

## RESEARCH ARTICLE

# The intercorrelation among CCT6A, CDC20, CCNB1, and PLK1 expressions and their clinical value in papillary thyroid carcinoma prognostication

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## Abstract

**Background:** CCT6A promotes several carcinomas' growth and invasion in multiple ways, and it relates to CCNB1 and PLK1 through its interaction with CDC20 via protein-protein interaction bioinformatics. This study aimed to explore the intercorrelation among CCT6A, CDC20, CCNB1, and PLK1, and their association with tumor features and prognosis in papillary thyroid carcinoma (PTC) patients.

**Methods:** CCT6A, CDC20, CCNB1, and PLK1 expressions in 186 tumor and 30 non-tumor specimens from PTC patients were determined by immunohistochemical (IHC). Clinical features, disease-free survival (DFS), and overall survival (OS) were retrieved.

**Results:** CCT6A, CDC20, CCNB1, and PLK1 expressions were upregulated in tumor tissues compared with non-tumor tissues (all  $p < 0.001$ ). CCT6A expression positively correlated with CDC20, CCNB1, and PLK1 expressions; besides, CDC20 expression positively associated with CCNB1 and PLK1 expressions, and CCNB1 expression was also positively related to PLK1 expression (all  $p < 0.05$ ). Moreover, elevated tumor CCT6A expression was correlated with extrathyroidal invasion ( $p = 0.015$ ), higher pT stage ( $p < 0.001$ ), pN stage ( $p = 0.046$ ), and pTNM stage ( $p = 0.042$ ); while tumor CDC20, CCNB1, and PLK1 expressions only correlated with some of these indexes (most  $p < 0.05$ ). Notably, CCT6A and CDC20 high expressions predicted worse DFS and OS (all  $p < 0.05$ ); CCNB1 positive expression only predicted poor DFS ( $p = 0.044$ ) but not OS ( $p = 0.152$ ); however, PLK1 expression failed to predict these two indexes (both  $p > 0.05$ ). After adjustment using multivariate Cox's regression, CCT6A expression (high vs. low) independently estimated shorter DFS ( $p = 0.010$ ) and OS ( $p = 0.006$ ).

**Conclusion:** CCT6A, CDC20, CCNB1, and PLK1 are intercorrelated, and they exhibit certain prognostic values in PTC patients.

## KEYWORDS

CCNB1, CCT6A, CDC20, papillary thyroid carcinoma, PLK1

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## 1 | INTRODUCTION

Papillary thyroid carcinoma (PTC), the major histological type of differentiated thyroid cancer, accounts for nearly 65%–70% of newly diagnosed thyroid carcinoma cases.<sup>1</sup> Luckily, most PTC patients exhibit a favorable prognosis after applying appropriate management (including surgery, postoperative iodine therapy, hormonal therapy, etc.).<sup>2</sup> However, some PTC patients present with extrathyroidal invasion when diagnosed, and they are more likely to develop lymph node and distal metastases further causing locoregional recurrence and impairing their prognosis.<sup>3,4</sup> Hence, it is critical and necessary to identify potential biomarkers, which might help the clinicians to realize the individualized treatment in PTC.

Chaperonin-containing tailless complex polypeptide 1 subunit 6A (CCT6A) is reported to be closely involved in the pathogenesis of hepatocellular carcinoma (HCC), non-small-cell lung carcinoma (NSCLC), and colon carcinoma; also CCT6A has been identified as one potential prognostic biomarker of carcinoma in the clinical filed.<sup>5–11</sup> Moreover, CCT6A is also reported to interact with various oncoproteins to participate in carcinoma development and progression. For instance, CCT6A interacts with the cell division cycle protein 20 (CDC20) during prometaphase in HeLa cells; CDC20 also interacts with cyclin B1 (CCNB1) and polo-like kinase 1 (PLK1); PLK1 is linked with CCNB1 in HCC cell lines.<sup>12–15</sup> Inspiringly, to determine the interactions of CCT6A with other proteins, the protein–protein interactions are explored using the STRING database (<https://cn.string-db.org/>).<sup>16</sup> Then it is revealed that CCT6A links with CCNB1 and PLK1 via its interaction with CDC20 (Figure 1), while this interaction has not been validated clinically, especially in PTC patients. Also, limited studies report the clinical value of CCT6A, CDC20, CCNB1, and PLK1 in PTC management.

Subsequently, this study aimed to explore the intercorrelation among CCT6A, CDC20, CCNB1, and PLK1, as well as to investigate their association with tumor features and prognosis in PTC patients.

