

# Expression and prognostic value of *CDK1*, *CCNA2*, and *CCNB1* gene clusters in human breast cancer

Journal of International Medical Research

49(4) 1–13

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DOI: 10.1177/0300060520980647

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## Abstract

**Objective:** Cell cycle-associated proteins play important roles in breast cancer (BRCA), based on evidence from cell lines, preclinical murine models, and human tissue samples.

**Methods:** Herein, we used the Onomine, GEPIA, Kaplan–Meier Plotter, and cBioPortal databases to examine transcriptional and survival data pertaining to cyclin-associated gene clusters (*CDK1*, *CCNA2*, and *CCNB1*) in BRCA patients.

**Results:** *CDK1*, *CCNA2*, and *CCNB1* gene expression levels were higher in BRCA compared with control tissue samples and were correlated with more-advanced tumor stage. Kaplan–Meier survival analyses confirmed that elevated *CDK1*, *CCNA2*, and *CCNB1* expression levels were associated with overall and post-progression survival and recurrence-free probability rates in patients with BRCA.

**Conclusion:** The results of this study implied that *CDK1*, *CCNA2*, and *CCNB1* gene clusters may provide potential therapeutic targets and prognostic biomarkers in patients with BRCA.

## Keywords

*CDK1*, *CCNA2*, *CCNB1*, prognosis, breast cancer, cell cycle

Date received: 7 May 2020; accepted: 19 November 2020

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## Introduction

Breast cancer (BRCA) remains the most common form of cancer and the main cause of cancer-related deaths affecting women globally.<sup>1</sup> Surgical resection is the



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standard therapy for BRCA. Patients are generally categorized according to a variety of clinical and pathological factors, including age, tumor size, tumor histological grade, and lymph node metastasis, to assess their prognosis and determine the appropriate treatment options. However, this conventional method often fails to provide a precise prognosis.<sup>2,3</sup> Additional prognostic markers and potential drug targets therefore need to be identified to enhance the prognosis and delivery of individualized treatments.

Cyclin-dependent kinase (CDK) 1 (*CDK1* gene) is the only essential CDK<sup>4,5</sup> required to facilitate the G2–M phase transition. It regulates G1 phase progression and the G1–S transition.<sup>6</sup> Alterations in CDK1 activity may lead to uncontrolled proliferation of tumor cells, which is a hallmark of malignant tumors. Cyclin A2 (*CCNA2* gene) is expressed in most human tissues and is a highly conserved member of the cyclin protein family. It plays a key role in controlling the G1–S and G2–M cell cycle transitions, in addition to being an important regulator of hematopoietic and embryonic cells.<sup>7</sup> Cyclin B1 (*CCNB1* gene) is also a highly conserved cyclin family protein that is ubiquitously expressed in humans, and which is purportedly involved in regulating tumor epithelial–mesenchymal transitions and metastasis.<sup>8</sup> However, the underlying mechanisms and unique roles of these three genes in BRCA remain unclear.

Dysregulated expression levels of *CDK1/CCNA2/CCNB1* gene clusters and their association with patient prognosis and findings in BRCA have been reported in some studies, but their expression profiles and prognostic relevance are still largely unknown. In the current study, we assessed *CDK1*, *CCNA2*, and *CCNB1* gene expression levels and mutations in BRCA patients to assess their potential functions, expression

patterns, and prognostic relevance using large publicly available datasets.

## Materials and methods

### Ethics statement

This study was approved by the Institutional Review Board of Chinese Academy of Medical Sciences and Peking Union Medical College (Permit No: NO.135) and conducted according to the ethical guidelines of the Declaration of Helsinki. All the datasets were extracted from online public databases and no additional consent was therefore required.

### Oncomine analysis

The Oncomine online database ([www.oncomine.org](http://www.oncomine.org)) is a public database that can be used to analyze gene expression in various cancers. We searched the database for the genes of interest and analyzed their expression levels in BRCA. We also assessed the expression levels of *CDK1*, *CCNA2*, and *CCNB1* in different cancers using the same database.

### Gene Expression Profiling Interactive Analysis (GEPIA) dataset

The GEPIA database combines The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression data, including RNA-seq results from 9736 tumor and 8587 normal samples, thus allowing the assessment of differential expression profiles, patient outcomes, and other factors.<sup>9</sup> These public bioinformatics platforms allowed analysis of the expression profiles of *CDK1*, *CCNA2*, and *CCNB1* in BRCA.

