Received: 2 March 2021 | Revised: 1 April 2021 | Accepted: 3 April 2021

DOI: 10.1002/jcla.23793

RESEARCH ARTICLE

WILEY

CCT6A may act as a potential biomarker reflecting tumor size, lymphatic metastasis, FIGO stage, and prognosis in cervical cancer patients

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Abstract

Objective: Chaperonin-containing tailless complex polypeptide subunit 6A (CCT6A) is a critical regulator and newly identified clinical biomarker of several cancers, while its correlation with the clinical characteristics and prognosis of cervical cancer patients is unclear. Therefore, this study aimed to explore this issue.

Methods: Chaperonin-containing tailless complex polypeptide subunit 6A expression in tumor and tumor-adjacent tissues from 198 cervical cancer patients who underwent resection were detected by immunohistochemistry assay and reverse transcription-quantitative polymerase chain reaction. Besides, the clinicopathological features and survival data of cervical cancer patients were collected.

Results: Chaperonin-containing tailless complex polypeptide subunit 6A protein and mRNA levels were both increased in tumor tissues compared with tumor-adjacent tissues (both $p < 0.001$). Receiver operating characteristic curves showed that CCT6A protein (AUC: 0.774, 95% CI: 0.729–0.819) and mRNA levels (AUC: 0.904, 95% CI: 0.874–0.934) well discriminated tumor tissues from tumor-adjacent tissues. Besides, correlation analyses found that CCT6A protein and mRNA levels were positively correlated with lymph node metastasis and FIGO stage (all $p < 0.05$), apart from which CCT6A mRNA level was also positively associated with tumor size ($p = 0.032$). In addition, CCT6A protein and mRNA levels were negatively correlated with accumulating disease-free survival (both $p < 0.05$); meanwhile CCT6A mRNA level was negatively associated with accumulating overall survival as well ($p = 0.010$).

Conclusion: Chaperonin-containing tailless complex polypeptide subunit 6A is elevated in tumor tissues, and its high expression associates with larger tumor size, lymph node metastasis, higher FIGO stage, and worse prognosis in cervical cancer patients.

KEYWORDS
CCT6A, cervical cancer, disease-free survival, overall survival, tumor characteristics

1 | INTRODUCTION

Cervical cancer is one of the most common gynecological malignancies.[1,2] Meanwhile, it is also listed as one of the leading causes of cancer-related mortality in women worldwide, which dramatically endangers human health.[3,4] Due to the advancement in several areas of cervical cancer including the screening programs, vaccines, neoadjuvant therapies, targeted treatment, and personalized medicine, the prevalence and prognosis of cervical cancer have been improved to a degree[5–7]; however, cervical cancer is still a huge threat to female health under such a circumstance.[4,7] Therefore, searching for clinical biomarkers might enhance the surveillance of patients with cervical cancer to potentially improve their prognosis.

Chaperonin-containing tailless complex polypeptide subunit 6A (CCT6A) is a key regulator of cytoskeletal organization and cell cycle.[8,9] Notably, CCT6A has been found to play vital roles in modulating the progression of several cancers. For instance, previous studies suggest that CCT6A regulates the cell cycle in hepatocellular carcinoma cells and breast cancer cells.[10,11] Clinically, it is revealed that CCT6A may be a potential prognostic biomarker in patients with breast cancer or non-small cell lung cancer.[11,12] However, whether CCT6A could also present prognostic value in patients with cervical cancer is still unclear. Therefore, the aim of this study was to detect the mRNA and protein expressions of CCT6A in tumor and tumor-adjacent tissues of cervical cancer patients by immunohistochemistry (IHC) assay and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), respectively, so as to investigate its correlation with tumor characteristics and prognosis in cervical cancer patients.