## 2 | METHODS

### 2.1 | Patients

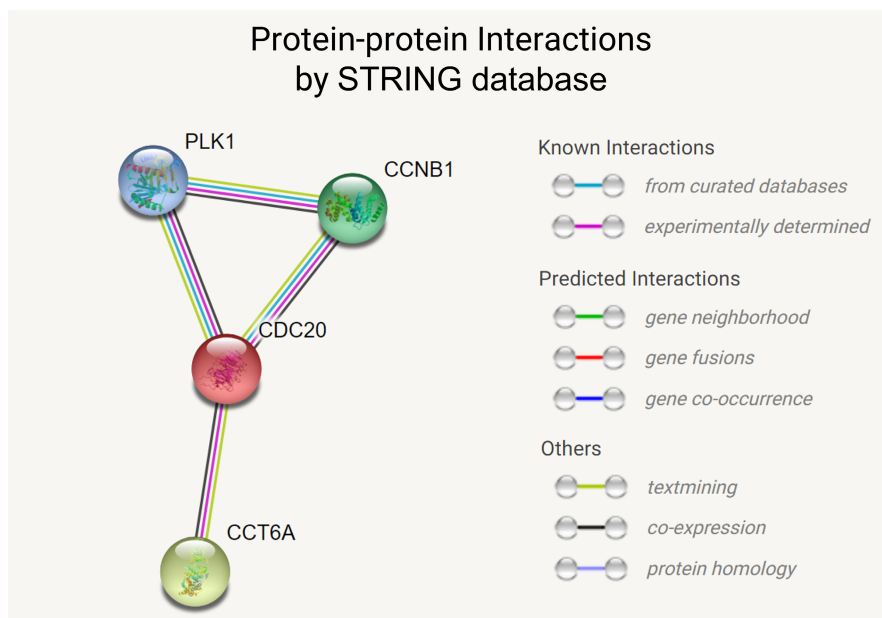
This retrospective study screened 186 cases of PTC patients who received surgical resection between January 2014 and December 2018. The screening criteria were: (i) pathological diagnosis of PTC; (ii) more than 18 years old; (iii) had available clinical data and follow-up information; (iv) had available formalin-fixed paraffin-embedded (FFPE) tumor specimens. Patients who had a prior history of other solid tumors or hematologic malignancies were ineligible for inclusion. The study was permitted by Ethics Committee of Guangdong Provincial People's Hospital Zhuhai Hospital.

### 2.2 | Data documentation

Clinical characteristics were collected for study analysis. Tumor-node-metastasis (TNM) stage was evaluated per the eighth version, and patients before 2016 were reclassified. Besides, standardized follow-up information of PTC patients was collected as well, and the last date of follow-up recording was December 2021. Patients without complete follow-up information were censored at the last date of contact.

### 2.3 | Specimen collection and examination

A total of 186 FFPE specimens of tumor tissues as well as 30 FFPE specimens of non-tumor tissues were obtained to examine the protein expression of CCT6A, CDC20, CCNB1, and PLK1 by immunohistochemistry (IHC), and the procedure of IHC assay was the same as a previous study.<sup>17</sup> The primary antibodies used in the study were: the rabbit polyclonal anti-CCT6A antibody (Cat.



**FIGURE 1** Protein–protein interaction by the STRING database displayed the interaction among CCT6A, CDC20, CCNB1, and PLK1. This figure was generated from the STRING database website (<https://cn.string-db.org/>).

ab110905, 1:100), the rabbit polyclonal anti-CDC20 antibody (Cat. ab183479, 1:1000), the rabbit polyclonal anti-CCNB1 antibody (Cat. ab215436, 1:100), the rabbit polyclonal anti-PLK1 antibody (Cat. ab109777, 1:500). The second antibody used in the study was: the goat antirabbit IgG (H&L) (ab150077; 1:1000). All antibodies were purchased from Abcam, UK.

## 2.4 | IHC evaluation

IHC results were assessed using a light microscope by two pathologists who were unaware of the patients' information. The protein expression of CCT6A, CDC20, and PLK1 was evaluated by a semiquantitative scoring method based on the intensity and density of positively stained cells, which referred to three studies, respectively.<sup>9,18,19</sup> The intensity of stained cells was scored 0–3, and the density of positively stained cells was scored 0–4. The final score of the IHC assay was the product of the intensity score and the density score, which was ranged from 0 to 12. For statistical analysis, the IHC score  $\leq 3$  was considered a low protein expression group; the IHC score  $> 3$  was considered a high protein expression group. The CCNB1 protein expression was evaluated based on the percentage of positive cells according to a previous study,<sup>20</sup> which was classified into 5 grades: grade 0, 0% positive cells; grade 1,  $< 1\%$  positive cells; grade 2, 1%–5% positive cells; grade 3, 6%–10% positive cells; grade 4,  $> 10\%$  positive. For statistical analysis, IHC results with 0% positive cells were considered a negative group; more than 0% positive cells were considered a positive group.

## 2.5 | Statistics

SPSS (v.24.0, IBM Corp., USA) was used for statistical analyses, and GraphPad Prism (v.7.01, GraphPad Software Inc., USA) was used for graph constructions. The protein expression of CCT6A, CDC20, CCNB1, and PLK1 between tumor tissues and non-tumor tissues was assessed using paired-samples *t*-test. Receiver operating characteristic (ROC) analysis was performed to evaluate the ability of variables in distinguishing different subjects. The correlation between two variables was determined using Spearman's rank correlation test. The protein expression between patients with different tumor features was analyzed using student's *t*-test. Disease-free survival (DFS) and overall survival (OS) were imputed based on follow-up information, which were presented using Kaplan–Meier curves and evaluated using log-rank test or Breslow test as appropriate. To further validate the correlation of CCT6A, CDC20, CCNB1, and PLK1 with DFS as well as OS, these expression-related survival data derived from the website KM plotter (<https://kmpplot.com/>) was obtained. Cox's proportional hazard regression analysis was applied for prognostic analysis, and all patients' clinical characteristics were included in the forward-stepwise multivariate regression analysis. *p* value  $< 0.05$  was considered significant.

## 3 | RESULTS

### 3.1 | Patients' features

PTC patients possessed a mean age of  $46.3 \pm 12.1$  years, consisting of 138 (74.2%) females and 48 (25.8%) males (Table 1). Moreover, there were 74 (39.8%) patients who occurred extrathyroidal invasion. In terms of the pathological tumor (pT) stage, 22 (11.8%), 52 (28.0%), 55 (29.6%), 39 (21.0%), and 18 (9.7%) patients were classified as pT1, pT2, pT3, pT4a, and pT4b, respectively. Furthermore, 98 (52.7%) patients received post-surgery radioiodine therapy, while 88 (47.3%) patients did not. The detailed clinical features of PTC patients were displayed in Table 1.