### RNA extraction and quantitative real-time-PCR

We verified the mRNA expression levels of *CDK1*, *CCNA2*, and *CCNB1* in eight pairs

of BRCA and adjacent non-tumor tissues collected from the Breast Cancer Center of the National Cancer Center (Beijing, China). Approximately 20 mg of tissue was obtained from each sample for RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. All PCR reactions were performed using an ABI Prism 5700 Sequence Detection System (PerkinElmer Applied Biosystems, Foster City, CA, USA).

### Human Protein Atlas

The Human Protein Atlas (<https://www.proteinatlas.org>) contains immunohistochemical expression data for nearly 20 common kinds of tumors, with 12 individual subtypes per tumor.<sup>10</sup> The database allows researchers to identify the expression patterns of certain proteins in a given type of tumor. We directly compared CDK1, cyclin A2, and cyclin B1 levels between normal and BRCA tissues using immunohistochemistry images.

### Kaplan–Meier (K–M) plotter

The prognostic relevance of *CDK1*, *CCNA2*, and *CCNB1* expression in BRCA was assessed using K–M Plotter ([www.kmplot.com](http://www.kmplot.com)),<sup>11</sup> which contains survival information and gene expression data for 6234 clinical BRCA patients. The median expression value was used to stratify patients into low- and high-expressing groups for each gene, and overall survival (OS), post-progression survival (PPS), and recurrence-free probability (RFP) rates were assessed in BRCA patients using K–M plotter, with corresponding 95% confidence intervals and *P*-values.

### Functional pathway enrichment analyses

The online STRING (<https://string-db.org/>) tool provides investigators with systematic

and comprehensive functional annotation tools to reveal the biological meaning behind an extensive list of genes. We conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for *CDK1*, *CCNA2*, and *CCNB1* using STRING, with a significance threshold of  $P < 0.05$ .

### TCGA and cBioPortal

TCGA contains sequencing and clinicopathologic data for 30 cancer types.<sup>12</sup> The Metastatic Breast Cancer Project (Provisional, October 2018) and Breast Invasive Carcinoma (TCGA PanCan 2018) were selected for further analyses of *CDK1*, *CCNA2*, and *CCNB1* using cBioPortal (<https://www.cbioportal.org>). Genomic profiles, including mutations and copy-number alterations, were calculated using cBioPortal's online tool.

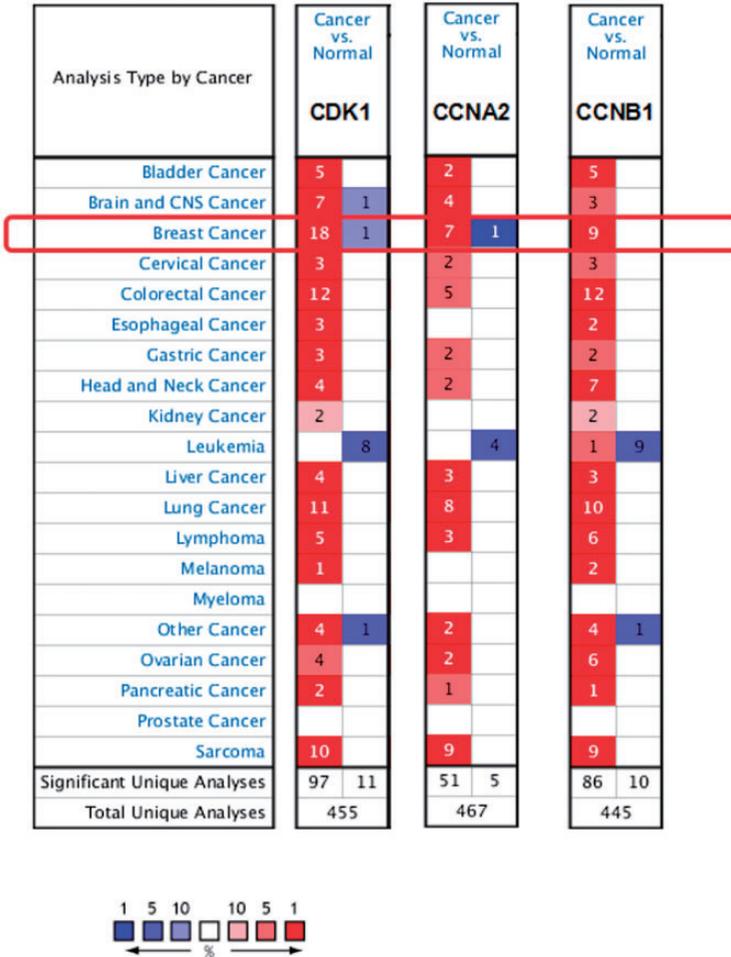
### Statistical analysis

Data from the online databases were processed as detailed above. Results derived from samples were analyzed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Continuous variables were compared between two groups using Student's *t*-test.  $P < 0.05$  was considered significant.