2 | MATERIALS AND METHODS

2.1 | Patients and specimen collection

After collection of the informed consents from the patients (or their families) and the approval by the Institutional Review Board, this study retrospectively collected 198 cervical cancer patients who underwent surgery in our hospital between January 2016 and December 2020. All 198 patients were pathologically diagnosed with cervical cancer with Federation International of Gynecology and Obstetrics (FIGO) stage I-IIA, and all of them underwent surgical resection without neoadjuvant therapy. Meanwhile, by reviewing the medical records of all patients, it was confirmed that none of them had a history of other carcinomas or malignancies. The clinicopathological features and survival data of patients were collected from the medical documents. A total of 198 pairs of tumor and tumor-adjacent tissue specimens were collected from the sample library, and all the specimens were available for immunohistochemistry (IHC) assay. In addition, among 198 pairs of the specimens, there were 176 pairs that were fresh-frozen in liquid nitrogen, which could be used not only for IHC but also for RNA isolation and analysis.

2.2 | IHC assay

Immunohistochemistry assay was carried out to assess the CCT6A protein expression in the 198 pairs of tumor and tumor-adjacent tissue specimens. The procedures of IHC were performed as same as that reported in a previous study.[12] The CCT6A Polyclonal Antibody (1:200 dilution, Invitrogen, Carlsbad, California, USA) was applied as the primary antibody, and the Goat anti-Rabbit IgG (H+L) Secondary Antibody (1:60 dilution, Invitrogen, Carlsbad, California, USA) was used as the secondary antibody. The IHC staining result was observed microscopically, and a semi-quantitative scoring method based on IHC staining intensity and density was used to evaluate the CCT6A protein expression in specimens, which was performed referring to a previous study.[13] In brief, the IHC staining intensity was scored from 0 to 3, and the density was scored from 0 to 4. The

TABLE 1 Clinicopathological characteristics of cervical cancer patients

Items	Cervical cancer patients (*N* = 198)
Age (years), mean±SD	50.9 ± 10.4
<50 years	90 (45.5)
≥50 years	108 (54.5)
HPV status, No. (%)	
Negative	38 (19.2)
Positive	160 (80.8)
Histological type, No. (%)	
ASC	11 (5.5)
ADC	31 (15.7)
SCC	156 (78.8)
Pathological differentiation grade, No. (%)	
G1	61 (30.8)
G2	80 (40.4)
G3	57 (28.8)
Tumor size, No. (%)	
<4 cm	110 (55.6)
≥4 cm	88 (44.4)
Lymph node metastasis, No. (%)	
Absent	163 (82.3)
Present	35 (17.7)
FIGO stage, No. (%)	
Stage I	126 (63.6)
Stage IIA	72 (36.4)

Abbreviations: ADC, adenocarcinoma; ASC, adenosquamous carcinoma; FIGO, federation international of gynecology and obstetrics; HPV, human papillomavirus; SCC, squamous cell carcinoma; SD, standard deviation.

product of both intensity score and density score was the total IHC score. A cutoff value of 3 was used to classify CCT6A low and high.[14]

2.3 | Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

Reverse transcription-quantitative polymerase chain reaction assay was conducted for relative quantitative analysis of CCT6A mRNA expression in the 176 pairs of fresh-frozen specimens. The PCR primers were designed referring to a previous study, and the procedures of RT-qPCR were also performed as same as that reported in the previous study.[10] TRIzol™ Reagent (Invitrogen, Carlsbad, California, USA) was used for separation of total RNA; iScript™ Reverse Transcription Supermix (Bio-Rad, Hercules, California, USA) was used for reverse transcription; QuantiNova SYBR Green PCR Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, Germany) was used for qPCR analysis (95°C, 5 min, 1 cycle; 95°C, 5 s, 61°C, 20 s, 40 cycles). GAPDH was severed as an internal reference gene. The relative expression of CCT6A mRNA was calculated with the use of $2^{-\Delta\Delta Ct}$ method. The PCR primer sequences were as follows: CCT6A, forward primer: 5′-TGACGACCTAAGTCCTGACTG-3′, and reverse primer: 5′-ACAGAACGAGGGTTGTTACATTT-3′; GAPDH, forward primer: 5′-TGCACCACCAACTGCTTAGC-3′ and reverse primer: 5′-GGCATGGACTGTGGTCATGAG-3′. The median value of the relative expression of CCT6A mRNA in the total tumor specimens (2.513 [1.848–4.251]) was used as the cutoff value to classify CCT6A mRNA low and high expression.