### 3.2 | Intercorrelation among CCT6A, CDC20, CCNB1, and PLK1

The positive and negative controls of antibodies were provided (Figure S1). CCT6A protein expression ( $p < 0.001$ ), CDC20 protein expression ( $p < 0.001$ ), CCNB1 protein expression ( $p < 0.001$ ), and PLK1 protein expression ( $p < 0.001$ ) were upregulated in tumor tissue compared to non-tumor tissue from PTC patients (Figure 2A–E). Moreover, CCT6A, CDC20, CCNB1, and PLK1 all exhibited fair value in distinguishing tumor tissue from non-tumor tissue (Figure S2A–D).

Further correlation analyses displayed that CCT6A protein expression was positively related to CDC20 ( $r = 0.307$ ,  $p < 0.001$ ), CCNB1 ( $r = 0.528$ ,  $p < 0.001$ ), and PLK1 ( $r = 0.438$ ,  $p < 0.001$ ) protein expressions in tumor tissue from PTC patients. Moreover, CDC20 was positively associated with CCNB1 ( $r = 0.301$ ,  $p < 0.001$ ) and PLK1 ( $r = 0.366$ ,  $p < 0.001$ ) protein expressions. Furthermore, CCNB1 protein expression was also positively related to PLK1 protein expression ( $r = 0.245$ ,  $p = 0.001$ ) (Table 2).

### 3.3 | The potential signaling pathways related to CCT6A, CDC20, CCNB1, and PLK1 in PTC

The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted by the DAVID web server. It was shown that CCT6A, CDC20, CCNB1, and PLK1 were potentially involved in cell cycle-related pathways, apoptosis, DNA replication, Fanconi anemia pathway, etc. (Figure S3).

### 3.4 | Correlation of tumor CCT6A, CDC20, CCNB1, and PLK1 with tumor features

Upregulated tumor CCT6A protein expression was related to extrathyroidal invasion ( $p = 0.015$ ), advanced pT stage ( $p < 0.001$ ), pathological node (pN) stage ( $p = 0.046$ ), and pathological tumor-node-metastasis (pTNM) stage ( $p = 0.042$ ). Besides, elevated CDC20 protein expression was associated with tumor size  $> 4$  cm ( $p = 0.038$ ),

TABLE 1 Clinical characteristics

| Items                             | PTC patients (N = 186) |
|-----------------------------------|------------------------|
| Age (years), mean ± SD            | 46.3 ± 12.1            |
| Gender, No. (%)                   |                        |
| Female                            | 138 (74.2)             |
| Male                              | 48 (25.8)              |
| Tumor size (cm), mean ± SD        | 3.6 ± 1.6              |
| Extrathyroidal invasion, No. (%)  |                        |
| No                                | 112 (60.2)             |
| Yes                               | 74 (39.8)              |
| pT stage, No. (%)                 |                        |
| pT1                               | 22 (11.8)              |
| pT2                               | 52 (28.0)              |
| pT3                               | 55 (29.6)              |
| pT4a                              | 39 (21.0)              |
| pT4b                              | 18 (9.7)               |
| pN stage, No. (%)                 |                        |
| pN0                               | 52 (28.0)              |
| pN1                               | 134 (72.0)             |
| pTNM stage, No. (%)               |                        |
| I                                 | 131 (70.4)             |
| II                                | 28 (15.1)              |
| III                               | 19 (10.2)              |
| IV                                | 8 (4.3)                |
| Post-surgery radioiodine, No. (%) |                        |
| No                                | 88 (47.3)              |
| Yes                               | 98 (52.7)              |

Abbreviations: pN, pathological node; pT, pathological tumor; PTC, papillary thyroid carcinoma; pTNM, pathological tumor-node-metastasis; SD, standard deviation.

higher pT stage ( $p = 0.006$ ) and pTNM stage ( $p = 0.002$ ). Meanwhile, increased CCNB1 protein expression was linked with a higher pT stage ( $p = 0.006$ ) and pathological node (pN) stage ( $p = 0.019$ ). While enhanced PLK1 protein expression was only related to the advanced pN stage ( $p = 0.001$ ). Finally, no correlation of CCT6A, CDC20, CCNB1, or PLK1 with other tumor features was observed (Table 3).

### 3.5 | Association of tumor CCT6A, CDC20, CCNB1, and PLK1 with DFS

The 1-year, 3-year, and 5-year DFS rates in total patients were 100%, 93.3%, and 88.7%, respectively. Tumor CCT6A high ( $p = 0.023$ , Figure 3A), CDC20 high ( $p = 0.043$ , Figure 3B), and CCNB1 positive ( $p = 0.044$ , Figure 3C) were related to shorter DFS, while tumor PLK1 high did not correlate with DFS in PTC patients ( $p = 0.341$ , Figure 3D). Further validation using the data from the website KM plotter (<https://kmplot.com>) showed that tumor CCT6A high

( $p = 0.002$ ), CDC20 high ( $p < 0.001$ ), CCNB1 high ( $p = 0.015$ ), and PLK1 high ( $p < 0.001$ ) were all related to shorter accumulating DFS (Figure S4A–D).