## Results

### *CDK1/CCNA2/CCNB1* gene expression in BRCA

We compared the gene expression levels of *CDK1*, *CCNA2*, and *CCNB1* in all cancers and normal tissues using OncoPrint (Figure 1). *CDK1* mRNA levels were significantly elevated in BRCA patients in 11 datasets, *CCNA2* was significantly upregulated in seven BRCA datasets, and *CCNB1* was significantly upregulated in nine BRCA datasets.

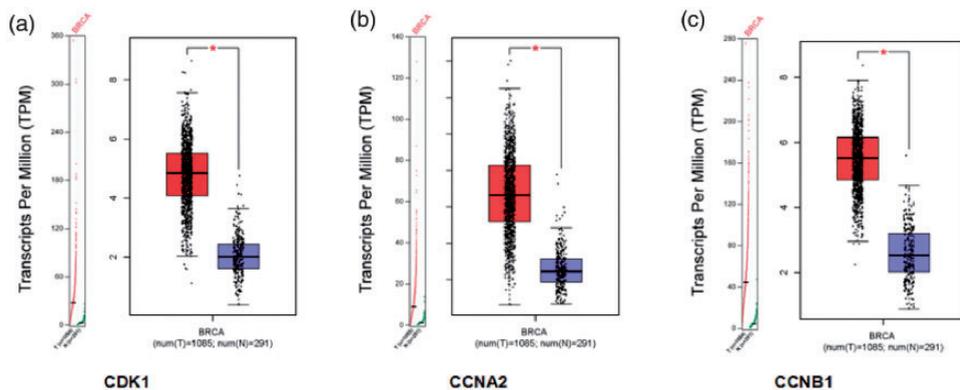


**Figure 1.** Transcription levels of *CDK1*, *CCNA2*, and *CCNB1* in different types of cancers (Oncomine). Red: over-expression; blue: under-expression.

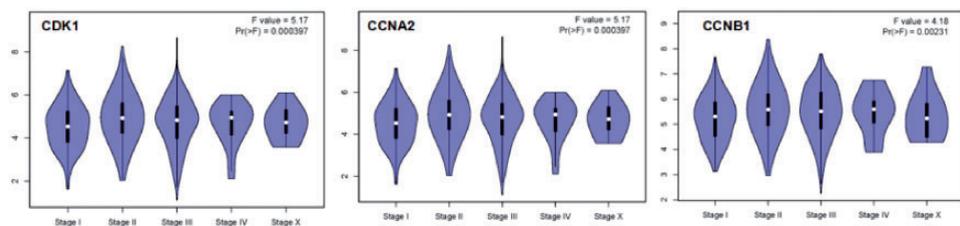
**Relationship between *CDK1/CCNA2/CCNB1* levels and clinicopathological findings in BRCA patients**

Gene expression levels of *CDK1*, *CCNA2*, and *CCNB1* were all higher in BRCA tumor tissues compared with normal tissues, as revealed by GEPIA (Figure 2). We also found a clear correlation between gene expression level and tumor stage, with

BRCA patients with more advanced stages tending to have higher expression levels of *CDK1*, *CCNA2*, and *CCNB1* (Figure 3). We also examined the protein expression patterns of CDK1, cyclin A2, and cyclin B1 based on the Human Protein Atlas. The results confirmed that levels of all three proteins were elevated in BRCA samples relative to normal controls (Figure 4). We verified the mRNA expression levels of



**Figure 2.** Correlation between expression levels of *CDK1*, *CCNA2*, and *CCNB1* and tumor stage in breast cancer (BRCA) patients (GEPIA) by box plots. Expression profiles (red = high expression; blue = low expression) and box plots of (a) *CDK1*, (b) *CCNA2*, and (c) *CCNB1* in BRCA patients (GEPIA). \* $P < 0.05$ .