2.4 | Statistical analysis

Descriptive analysis was performed for the clinicopathological features. Difference analysis of CCT6A IHC score and mRNA expression was completed using paired *t* test and Wilcoxon-signed rank test. Correlation analysis was carried out with the use of Student's *t* test and Wilcoxon rank sum test. Receiver operating characteristic (ROC) curve analysis was performed to estimate the feasibility of CCT6A in distinguishing tumor from tumor-adjacent tissue. Disease-free survival (DFS) was estimated from the surgery to disease relapse or the death of patients. Overall survival (OS) was estimated from the surgery to the death of patients. Patients with follow-up data missing were not analyzed in the study. Survival data was analyzed by Kaplan-Meier curve and Log-rank test. Cox's proportional hazards regression model analysis was used to evaluate the factors associated with DFS or OS. Data analysis and graph forming were completed using SPSS 21.0 (IBM, Chicago, Illinois, USA) and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, California, USA), respectively. Statistical significance was concluded by a p value <0.05.

3 | RESULTS

3.1 | Patients' characteristics

The mean age of the cervical cancer patients was 50.9 ± 10.4 years. There were 110 (55.6%) patients with tumor size <4 cm and 88 (44.4%) patients with tumor size ≥4 cm. Besides, 35 (17.7%) patients