After adjustment using multivariate Cox's regression analysis, tumor CCT6A protein expression (high vs. low) (hazard ratio [HR]: 5.146,  $p = 0.010$ ) was independently related to reduced DFS in PTC patients. Moreover, age ( $\geq 55$  years vs.  $< 55$  years) (HR: 9.611,  $p < 0.001$ ), higher pT stage (HR: 2.313,  $p < 0.001$ ), and higher pN stage (HR: 12.318,  $p = 0.017$ ) could also serve as independent prognostic factors for declined DFS in PTC patients (Table 4).

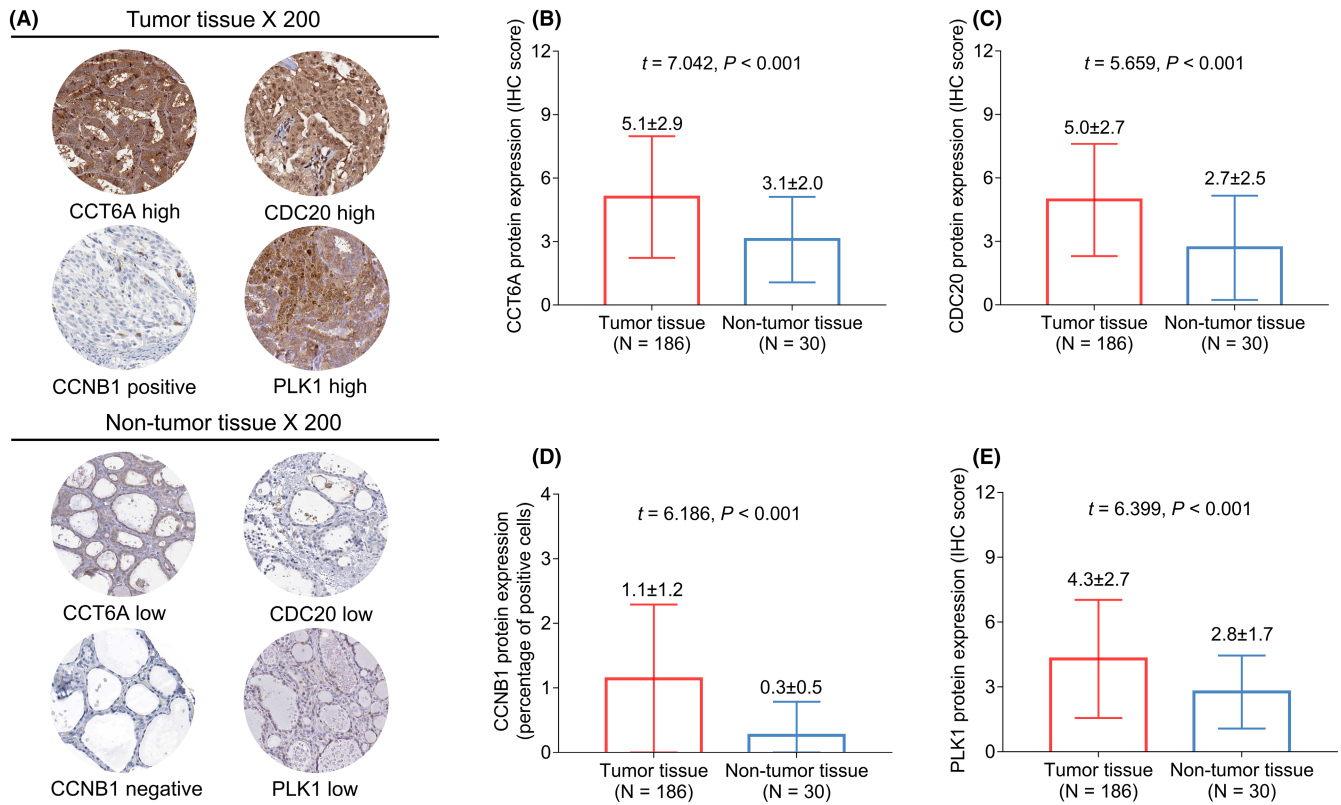
### 3.6 | Association of tumor CCT6A, CDC20, CCNB1, and PLK1 with OS

The 1-year, 3-year, and 5-year OS rates in total patients were 100%, 97.7%, and 91.5%, respectively. Only tumor CCT6A high ( $p = 0.031$ , Figure 4A) and CDC20 high ( $p = 0.015$ , Figure 4B) were related to reduced OS in PTC patients. While no correlation of CCNB1 positive ( $p = 0.152$ , Figure 4C) or PLK1 high ( $p = 0.313$ , Figure 4D) with OS was observed. However, none of these proteins were related to accumulating OS in the validation using the data from the website KM plotter (<https://kmplot.com/>) (all  $p > 0.05$ ) (Figure S5A–D).

After adjustment using multivariate Cox's regression analysis, tumor CCT6A protein expression (high vs. low) (HR: 18.006,  $p = 0.006$ ) was independently related to reduced OS in PTC patients. Furthermore, extrathyroidal invasion (yes vs. no) (HR: 8.270,  $p = 0.047$ ) could independently forecast declined OS in PTC patients as well (Table 5).