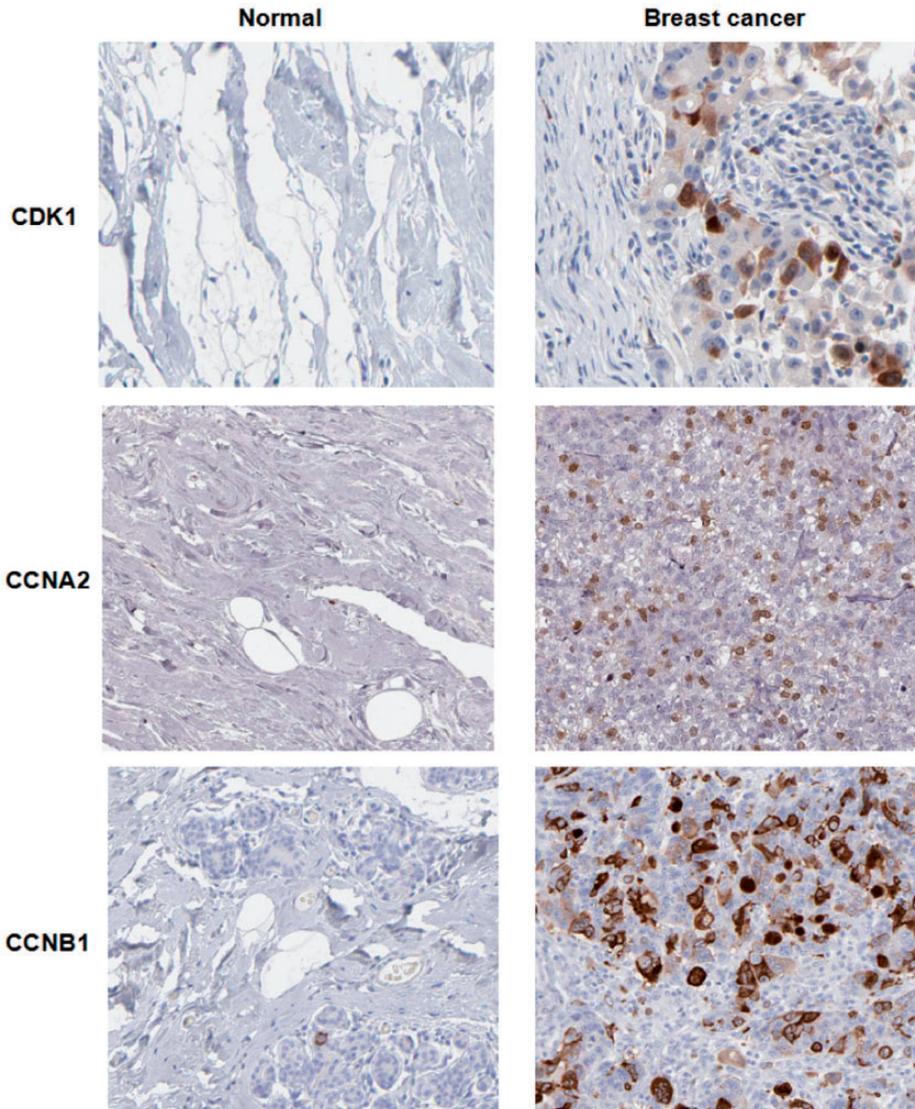
**Figure 3.** Correlation between expression levels of *CDK1*, *CCNA2*, and *CCNB1* and tumor stage in breast cancer (BRCA) patients (GEPIA) by violin plots. Violin plots of correlations between expression of (a) *CDK1*, (b) *CCNA2*, and (c) *CCNB1* and tumor stage in patients with BRCA.  $P < 0.05$ .

*CDK1*, *CCNA2*, and *CCNB1* in eight pairs of BRCA tissues and adjacent non-tumor tissues collected from the Breast Cancer Center of the National Cancer Center (Beijing, China). mRNA expression levels of all three genes were significantly higher in BRCA compared with normal tissues (Fig. S1). Overall, these results showed that transcriptional and proteomic expression levels of *CDK1*, *CCNA2*, and *CCNB1* were upregulated in patients with BRCA.

### Genetic alterations of *CDK1/CCNA2/CCNB1* gene clusters and neighboring-gene network in BRCA patients

The frequencies of *CDK1*, *CCNA2*, and *CCNB1* mutations in BRCA were assessed

using cBioPortal, with 1321 patients from the Metastatic Breast Cancer Project (Provisional, October 2018) and Breast Invasive Carcinoma (TCGA PanCan 2018). The percentages of genetic variations in *CDK1*, *CCNA2*, and *CCNB1* in BRCA varied from 0.8% to 4% for individual genes according to these two datasets (*CDK1*, 4%; *CCNA2*, 0.8%; *CCNB1*, 1.6%; Figure 5a). Seventy-seven (6%) samples exhibited gene set/pathway alterations, with mutation rates of 14.35% and 3.97% for the analyzed gene sets (Figure 5b). We explored the relationships among these genes in BRCA by protein–protein interaction (PPI) enrichment analysis using NEST. The PPI network was constructed using STRING. The results showed that