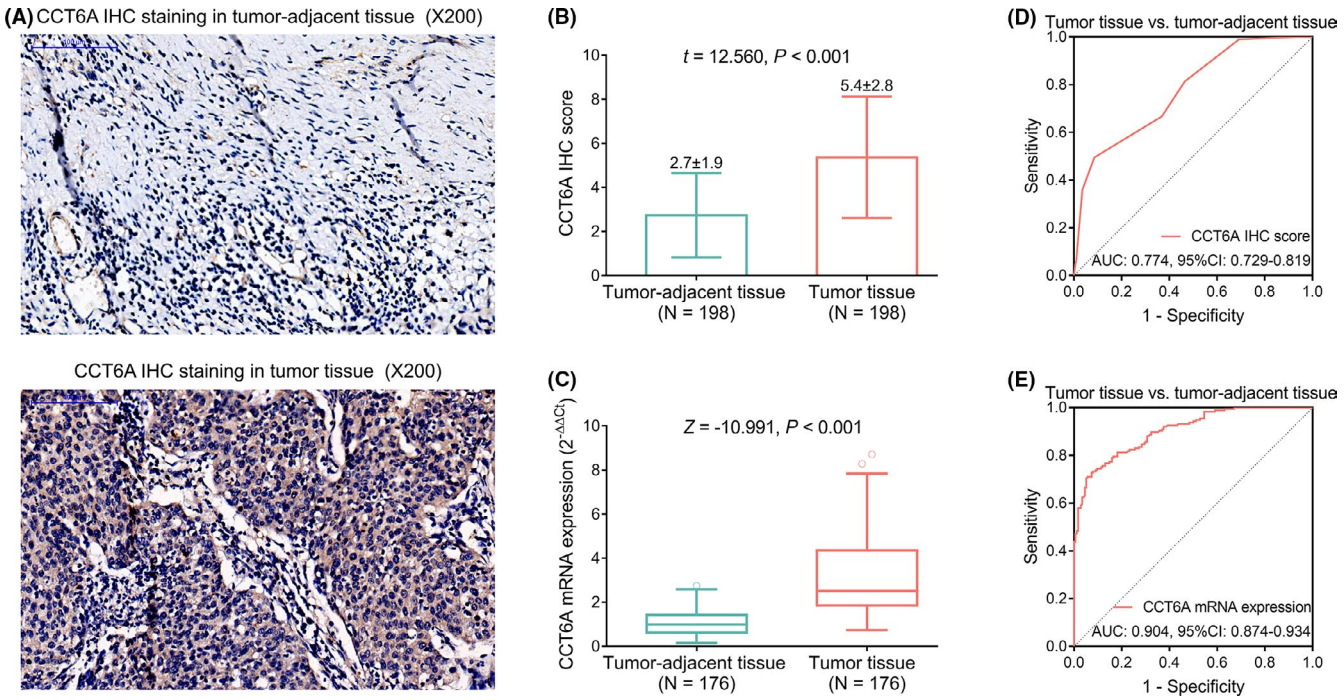

FIGURE 1 Comparison of CCT6A between tumor tissues and tumor-adjacent tissues in cervical cancer patients. (A) Representative images of CCT6A detection by IHC assay; (B) Comparison of CCT6A IHC score between tumor tissues and tumor-adjacent tissues; (C) Comparison of CCT6A mRNA level between tumor tissues and tumor-adjacent tissues; (D) Value of CCT6A IHC score in discriminating tumor tissues from tumor-adjacent tissues; (E) Value of CCT6A mRNA level in discriminating tumor tissues from tumor-adjacent tissues. CCT6A, chaperonin-containing tailless complex polypeptide subunit 6A; IHC, immunohistochemistry; AUC, area under curve; CI, confidence interval