## 4 | DISCUSSION

CCT6A is identified as one prognostic biomarker in clinical oncology. For instance, upregulated CCT6A expression is reported in tumor tissue harvested from HCC patients, cervical carcinoma patients, NSCLC patients, and gastric carcinoma patients.<sup>8–11</sup> Moreover, CCT6A is positively related to pTNM stage in gastric carcinoma patients.<sup>11</sup> Furthermore, CCT6A protein high (vs. low) is independently related to shorter DFS in HCC patients, gastric carcinoma patients, and NSCLC patients.<sup>8,10,11</sup> While the clinical role of CCT6A measurement in PTC patients remains elusive. In the current study, it was noticed that CCT6A protein expression was upregulated in tumor tissue compared with non-tumor tissue in PTC patients, which could be explained as that: CCT6A promoted cell proliferation, invasion, and malignant behaviors in PTC, which were the hallmarks of cancer, thus an increase of CCT6A in tumor tissue from PTC patients was observed.<sup>5–7,21</sup> Moreover, higher CCT6A expression was related to extrathyroidal invasion and advanced pathological stages in PTC patients, which could be explained as that CCT6A activated the transforming growth factor (TGF)- $\beta$  signaling pathway to promote PTC cell proliferation and cell invasion, thus led to a positive correlation of tumor invasion as well as advanced pT and pTNM stage in



**FIGURE 2** CCT6A, CDC20, CCNB1, and PLK1 were upregulated in PTC tissue. Presentation of CCT6A, CDC20, CCNB1, and PLK1 protein expressions in tumor tissue and non-tumor tissue from PTC patients by IHC images (A). Comparison of CCT6A protein expression by IHC score (B), CDC20 protein expression by IHC score (C), CCNB1 protein expression by the percentage of positive cells (D), and PLK1 protein expression by IHC score (E) between tumor tissue and non-tumor tissue from PTC patients. The paired-sample *t*-test was applied for statistical analysis.

**TABLE 2** Correlation among CCT6A, CDC20, CCNB1, and PLK1 in tumor tissue

| Items                           | CDC20 protein expression | CCNB1 protein expression | PLK1 protein expression |
|---------------------------------|--------------------------|--------------------------|-------------------------|
| <b>CCT6A protein expression</b> |                          |                          |                         |
| <i>r</i> value                  | 0.307                    | 0.528                    | 0.438                   |
| <i>p</i> value                  | <0.001                   | <0.001                   | <0.001                  |
| <b>CDC20 protein expression</b> |                          |                          |                         |
| <i>r</i> value                  | -                        | 0.301                    | 0.366                   |
| <i>p</i> value                  | -                        | <0.001                   | <0.001                  |
| <b>CCNB1 protein expression</b> |                          |                          |                         |
| <i>r</i> value                  | -                        | -                        | 0.245                   |
| <i>p</i> value                  | -                        | -                        | 0.001                   |

Note: The spearman's rank correlation test was applied for statistical analysis.

Abbreviations: CCNB1, cyclin B1; CCT6A, chaperonin-containing tailless complex polypeptide 1 subunit 6A; CDC20, cell division cycle protein 20; PLK1, polo-like kinase 1.

PTC patients.<sup>6,22</sup> Notably, it was also discovered that tumor CCT6A high could serve as an independent factor for reduced DFS and OS in PTC patients, indicating its potential prognostic values in PTC management.

Apart from the prognostic value of CCT6A, the occurrence of extrathyroidal invasion could also serve as an independent factor for estimating shorter OS in PTC patients as shown in our study. The possible reason to explain this finding was that: PTC cancer cells might be able to achieve epithelial-to-mesenchymal transition and cause the outgrowth of disseminated tumor cells in the distal tissue aside from the thyroid gland, which further enhanced the risk of angiogenesis from distal tumor tissue causing further growth of tumors and might cause an unfavorable prognosis in PTC patients.<sup>23,24</sup>

In order to validate the interaction among CCT6A, CDC20, CCNB1, and PLK1, multiple correlation analyses were conducted in the current study. Then, it was observed that CCT6A was positively correlated with CDC20, CCNB1, and PLK1; also, CDC20 was positively related to CCNB1 and PLK1 in PTC patients as well. However, no correlation of PLK1 with CCNB1 in PTC patients was observed. These findings indicated that CCT6A was related to CCNB1 and PLK1 via its interaction with CDC20 despite the weak connection between PLK1 and CCNB1.

