21	−0. 06	0. 19	−0. 22	0. 17	−0. 27	0. 19	1	CDK20
CDK1	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9	CDK20	

Fig. 5. Analysis of alterations and correlation between members of CDKs in BC. (A) Gene expression and alteration analysis of CDKs in BC (cBioPortal). (B) Correlation analysis between different CDKs in BC. cBioportal (https://www.cbioportal.org/).

Physics CDKs' function in the cell cycle

Cell cycle progression is dependent on cyclins, which are regulated by CDKs. As shown in Figure 6, cyclin D is phosphorylated by CDK2, CDK4, and CDK6, driving cell cycle progression to G1. However, p16INK4a, p15INK4b, p18INK4c and p19INK4d in the INK4 family interfere with CDK4 and CDK6 and prevent cyclin D phosphorylation and progression toward G1. CDK2/Cycline E regulates the G1/S transition of the cell cycle and CDK2-cycline A controls the S phase of the cell cycle. cdk1/Cycline B regulates G2/M. The kinase suppressor protein (KIP) family, which includes p21CIP1, p27KIP1, and p57KIP2, binds and inactivates CDK. Binding of cdk2 to p27KIP1 delays activation of the cdk2/Cycline E complex, resulting in SKP1/SKP2 ubiquitination of cdk2 and destruction by the proteasome. As shown in Figure 6, CDK1, CDK2, CDK4, CDK6, CDK7, and CDK14 are involved in the cell cycle process.

MiRNA targeted CDKs

ENCORI database was used to screen miRNAs targeting CDKs. As shown in Table 2, there were 42 miRNAs targeting cdk1, of which 21 were negatively correlated. One hundred miRNAs targeted cdk2, 36 of which were negatively correlated. Two miRNAs target cdk3, one of which is negatively correlated. Fifty-one miRNAs targeted cdk4, of which 20 were negatively correlated. Nine miRNAs targeted cdk5, three of which were negatively correlated. 368 miRNAs targeted cdk6, of which 94 were negatively correlated. Seven miRNAs targeted cdk7, all of which were negatively correlated. 116 miRNAs targeted cdk8, 35 of which were negatively correlated. There were 68 miRNAs targeting cdk9, 45 of which were negatively correlated. There were 8 miRNAs targeting cdk20, 5 of which were negatively correlated. The above miRNA capable of targeting CDKs may serve as potential biologic drugs for the treatment of breast cancer progression.