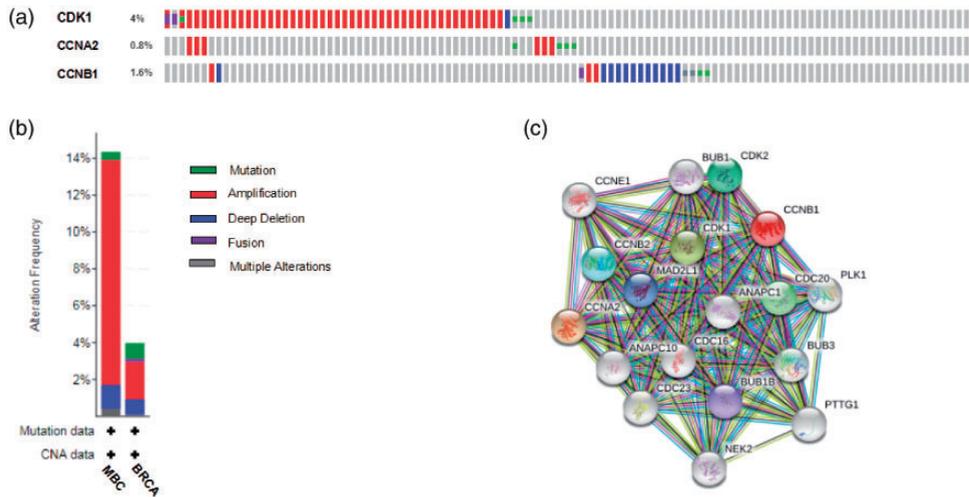


**Figure 4.** Protein expression levels of cyclin-dependent kinase I (CDK1), cyclin A2 (CCNA2), and cyclin B1 (CCNB1) in breast cancer (BRCA) tissues from the Human Protein Atlas. CDK1, cyclin A2, and cyclin B1 were not expressed in normal breast tissues but were highly expressed in BRCA tissues.

*CCNE1, BUB1, CDK2, CCNB2, MAD2L1, ANAPC1, CDC20, PLK1, ANAPC10, CDC16, BUB3, BUB1B, CDC23, NEK2, and PTTG1* were closely associated with *CDK1/CCNA2/CCNB1* gene clusters and functions (Figure 5c).

#### *Functional enrichment analysis of CDK1/CCNA2/CCNB1 gene clusters in BRCA patients*

We explored the functions of *CDK1, CCNA2,* and *CCNB1* using GO and



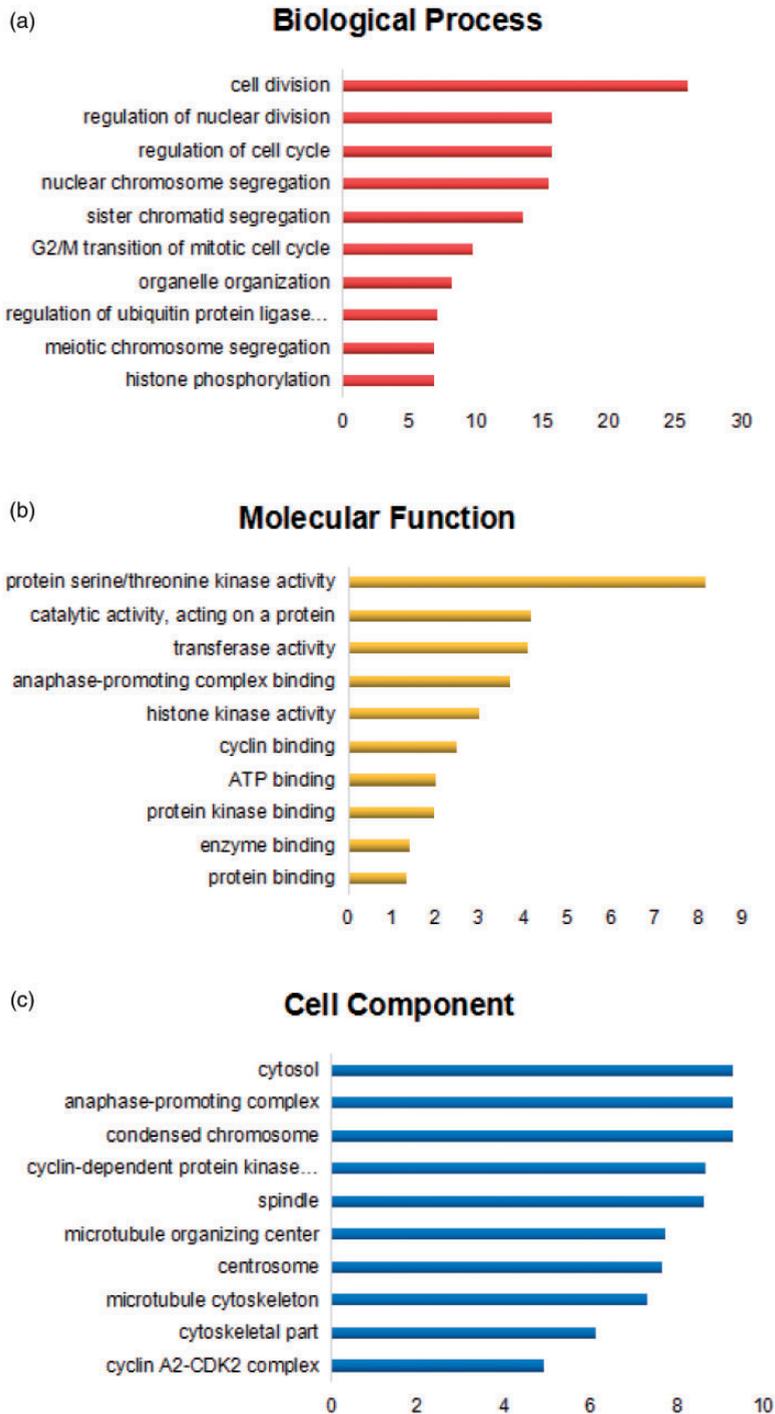
**Figure 5.** Frequencies of gene alterations in *CDK1*, *CCNA2*, and *CCNB1* and neighboring-gene network in breast cancer (BRCA) (cBioPortal). (a) Summary of alterations in *CDK1*, *CCNA2*, and *CCNB1*. (b) OncoPrint visual summary of alterations based on query of *CDK1*, *CCNA2*, and *CCNB1*. (c) Protein-protein interaction network of cyclin-dependent kinase 1, cyclin A2, and cyclin B1. CNA, copy-number alterations; MBC, metastatic breast cancer.