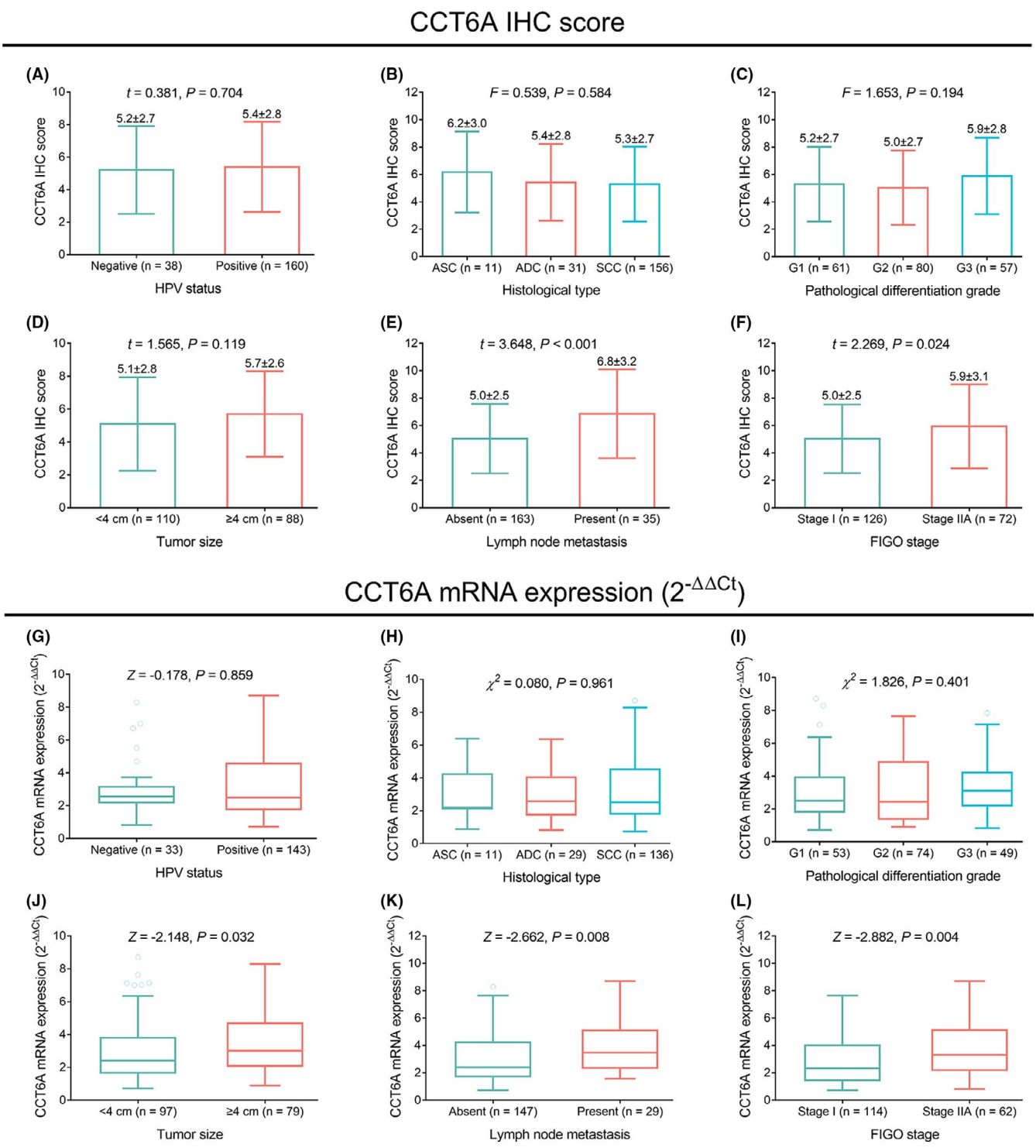

FIGURE 2 Correlation of CCT6A with the clinicopathological characteristics in cervical patients. Correlation of CCT6A IHC score with HPV status (A), histological type (B), pathological differentiation grade (C), tumor size (D), lymph node metastasis (E), and FIGO stage (F). Correlation of CCT6A mRNA level with HPV status (G), histological type (H), pathological differentiation grade (I), tumor size (J), lymph node metastasis (K), and FIGO stage (L). CCT6A, chaperonin-containing tailless complex polypeptide subunit 6A; IHC, immunohistochemistry; HPV, human papillomavirus; ASC, adenosquamous carcinoma; ADC, adenocarcinoma; SCC, squamous cell carcinoma; FIGO, federation international of gynecology and obstetrics

had lymph node metastasis, while 163 (82.3%) patients did not. In addition, 126 (63.6%) patients were of FIGO stage I, and 72 (36.4%) patients were of FIGO stage IIA. The detailed clinical characteristics of cervical cancer patients were presented in Table 1.

3.2 | CCT6A expression

The protein level of CCT6A was detected by IHC staining (Figure 1A), which showed that CCT6A IHC score was increased in tumor tissues

compared with tumor-adjacent tissues ($p < 0.001$) (Figure 1B); meanwhile, ROC curve analysis revealed CCT6A IHC score showed good ability in discriminating tumor tissues from tumor-adjacent tissues (AUC: 0.774, 95% CI: 0.729–0.819) (Figure 1D). Besides, CCT6A mRNA level also showed a similar trend ($p < 0.001$) (Figure 1C), and its ability in discriminating tumor tissues from tumor-adjacent tissues was higher than CCT6A IHC score (AUC: 0.904, 95% CI: 0.874–0.934) (Figure 1E).

3.3 | Correlation of CCT6A with tumor characteristics

Correlation analysis found that both CCT6A IHC score and mRNA level were positively correlated with the presence of lymph node metastasis and FIGO stage IIA (vs. I) (all $p < 0.05$). In addition, CCT6A mRNA level was positively associated with tumor size ≥4 cm (vs. <4 cm) ($p = 0.032$) (Figure 2A–L).

3.4 | Correlation of CCT6A with prognosis

Chaperonin-containing tailless complex polypeptide subunit 6A high IHC score was correlated with reduced accumulating DFS ($p = 0.015$), but not accumulating OS ($p = 0.051$) (Figure 3A–B). Besides, CCT6A high mRNA expression was correlated with decreased accumulating DFS ($p = 0.018$) and OS ($p = 0.010$) (Figure 3C–D).

Moreover, univariate Cox's regression analyses showed that CCT6A (high IHC score vs. low IHC score) ($p = 0.018$, HR = 2.087) was only correlated with worse DFS, but not OS ($p = 0.057$, HR = 2.079) (Table 2, Table 3); further multivariate Cox's analyses found CCT6A could not independently predict DFS or OS (Table 2, Table 3).