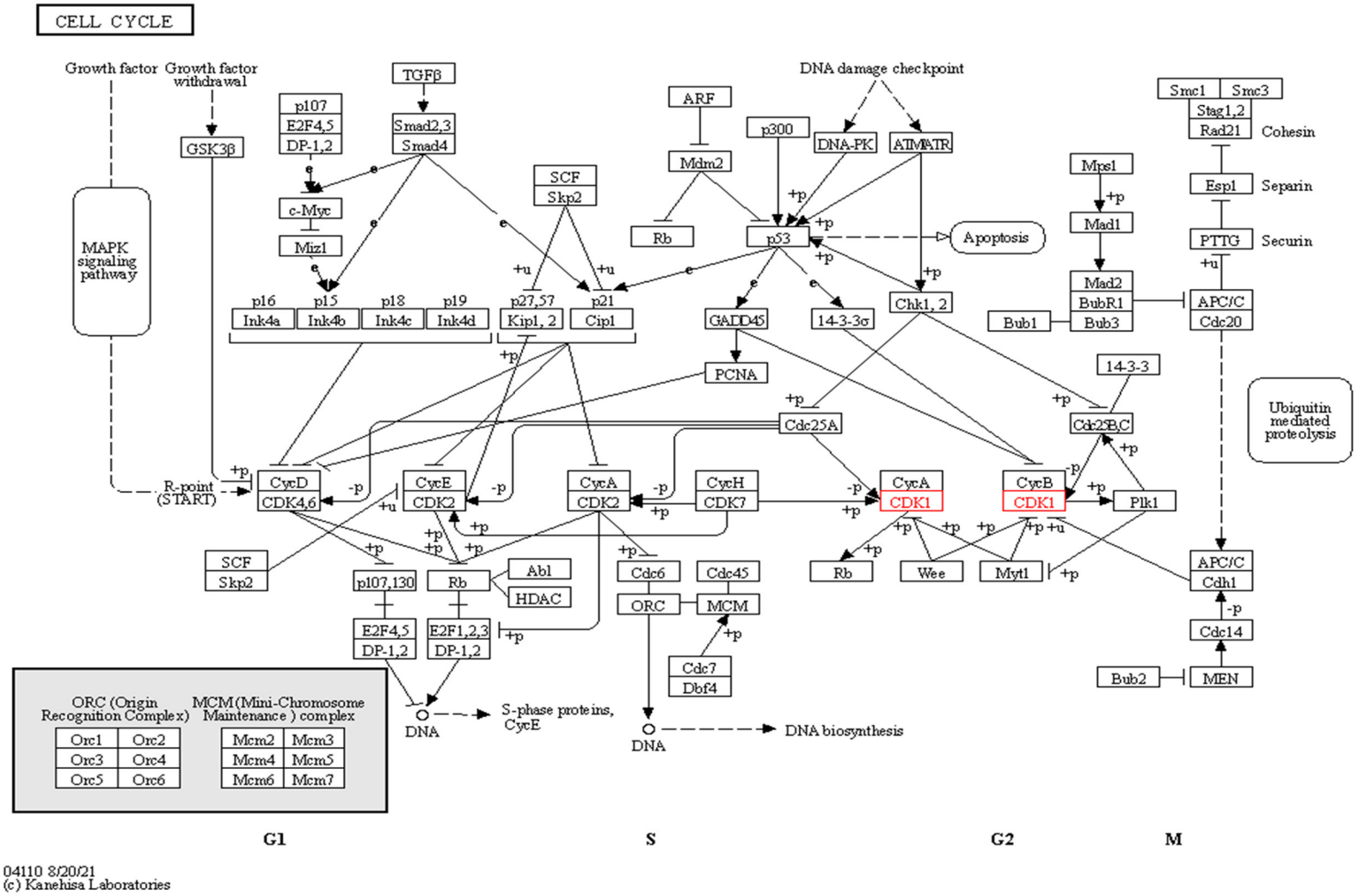


Fig. 6. Visualization genes on cell cycle map. KEGG database (https://www.genome.jp/kegg/).

TABLE 2. Analysis of miRNAs targeting CDKs.

	Negative regulation number	*Positive regulation number*	*miRNA factor that targets the CDK gene*
CCDK1	21	21	hsa-miR-329-3p, hsa-miR-410-3p, hsa-miR-944
CDK2	36	64	hsa-miR-29c-3p, hsa-miR-199a-5p, hsa-miR-664b-3p
CDK3	1	1	hsa-miR-628-5p
CDK4	20	31	hsa-miR-497-5p, hsa-miR-654-5p, hsa-miR-663a
CDK5	3	6	hsa-miR-24-3p, hsa-miR-1287-5p, hsa-miR-1295a
CDK6	94	274	hsa-miR-190b, hsa-miR-29c-3p, hsa-miR-375
CDK7	7	0	hsa-miR-139-5p, hsa-miR-362-5p, hsa-miR-500b-5p
CDK8	35	78	hsa-miR-7b-5p, hsa-miR-30a-5p, hsa-miR-5691
CDK9	45	23	hsa-miR-23a-3p, hsa-miR-23b-3p, hsa-miR-455-3p
CDK20	5	3	hsa-miR-24-3p, hsa-miR-574-3p, hsa-miR-4739

DISCUSSION

We present a detailed analysis of CDKs expression, clinicopathological features, gene mutations, and prognosis in breast cancer. According to our results, the expression levels of CDK1, CDK5 and CDK20 in breast cancer samples were significantly up-regulated (Figure 2AB). The data showed that there were significant differences in CDK1 in different breast cancer stages (Figure 3). The prognosis of breast cancer patients with high expression of CDK1, CDK3, CDK4, CDK8 and CDK20 is poor (Figure 4). Correlation analysis of CDKs mRNA expression in breast cancer showed a correlation between CDKs and CDKs. CDK1, CDK2, CDK4, CDK, 6, CDK7 and CDK14 are involved in cell cycle processes (Figure 6). MiRNA targeting CDKs may be potential biologic drugs for the treatment of breast cancer progression. In conclusion, CDK1, CDK2c, CDK4, CDK5, CDK7, CDK8 and CDK20 can be used as potential targets for the treatment of breast cancer targeting CDKs.