KEGG analyses. GO analysis assesses the biological process, molecular function, and cellular component annotations of the genes of interest. The *CDK1/CCNA2/CCNB1* gene clusters and neighboring genes were primarily enriched for histone phosphorylation, meiotic chromosome segregation, regulation of ubiquitin protein ligase activity, sister chromatid segregation, organelle organization, nuclear chromosome segregation, G2/M transition, cell division, and regulation of cell cycle and nuclear division (Figure 6a). Enriched molecular functions included transcription regulation by protein binding, enzyme binding, ATP binding, protein kinase binding, cyclin binding, histone kinase activity, anaphase-promoting complex binding, transferase activity, catalytic activity, acting on a protein, and protein serine/threonine kinase activity (Figure 6b). Cellular component annotations for these genes included cyclin A2-CDK2 complex, cytoskeletal part, microtubule cytoskeleton,

centrosome, microtubule organizing center, spindle, CDK holoenzyme complex, condensed chromosome, anaphase-promoting complex, and cytosol (Figure 6c). The KEGG pathways for these genes are shown in Table 1. Among these pathways, cell cycle, p53 signaling, progesterone-mediated oocyte maturation, oocyte meiosis, HTLV-I infection, cellular senescence, ubiquitin-mediated proteolysis, viral carcinogenesis, FoxO signaling, and phosphoinositide 3-kinase-Akt signaling were involved in the tumor development and pathogenesis of BRCA.

### Prognostic values of *CDK1/CCNA2/CCNB1* gene clusters in BRCA patients

We evaluated the prognostic significance of the *CDK1/CCNA2/CCNB1* gene clusters in BRCA patients using K-M plotter. Increased expression levels of *CDK1*, *CCNA2*, and *CCNB1* were strongly associated with poor OS, RFP, and PPS (Figure 7).



**Figure 6.** Functional enrichment analysis of *CDK1*, *CCNA2*, and *CCNB1* in patients with breast cancer. Gene Ontology enrichment analysis predicted the functions of the target genes in terms of (a) biological processes, (b) molecular functions, and (c) and cellular components.