4 | DISCUSSION

Chaperonin-containing tailless complex polypeptide subunit 6A has already been identified as a potential biomarker in several cancers. For example, in non-small cell lung cancer, CCT6A protein level is elevated in tumor tissues compared with non-cancerous adjacent tissues, and its high expression in the tumor correlates with lymph node metastasis, higher TNM stage, and abnormal level of carcinoembryonic antigen.[12] Besides, another study focusing on breast cancer reveals that both CCT6A protein and mRNA levels are dramatically enhanced in tumor tissues compared with adjacent tissues, and its high expression is associated with unfavorable survival in breast cancer patients[11]; in addition, similar information is also reported in Ewing sarcoma.[15] In the current study, we found that CCT6A was elevated in tumor tissues compared with tumor-adjacent tissues in cervical cancer patients. One possible explanation for these data might be that: CCT6A high expression could regulate cell cycle (as in hepatocellular carcinoma cells[10]) to facilitate the malignant proliferation of cervical epithelial cells, which increased cervical carcinogenesis.[16] Apart from that, we also found that CCT6A high expression was correlated with larger tumor size, presence of lymph node metastasis and increased FIGO stage. The possible explanations were listed as follows: (1) CCT6A high expression might regulate cell cycle[10] to improve the proliferation of cervical cancer cells, thus it was correlated with larger tumor size; (2) CCT6A high expression could activate the transforming growth factor-β signaling (as in hepatocellular carcinoma cells[17]) to enhance the metastatic potential of cervical cancer cells, thus it was correlated with the presence of lymph node metastasis; (3) CCT6A high expression might comprehensively enhance the progression of cervical cancer, thus it was positively associated with FIGO stage.

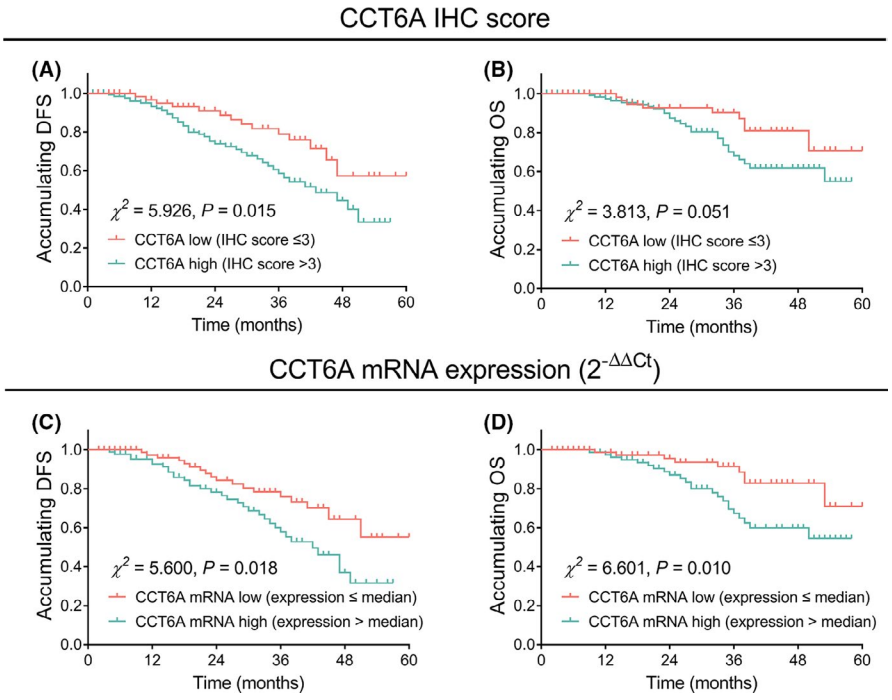

FIGURE 3 Correlation of CCT6A with accumulating DFS and OS in cervical patients. Correlation of CCT6A IHC score with accumulating DFS (A) and accumulating OS (B); Correlation of CCT6A mRNA level with accumulating DFS (C) and accumulating OS (D). CCT6A, chaperonin-containing tailless complex polypeptide subunit 6A; IHC, immunohistochemistry; DFS, disease-free survival; OS, overall survival

As mentioned above, CCT6A shows potentially prognostic value in patients with breast cancer or Ewing sarcoma.[11,15] In the present study, it was found that CCT6A high expression was correlated with worse DFS and OS. Possible explanations for these data could be that: (1) CCT6A high expression was associated with worse tumor characteristics (mentioned above), which exacerbated disease severity of cervical cancer patients and resulted in unfavorable prognosis in cervical cancer patients; (2) CCT6A high expression might promote the stemness of cervical cancer cells, which enhanced the recurrence of cervical cancer,[18] thus it was correlated with worse DFS; (3) CCT6A high expression could improve the chemoresistance of cervical cancer cell, thus indirectly affecting the prognosis of cervical cancer patients.[19] Further multivariate Cox's regression analyses found that CCT6A was not an independent risk factor for DFS or OS, implying it might interact with other factors such as FIGO stage and tumor size to affect the prognosis of cervical cancer patients.