CDK1 promotes cell cycle G2/M and G1/s conversion and G1 progression [7,8]. CDK1 is the key driver of unlimited proliferation of malignant tumors [9]. The prognosis of various malignant cancers is tightly correlated with the upregulation of CDK1 protein. For example, CDK1 is significantly overexpressed in epithelial ovarian cancer. The early detection of ovarian cancer may benefit from identifying changes in CDK1 content in ovarian tissue [10]. Additionally, CDK1 is also overexpressed in endometrial carcinoma, and the accumulation of CDK1 is related to the histological grade of endometrial carcinoma [11]. At present, there has been some progress in targeting CDK1 for tumor therapy. At DNA structural checkpoints, CDK1 inhibitors can stop tumor cells from progressing through the cell cycle, causing G2/M phase cell cycle arrest [12]. In addition to drugs that directly target the activity of CDK1, the anticancer effects of many drugs are considered to be achieved at least in part by inhibiting CDK1, including 5MeOlndox, HDAC inhibitors and flavonoids [13,14]. Combined with our study results, CDK1 is overexpressed in breast cancer, and CDK1 is directly related to the prognosis of breast cancer patients (Figure 2, Figure 4). Drugs targeting CDK1 can inhibit the level of CDK1 and inhibit the development or progression of breast cancer.

The activation or expression disorder of CDK2 plays a key regulatory role in the progression of gastric cancer cell cycle, and the upregulation of CDK2 may play a crucial role in promoting the proliferation and cell phase transformation of gastric cancer cells [15-17]. Current studies have shown that triple negative breast cancer (TNBC) cell migration is inhibited when CDK2 is inhibited, and the high expression of CDK2 can significantly enhance the apoptosis of cancer cells [18]. Our results also show that CDK2 is highly expressed in breast cancer tissues, that CDK2 is positively correlated with CDK4/6/8 expression, and that CDK2 is involved in cell cycle progression (Figure 5, Figure 6). Therefore, CDK2 inhibitors may have the potential to be developed into effective anticancer drugs. Since 1990, more than a dozen CDK2 inhibitors have entered clinical studies. First-generation CDK2 inhibitors, such as flavonol, (R)-roscovitine, ssn-032, and PHA-793887, were stopped in Phase II or III trials due to their pharmacological side effects and low specificity [1,19]. The second generation of CDK2 inhibitors has been developed.

CDK3 is involved in cell cycle transition and is critical for the regulation of G0/G1 and G1/S

phases of the cell cycle. Studies have shown that CDK3 has the ability to promote cell growth and transformation and can be used as tumor initiation [20]. CDK3 is overexpressed in many cancer cells and is essential for cell proliferation and malignant transformation [21]. However, CDK3 may also inhibit migration and invasion, and overexpression of CDK3 can prevent the migration and invasion of breast cancer cells [20]. In contrast to the above results, CDK3 promotes epithelial-mesenchymal transition (EMT) and metastasis in rectal cancer [22]. In addition, our results show that breast cancer patients with high expressions of CDK3 have a poorer prognosis (Figure 4). Targeting CDK3 can inhibit breast cancer metastasis by inhibiting the Wnt/β-catenin pathway [8], and CDK3 may play a role as a potential target for breast cancer.

CDK4/6 has 71% amino acid similarity and is a key regulator of the cell cycle. CDK4/6 interacts with cyclin D1, cyclin D2 and cyclin D3 [2]. Overexpression of CDK4/6 can promote G1/S transformation through direct or indirect phosphorylation of Rb, thus promoting tumorigenesis [23]. Studies have shown that CDK4/6 is highly expressed in a variety of cancers, such as breast cancer, preglioblastoma [24], melanoma [25], and epithelial ovarian cancer [26]. Our results also show breast cancer patients with high expressions of CDK4 have a poorer prognosis (Figure 4). Correlation analysis of CDKs mRNA expression in breast cancer showed that CDK4 and CDK6 are involved in the cell cycle process (Figure 6). To date, three cyclin dependent kinase 4/6 inhibitors are in various stages of clinical development: PD0332991 (palbociclib), LEE011 (ribociclib) and LY2835219 (abemaciclib) [27]. Results from current phase I, II and III trials in breast cancer are encouraging, showing convincing efficacy and tolerable side effects. Future and ongoing clinical trials could expand the potential use of these drugs. In conclusion, CDK4/6i is an exciting compound that may transform the treatment landscape of breast cancer. Therefore, CDK4/6 is considered a key therapeutic target for a variety of cancers. In addition, the FDA has approved CDK4/6 inhibitor drugs (ribociclib, palbociclib and abemaciclib) for marketing [23].