**Table 1.** Kyoto Encyclopedia of Genes and Genomes pathway analysis of *CDK1*, *CCNA2*, and *CCNB1* in breast cancer.

Pathway ID	Pathway name	Gene count	False discovery rate	Genes
04110	Cell cycle	17	5.01E-35	<i>ANAPC1</i> , <i>ANAPC10</i> , <i>BUB1</i> , <i>BUB1B</i> , <i>BUB3</i> , <i>CCNA2</i> , <i>CCNB1</i> , <i>CCNB2</i> , <i>CCNE1</i> , <i>CDC16</i> , <i>CDC20</i> , <i>CDC23</i> , <i>CDK1</i> , <i>CDK2</i> , <i>MAD2L1</i> , <i>PLK1</i> , <i>PTTG1</i>
04114	Oocyte meiosis	14	5.40E-27	<i>ANAPC1</i> , <i>ANAPC10</i> , <i>BUB1</i> , <i>CCNB1</i> , <i>CCNB2</i> , <i>CCNE1</i> , <i>CDC16</i> , <i>CDC20</i> , <i>CDC23</i> , <i>CDK1</i> , <i>CDK2</i> , <i>MAD2L1</i> , <i>PLK1</i> , <i>PTTG1</i>
04914	Progesterone-mediated oocyte maturation	12	4.61E-23	<i>ANAPC1</i> , <i>ANAPC10</i> , <i>BUB1</i> , <i>CCNA2</i> , <i>CCNB1</i> , <i>CCNB2</i> , <i>CDC16</i> , <i>CDC23</i> , <i>CDK1</i> , <i>CDK2</i> , <i>MAD2L1</i> , <i>PLK1</i>
05166	HTLV-I infection	10	3.28E-14	<i>ANAPC1</i> , <i>ANAPC10</i> , <i>BUB1B</i> , <i>BUB3</i> , <i>CCNB2</i> , <i>CDC16</i> , <i>CDC20</i> , <i>CDC23</i> , <i>MAD2L1</i> , <i>PTTG1</i>
04115	p53 signaling pathway	5	2.30E-08	<i>CCNB1</i> , <i>CCNB2</i> , <i>CCNE1</i> , <i>CDK1</i> , <i>CDK2</i>
04218	Cellular senescence	6	2.30E-08	<i>CCNA2</i> , <i>CCNB1</i> , <i>CCNB2</i> , <i>CCNE1</i> , <i>CDK1</i> , <i>CDK2</i>
04120	Ubiquitin-mediated proteolysis	5	4.38E-07	<i>ANAPC1</i> , <i>ANAPC10</i> , <i>CDC16</i> , <i>CDC20</i> , <i>CDC23</i>
05203	Viral carcinogenesis	5	1.72E-06	<i>CCNA2</i> , <i>CCNE1</i> , <i>CDC20</i> , <i>CDK1</i> , <i>CDK2</i>
04068	FoxO signaling pathway	4	1.52E-05	<i>CCNB1</i> , <i>CCNB2</i> , <i>CDK2</i> , <i>PLK1</i>
04151	PI3K-Akt signaling pathway	2	0.0489	<i>CCNE1</i> , <i>CDK2</i>

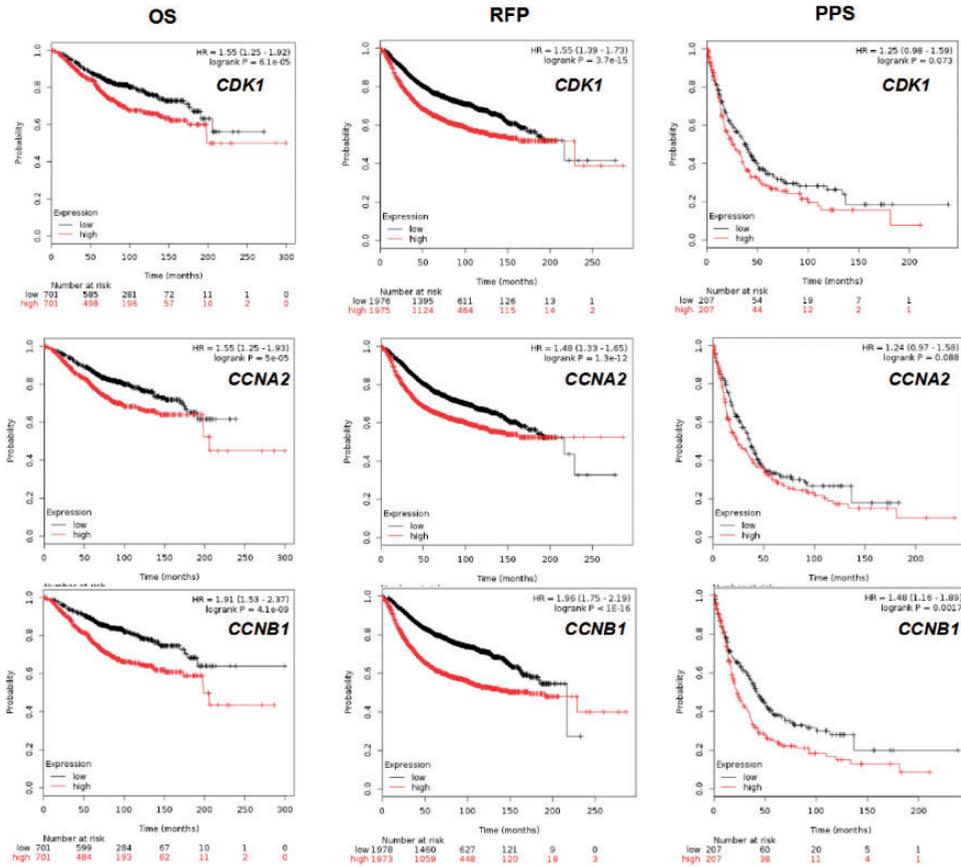
HTLV, human T-lymphotropic virus; PI3K, phosphoinositide 3-kinase.