TABLE 3 Correlation of tumor CCT6A, CDC20, CCNB1, and PLK1 with tumor features

| Items                   | CCT6A protein expression (IHC score) |           |                | CDC20 protein expression (IHC score) |           |              | CCNB1 protein expression (percentage of positive cells, %) |           |              | PLK1 protein expression (IHC score) |           |              |
|-------------------------|--------------------------------------|-----------|----------------|--------------------------------------|-----------|--------------|--|-----------|--------------|-------------------------------------|-----------|--------------|
|                         | Mean $\pm$ SD                        | t/r value | p value        | Mean $\pm$ SD                        | t/r value | p value      | Mean $\pm$ SD  | t/r value | p value      | Mean $\pm$ SD                       | t/r value | p value      |
| Tumor size              |                                      |           |                |                                      |           |              |  |           |              |                                     |           |              |
| ≤4 cm                   | 4.8 $\pm$ 2.9                        | -1.748    | 0.082          | 4.6 $\pm$ 2.5                        | -2.095    | 0.038        | 1.0 $\pm$ 1.1  | -1.497    | 0.136        | 4.2 $\pm$ 2.7                       | -0.416    | 0.678        |
| >4 cm                   | 5.6 $\pm$ 2.9                        |           |                | 5.5 $\pm$ 2.8                        |           |              | 1.3 $\pm$ 1.2  |           |              | 4.4 $\pm$ 2.8                       |           |              |
| Extrathoroidal invasion |                                      |           |                |                                      |           |              |  |           |              |                                     |           |              |
| No                      | 4.7 $\pm$ 2.7                        | -2.468    | <b>0.015</b>   | 4.8 $\pm$ 2.7                        | -1.187    | 0.237        | 1.0 $\pm$ 1.1  | -1.518    | 0.131        | 4.5 $\pm$ 2.9                       | 1.065     | 0.288        |
| Yes                     | 5.8 $\pm$ 3.1                        |           |                | 5.2 $\pm$ 2.6                        |           |              | 1.3 $\pm$ 1.2  |           |              | 4.0 $\pm$ 2.4                       |           |              |
| pT stage                |                                      |           |                |                                      |           |              |  |           |              |                                     |           |              |
| pT1                     | 4.0 $\pm$ 2.6                        | 0.314     | < <b>0.001</b> | 3.8 $\pm$ 2.4                        | 0.200     | <b>0.006</b> | 0.8 $\pm$ 1.1  | 0.202     | <b>0.006</b> | 3.8 $\pm$ 2.9                       | 0.035     | 0.639        |
| pT2                     | 4.2 $\pm$ 2.4                        |           |                | 4.6 $\pm$ 2.5                        |           |              | 1.0 $\pm$ 1.2  |           |              | 4.5 $\pm$ 2.8                       |           |              |
| pT3                     | 5.3 $\pm$ 2.8                        |           |                | 5.4 $\pm$ 2.6                        |           |              | 1.2 $\pm$ 1.1  |           |              | 4.3 $\pm$ 2.9                       |           |              |
| pT4a                    | 5.7 $\pm$ 3.1                        |           |                | 4.8 $\pm$ 2.6                        |           |              | 1.1 $\pm$ 1.1  |           |              | 4.0 $\pm$ 2.3                       |           |              |
| pT4b                    | 7.2 $\pm$ 3.1                        |           |                | 6.3 $\pm$ 3.0                        |           |              | 2.0 $\pm$ 1.4  |           |              | 4.9 $\pm$ 2.6                       |           |              |
| pN stage                |                                      |           |                |                                      |           |              |  |           |              |                                     |           |              |
| pN0                     | 4.4 $\pm$ 3.0                        | -2.011    | <b>0.046</b>   | 4.5 $\pm$ 2.5                        | -1.321    | 0.188        | 0.9 $\pm$ 0.9  | -2.374    | <b>0.019</b> | 3.4 $\pm$ 2.0                       | -3.492    | <b>0.001</b> |
| pN1                     | 5.4 $\pm$ 2.8                        |           |                | 5.1 $\pm$ 2.7                        |           |              | 1.2 $\pm$ 1.2  |           |              | 4.7 $\pm$ 2.9                       |           |              |
| pTNM stage              |                                      |           |                |                                      |           |              |  |           |              |                                     |           |              |
| I                       | 4.9 $\pm$ 2.8                        | 0.150     | <b>0.042</b>   | 4.7 $\pm$ 2.6                        | 0.221     | <b>0.002</b> | 1.0 $\pm$ 1.1  | 0.096     | 0.190        | 4.2 $\pm$ 2.7                       | 0.027     | 0.712        |
| II                      | 5.5 $\pm$ 3.1                        |           |                | 5.3 $\pm$ 2.6                        |           |              | 1.5 $\pm$ 1.2  |           |              | 4.9 $\pm$ 3.0                       |           |              |
| III                     | 5.6 $\pm$ 3.0                        |           |                | 5.6 $\pm$ 2.9                        |           |              | 1.0 $\pm$ 1.2  |           |              | 4.1 $\pm$ 2.3                       |           |              |
| IV                      | 6.6 $\pm$ 3.5                        |           |                | 7.4 $\pm$ 2.0                        |           |              | 1.6 $\pm$ 1.4  |           |              | 4.5 $\pm$ 2.5                       |           |              |

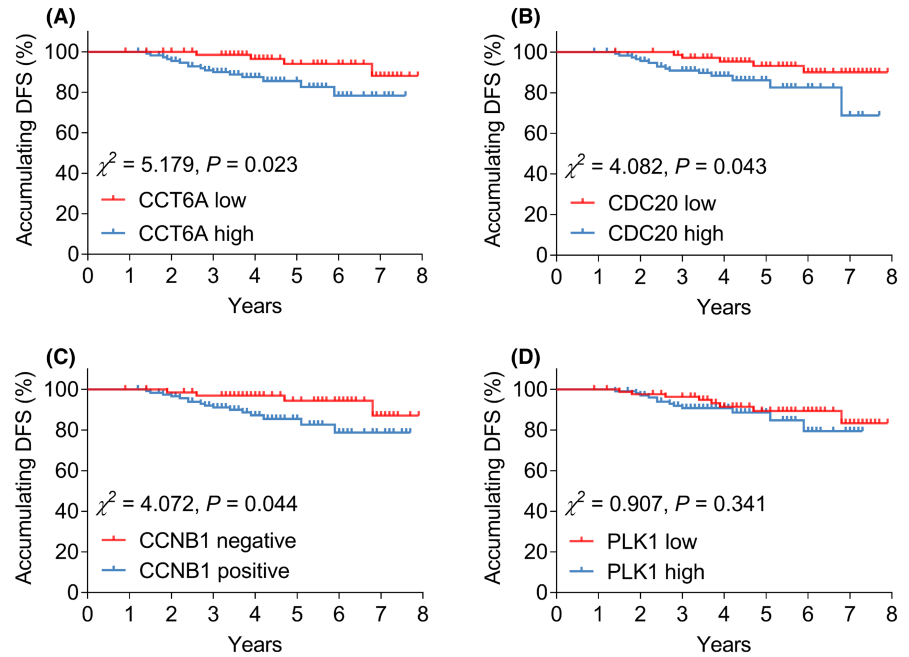
Note: Student's t-test and spearman's rank correlation test were applied for statistical analysis.