As an oncogene driver, CDK5 can be activated by Cyclin I to promote MEK/ERK signaling pathway [28]. High CDK5 expression is associated with poor prognosis, cell proliferation, migration, and invasion. Studies have shown that the expression and activity of CDK5 in human liver cancer tissues is increased compared with normal liver tissues. When CDK5 is knocked out in liver cancer cells, the proliferation ability is greatly reduced, as is the cloning survival [8]. Down-regulating CDK5 in gastric cancer can improve the prognosis of gastric cancer. Our results showed that the expression levels of CDK5 was significantly upregulated in breast cancer clinical samples compared with normal healthy tissues (Figure 2AB). Therefore, CDK5 regulation is a potential target for cancer therapy [29]. These studies demonstrate the potential value of CDK5 as a molecular marker for cancer. At present, a variety of anti-tumor drugs targeting CDK5, such as roscovitine and olomoucine, have entered preclinical trials [30].

In addition to activating CDK1 and CDK2, CDK7 is also involved in regulating CDK4 and CDK6 to maintain their activity and control cell cycle and gene transcription [31,32]. CDK7 is directly involved in cell cycle and transcriptional regulation, promoting tumor development. Studies have shown that the incidence and prognosis of epithelial ovarian cancer patients are closely related to the high expression of CDK7 [33]. In addition, studies have found that CDK7 expression is significantly upregulated in gastric cancer, and CDK7 is positively correlated with tumor grade and depth of invasion [34]. Our results showed that CDK7 was highly expressed in breast cancer and that CDK7 was involved in the cell cycle process (Figure 2AB, Figure 6). At present, cdk7 specific inhibitors, including non-covalent inhibitors BS-181, ICEC0942, LDC4297 and QS1189, as well as covalent inhibitors THZ1, THZ2 and YKL-5-124, have strong antitumor effects [35]. ICEC0942, an oral antitumor drug targeting CDK7, may have a better therapeutic effect on breast cancer when used alone or in combination with hormone therapy [36]. These results indicate that CDK7 is an ideal target for novel anti-tumor.

The CDK8/Cyclin C complex phosphorylates RNA polymerase II to regulate transcription [37], and CDK8/Cyclin C is also able to phosphorylate Cyclin H to block the activity of the active kinase CAK [38]. CDK8 is widely expressed in colon, colorectal and breast cancers. Reducing CDK8 expression can stop cancer cell proliferation, migration, and cell cycle G0/G1 phase progression [39-41]. In addition, inhibition of CDK8 expression can stop the proliferation of colon cancer cells [40]. However, one study found that CDK8 expression was negatively correlated with many important characteristics of endometrial cancer cells, such as cell proliferation, migration and invasion, and tumor progression in vivo, suggesting that CDK8 has an oncogenic role in endometrial cancer [42]. Our study showed that breast cancer patients with high expressions of CDK8 have a poorer prognosis (Figure 4). CDK8 is highly expressed in breast cancer, and the expression of CDK8 is positively correlated with the levels of CDK1, CDK2, CDK4, and CDK6 (Figure 2AB, Figure 5B). Thus, CDK8

is a promising new target for tumors. Recent studies have shown that CDK8 kinase activity is required for estrogen-induced transcription and that CDK8 is a possible therapeutic target for HR-positive breast cancer cells [43].

CDK9 is involved in transcription initiation, elongation, and termination of RNA polymerase II (pol II) and has a crucial role in development, differentiation, and cell fate [44]. CDK9 is dysregulated in a variety of malignancies [45,46], such as breast and cervical cancers [47]. Our results show that CDK9 is positively correlated with the levels of CDK3, CDK5, and CDK20 (Figure 5B). Current antitumor drugs developed to target CDK9 include Fadraciclib, AZD-4573, CDKI-73, and MC180295. Preclinical studies have confirmed the strong anticancer activity of all these drugs [35]. In addition, CDK9 inhibitors have been shown to halt the progression of breast cancer [48-51].