## Discussion

Dysregulation of cell cycle-related proteins has been well-documented in several cancers.<sup>13</sup> However, although the functions of *CDK1*, *CCNA2*, and *CCNB1* in tumorigenesis and prognosis have been partially confirmed,<sup>14-16</sup> additional bioinformatics analyses in BRCA samples are warranted. Herein, we explored the expression levels and prognostic relevance (OS, RFP, and PPS) of *CDK1*, *CCNA2*, and *CCNB1* in BRCA. The results advance our

understanding of BRCA and may provide useful information to improve treatment design and prognostic accuracy in BRCA patients.

CDKs are important proteins involved in cell cycle regulation.<sup>17</sup> Inhibition of CDK1 significantly impaired tumor growth and promoted tumor cell apoptosis in triple-negative BRCA.<sup>18</sup> In addition, BRCA patients with high CDK1 expression had poor 5-year recurrence-free survival compared with patients with low CDK1 expression,<sup>17</sup> consistent with our current findings.



**Figure 7.** Prognostic values of *CDK1*, *CCNA2*, and *CCNB1* expression in breast cancer (BRCA) patients. (a) Overall survival (OS), (b) recurrence-free probability (RFP), and (c) post-progression survival (PPS) in BRCA patients with high (red) and low (black) expression levels of *CDK1*, *CCNA2*, and *CCNB1* plotted using Kaplan–Meier plotter database at a threshold of  $P < 0.05$ . HR, hazard ratio.

Similar results were reported for other tumors, such as renal cell carcinoma,<sup>19</sup> colorectal cancer,<sup>20</sup> and lung cancer.<sup>21</sup> *CCNA2* regulates the G1–S and G2–M transitions of the cell cycle, and is a known prognostic biomarker for BRCA patient survival, as well as being linked to tamoxifen resistance.<sup>22,23</sup> Gan et al.<sup>24</sup> found that knockout of the *CCNA2* gene prevented G2–M transition, while small interfering RNA-mediated *CCNA2* knockdown increased the apoptosis rate, indicating that knockout of the *CCNA2*

gene might disrupt the cell cycle and induce cell apoptosis.<sup>24</sup> *CCNB1* is expressed predominantly in G2/M phase and participates in the regulation of mitosis. *CCNB1* is a potential prognostic factor predicting BRCA patient survival, in addition to being linked with the treatment of resistance.<sup>25</sup> In the current study, *CDK1*, *CCNA2*, and *CCNB1* were expressed at markedly higher levels in BRCA tissue samples compared with normal tissues, with expression levels positively correlated with tumor stage.

Interestingly, high *CDK1*, *CCNA2*, and *CCNB1* levels correlated well with reduced OS, RFP, and PPS in BRCA.

*CDK1*, *CCNA2*, and *CCNB1* gene clusters play a key role in regulating the mitotic cycle, and disruptions in the cell cycle are common factors involved in the occurrence and development of cancer; however, the mechanism remains unclear. Previous findings, together with the current results, support the vital roles of *CDK1*, *CCNA2*, and *CCNB1* in governing the cell cycle and cell differentiation, and indicate that upregulated expression of these genes contributes to breast cancer development and poorer differentiation.

The current study had several limitations. First, the data were derived from various public databases rather than being generated specifically for the study. Second, the results were web-based and were not verified by biological experiments. Further mechanistic studies are therefore needed to confirm our findings.

Although previous studies have revealed the role of cell cycle-related genes in BRCA, most focused on single genes.<sup>26,27</sup> In contrast, the current study systematically analyzed the expression of cyclin-associated gene clusters (*CDK1*, *CCNA2*, and *CCNB1*) in BRCA and their relationship with patient prognosis. Our results suggest that *CDK1*, *CCNA2*, and *CCNB1* may be possible therapeutic targets as well as potential biomarkers for identifying high-risk populations among BRCA patients. These results warrant further studies of the therapeutic potentials of various cell cycle inhibitors.

#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

#### Funding

This work was supported by the Fundamental Research Funds for the Central Universities [3332020026].

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#### Supplemental material

Supplemental material for this article is available online.

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