Bold indicates statistically significant p-value ( $p < 0.05$ ).

Abbreviations: CCT6A, chaperonin-containing tailless complex polypeptide 1 subunit 6A; CDC20, cell division cycle protein 20; CCNB1, cyclin B1; PLK1, polo-like kinase 1; IHC, immunohistochemistry; pT, pathological tumor; pN, pathological node; pTNM, pathological tumor-node-metastasis; SD, standard deviation.



**FIGURE 3** Tumor CCT6A high, CDC20 high, and CCNB1 positive were related to shorter DFS. Association of CCT6A high (A), CDC20 high (B), CCNB1 positive (C), and PLK1 high (D) with accumulating DFS in PTC patients. The log-rank test or Breslow test was applied for statistical analysis.



**TABLE 4** Cox's proportional hazards regression analysis for DFS

| Items  | p value          | HR     | 95% CI |        |
|--|------------------|--------|--------|--------|
|  |                  |        | Lower  | Upper  |
| <b>Univariable Cox's regression analysis</b>     |                  |        |        |        |
| CCT6A protein expression (high vs. low)          | <b>0.031</b>     | 3.386  | 1.114  | 10.289 |
| CDC20 protein expression (high vs. low)          | 0.052            | 2.789  | 0.992  | 7.840  |
| CCNB1 protein expression (positive vs. negative) | 0.054            | 2.957  | 0.981  | 8.911  |
| PLK1 protein expression (high vs. low)           | 0.365            | 1.519  | 0.614  | 3.757  |
| Age ( $\geq 55$ years vs. $< 55$ years)          | <b>&lt;0.001</b> | 7.570  | 2.748  | 20.854 |
| Gender (male vs. female)                         | 0.612            | 1.273  | 0.500  | 3.243  |
| Tumor size ( $> 4$ cm vs. $\leq 4$ cm)           | <b>0.002</b>     | 4.452  | 1.694  | 11.702 |
| Extrathyroidal invasion (yes vs. no)             | <b>0.001</b>     | 6.327  | 2.115  | 18.926 |
| Higher pT stage                                  | <b>&lt;0.001</b> | 2.396  | 1.537  | 3.736  |
| Higher pN stage                                  | <b>0.042</b>     | 8.045  | 1.077  | 60.114 |
| Higher pTNM stage                                | <b>&lt;0.001</b> | 2.778  | 1.902  | 4.056  |
| <b>Multivariable Cox's regression analysis</b>   |                  |        |        |        |
| CCT6A protein expression (high vs. low)          | <b>0.010</b>     | 5.146  | 1.489  | 17.781 |
| Age ( $\geq 55$ years vs. $< 55$ years)          | <b>&lt;0.001</b> | 9.611  | 3.241  | 28.500 |
| Higher pT stage                                  | <b>&lt;0.001</b> | 2.313  | 1.459  | 3.667  |
| Higher pN stage                                  | <b>0.017</b>     | 12.318 | 1.571  | 96.596 |

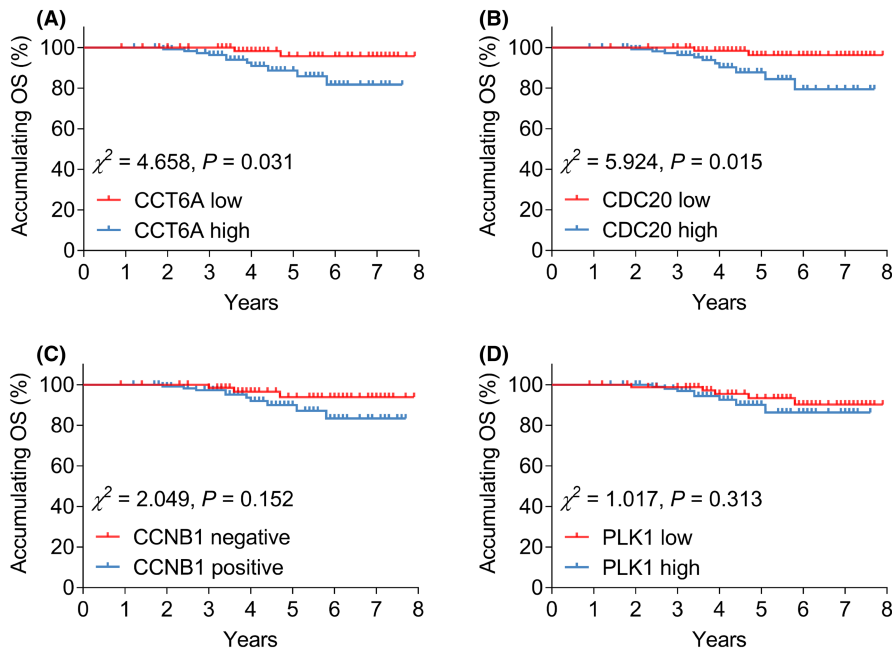
Note: The univariate and multivariate Cox's hazard regression was applied for analysis.

Bold indicates statistically significant  $p$ -value ( $p < 0.05$ ).

Abbreviations: CCNB1, cyclin B1; CCT6A, chaperonin-containing tailless complex polypeptide 1 subunit 6A; CDC20, cell division cycle protein 20; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; PLK1, polo-like kinase 1; pT, pathological tumor; pN, pathological node; pTNM, pathological tumor-node-metastasis.