The CDK family protein CDK20 gene was recently identified [52,53]. CDK20 has sequence similarity to the CDK7 gene, and the resulting homology analysis indicates that it has CAK activity [54]. In addition, several studies have shown that CDK20 is highly expressed in a variety of tumors including glioblastoma [55], and hepatocellular carcinoma [56-58], and reducing the expression of CDK20 can stop the spread of cancer cells. Our study showed that CDK20 is highly expressed in breast cancer, and breast cancer patients with high expressions of CDK20 have a poorer prognosis (Figure 1 and 4). CDK20 has also been suggested as a target for the prevention of cancer chemoresistance. A major hallmark of cancer is aberrant cell proliferation, which is uncontrolled unlimited cell division. In contrast, the complex composed of CDKs/Cyclins is mainly responsible for controlling cell division. Therefore, it is crucial to figure out the expression, role, and function of CDKs in tumors and to develop targeted drugs for cancer therapy against CDKs. Scientists have developed a variety of CDK inhibitors after extensive preliminary research base, and these targeted drugs have shown significant tumor suppressive effects, and good ability to cause apoptosis of tumor cells. Since the 1990s, researchers have conducted research and development of antitumor drugs targeting CDKs. The first generation of inhibitors of CDKs blocked cell division and cell cycle by inhibiting the activity of CDKs enzymes. The first generation of inhibitors targeting CDKs was developed as pan-CDK inhibitors, and these inhibitors include heteroenolic scaffolds, including flavonoids, purines, ninhydrin, aminopyrimidines, aminothiazoles, ninhydrin, hydantoin aspartates, and paladin derivatives [59]. However, the first generation of pan-CDK inhibitors exhibited high toxicity and low selectivity, invariably with detrimental effects on normal cells. To optimize the first-generation CDKs antitumor agents, researchers have developed several second-generation CDKs antitumor agents with high specificity and weak adverse effects, including dinaciclib P276-00, AT7519, TG02, roniciclib, RGB-286638, and others [35]. Preclinical and clinical studies have been conducted, and second-generation CDK inhibitors have shown efficient anticancer efficacy. To date, 62 small molecule protein kinase inhibitors have been approved by the U.S. Food and Drug Administration (FDA) in the United States. Only antitumor drugs targeting CDK4/6 are currently on the market, but the market demand for antitumor drugs remains singularly short with the current increasing number of oncology patients. Therefore, the development of more and more effective antitumor drugs targeting CDKs is a real need, and the research and creation of CDKs inhibitors will continue to progress and improve in the coming years. In addition, antitumor drugs targeting CDKs may also improve survival and prognosis of breast cancer patients. However, our study still has some limitations. For example, the predictive significance of CDKs in breast cancer was only assessed using the Kaplan Meier mapper. Given the substantial role played by CDKs in this disease, identification of CDKs as breast cancer biomarkers and targets will provide a diagnostic basis, and therapeutic measures for oncology patients.

CONCLUSIONS

In this study, the expression degree, clinicopathological features, gene recombination and prognosis of CDKs in breast cancer were comprehensively analyzed. Our results showed that the expression levels of CDK1, CDK5 and CDK20 were significantly upregulated in breast cancer clinical samples compared with normal healthy tissues. CDK1 had significant differences in different stages of breast cancer. Breast cancer patients with high expressions of CDK1, CDK3, CDK4, CDK8, and CDK20 have a poorer prognosis. Analysis of CDKs gene mutations or recombination using the CBioPortal database showed that 127 (13%) of 1084 breast cancer samples had CDK mutations. Correlation analysis of CDKs mRNA expression in breast cancer showed that CDK1, CDK2, CDK4, CDK6, CDK7, and CDK14 are involved in the cell cycle process. MiRNA capable of targeting CDKs may serve as potential biologic drugs for the treatment of breast cancer progression. In conclusion, CDK1, CDK2c, CDK4,

CDK5, CDK7, CDK8 and CDK20 can be used as molecular markers for breast cancer patients, or as potential targets for breast cancer therapy by targeting CDKs.

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Conflict of Interest:

No author has conflict of interest with the contents of this article.

Data availability statement :

The source of the raw/processed data needed to reproduce these findings is in the "Methods" section.

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