In terms of CDC20, CCNB1, and PLK1, they also have been indicated as potential prognostic markers in carcinoma patients as well.<sup>25-27</sup> For instance, CDC20 is upregulated in tumor tissue compared to non-tumor tissue from HCC patients, also increased

CDC20 is associated with lymph node metastasis, advanced pTNM stage, and reduced OS in HCC patients.<sup>25</sup> Besides, CCNB1 is reported to be upregulated in tumor tissue from HCC patients, and enhanced CCNB1 is related to declined DFS and OS in HCC



**FIGURE 4** Tumor CCT6A high and CDC20 high were linked with shorter OS. Correlation of CCT6A high (A), CDC20 high (B), CCNB1 positive (C), and PLK1 high (D) with accumulating OS in PTC patients. The log-rank test was applied for statistical analysis.

**TABLE 5** Cox's proportional hazards regression analysis for OS

| Items  | p value           | HR     | 95% CI |          |
|--|-------------------|--------|--------|----------|
|  |                   |        | Lower  | Upper    |
| <b>Univariable Cox's regression analysis</b>     |                   |        |        |          |
| CCT6A protein expression (high vs. low)          | <b>0.049</b>      | 4.563  | 1.006  | 20.696   |
| CDC20 protein expression (high vs. low)          | <b>0.029</b>      | 5.430  | 1.188  | 24.824   |
| CCNB1 protein expression (positive vs. negative) | 0.167             | 2.496  | 0.683  | 9.119    |
| PLK1 protein expression (high vs. low)           | 0.364             | 1.687  | 0.546  | 5.214    |
| Age ( $\geq 55$ years vs. $< 55$ years)          | <b>0.001</b>      | 28.151 | 3.659  | 216.568  |
| Gender (male vs. female)                         | 0.978             | 0.983  | 0.301  | 3.217    |
| Tumor size ( $> 4$ cm vs. $\leq 4$ cm)           | <b>0.005</b>      | 6.378  | 1.738  | 23.398   |
| Extrathyroidal invasion (yes vs. no)             | <b>0.006</b>      | 17.571 | 2.284  | 135.161  |
| Higher pT stage                                  | <b>0.001</b>      | 2.700  | 1.517  | 4.806    |
| Higher pN stage                                  | 0.169             | 34.662 | 0.221  | 5433.744 |
| Higher pTNM stage                                | <b>&lt; 0.001</b> | 3.470  | 2.132  | 5.646    |
| <b>Multivariable Cox's regression analysis</b>   |                   |        |        |          |
| CCT6A protein expression (high vs. low)          | <b>0.006</b>      | 18.006 | 2.292  | 141.437  |
| Extrathyroidal invasion (yes vs. no)             | <b>0.047</b>      | 8.270  | 1.027  | 66.605   |

Note: The univariate and multivariate Cox's hazard regression was applied for analysis.

Bold indicates statistically significant p-value ( $p < 0.05$ ).

Abbreviations: CCNB1, cyclin B1; CCT6A, chaperonin-containing tailless complex polypeptide 1 subunit 6A; CDC20, cell division cycle protein 20; CI, confidence interval; HR, hazard ratio; OS, overall survival; PLK1, polo-like kinase 1; pN, pathological node; pT, pathological tumor; pTNM, pathological tumor-node-metastasis.

patients as well.<sup>26</sup> Moreover, PLK1 is related to reduced DFS and OS in ovarian carcinoma patients, also it is independently associated with shorter OS by multivariate Cox's regression analysis.<sup>27</sup> In the current study, it was discovered that CDC20, CCNB1, and PLK1 were all increased in tumor tissue compared to non-tumor tissue in PTC patients, which could be explained that these three biomarkers were involved in various carcinogenesis pathways

and the occurrence of carcinoma, thus CDC20, CCNB1, and PLK1 might be upregulated in PTC patients.<sup>28–30</sup> Furthermore, CDC20, CCNB1, and PLK1 were related to some clinical features in PTC patients, which might be explained that these three proteins were able to promote local invasion and metastasis of cancers, which further contributed to the development of extrathyroidal invasion and eventually led to advanced pathological stages in PTC



patients.<sup>29-31</sup> Also, only CDC20 and CCNB1 were linked with prognosis in PTC patients, while PLK1 did not. These findings suggested the potential prognostic values of these biomarkers in PTC patients.

However, this study exhibited some limitations. For instance, the current study only detected the protein expression of CCT6A, CDC20, CCNB1, and PLK1 from tumor tissue and non-tumor tissue, and further study could determine their mRNA expressions. Moreover, due to the nature of the retrospective study, there were some biases in the selection of patients, and forthcoming prospective studies were also warranted. Besides, further studies could detect the expressions of CCT6A, CDC20, CCNB1, and PLK1 from other biological samples (i.e., serum, exosome) to explore their association with tumor features and prognosis in PTC patients.

In conclusion, CCT6A, CDC20, CCNB1, and PLK1 are intercorrelated, and they exhibit certain prognostic values in PTC patients.

## ACKNOWLEDGMENTS

None.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Peng W, Li W, Zhang X, Cen W, Liu Y. The intercorrelation among CCT6A, CDC20, CCNB1, and PLK1 expressions and their clinical value in papillary thyroid carcinoma prognostication. *J Clin Lab Anal.* 2022;36:e24609. doi: [10.1002/jcla.24609](https://doi.org/10.1002/jcla.24609)