There were several limitations in this study. First, this study did not enroll the unresectable cervical cancer patients, and the prognostic value of CCT6A in these patients could be investigated further. Second, the clinical values of the other members from the chaperonin-containing tailless complex polypeptide (CCT) family were not included in this study. Third, the molecular mechanisms of CCT6A regulating the progression and cell cycle in cervical cancer could be explored in further studies.

TABLE 2 Analysis of factors associated with DFS

	Univariate Cox's regression				Multivariate Cox's regression			
			95% CI				95% CI	
Parameters	*p* value	HR	Lower	Upper	*p* value	HR	Lower	Upper
CCT6A								
Low (IHC score <3)	Ref				Ref			
High (IHC score ≥3)	0.018	2.087	1.137	3.833	0.121	1.645	0.877	3.085
Age								
<50 years	Ref				Ref			
≥50 years	0.158	1.471	0.861	2.513	0.118	1.555	0.894	2.706
HPV status								
Negative	Ref				Ref			
Positive	0.844	0.939	0.502	1.756	0.963	1.015	0.538	1.914
Histological type								
ASC	Ref				Ref			
ADC	0.028	10.043	1.282	78.668	0.033	11.027	1.219	99.777
SCC	0.138	4.492	0.618	32.649	0.111	5.713	0.670	48.728
Pathological differentiation grade								
G1	Ref				Ref			
G2	0.190	1.716	0.765	3.853	0.791	1.125	0.470	2.691
G3	<0.001	4.269	2.024	9.006	0.329	1.554	0.641	3.766
Tumor size								
<4 cm	Ref				Ref			
≥4 cm	0.033	1.783	1.049	3.030	0.091	1.657	0.923	2.975
Lymph node metastasis								
Absent	Ref				Ref			
Present	<0.001	3.209	1.815	5.674	0.125	1.728	0.860	3.474
FIGO stage								
Stage I	Ref				Ref			
Stage IIA	<0.001	4.593	2.608	8.086	0.005	2.701	1.354	5.388

Abbreviations: ADC, adenocarcinoma; ASC, adenosquamous carcinoma; CCT6A, chaperonin-containing tailless complex polypeptide subunit 6A; CI, confidence interval; DFS, disease-free survival; FIGO, federation international of gynecology and obstetrics; HPV, human papilloma virus; HR, hazards ratio; IHC, immunohistochemistry; SCC, squamous cell carcinoma.

TABLE 3 Analysis of factors associated with OS

	Univariate Cox's regression				Multivariate Cox's regression			
			95% CI				95% CI	
Parameters	*p* value	HR	Lower	Upper	*p* value	HR	Lower	Upper
CCT6A								
Low (IHC score <3)	Ref				Ref			
High (IHC score ≥3)	0.057	2.079	0.978	4.419	0.431	1.375	0.623	3.034
Age								
<50 years	Ref				Ref			
≥50 years	0.166	1.604	0.822	3.129	0.410	1.350	0.662	2.754
HPV status								
Negative	Ref				Ref			
Positive	0.125	0.582	0.292	1.162	0.108	0.552	0.268	1.138
Histological type								
ASC	Ref				Ref			
ADC	0.072	6.752	0.843	54.105	0.142	5.754	0.558	59.384
SCC	0.335	2.673	0.362	19.764	0.319	3.165	0.328	30.512
Pathological differentiation grade								
G1	Ref				Ref			
G2	0.305	1.774	0.593	5.307	0.269	1.922	0.603	6.128
G3	0.001	5.153	1.958	13.563	0.740	1.228	0.365	4.128
Tumor size								
<4 cm	Ref				Ref			
≥4 cm	0.012	2.385	1.213	4.687	0.037	2.186	1.049	4.557
Lymph node metastasis								
Absent	Ref				Ref			
Present	0.001	3.083	1.584	6.000	0.230	1.662	0.725	3.808
FIGO stage								
Stage I	Ref				Ref			
Stage IIA	<0.001	5.228	2.466	11.084	0.027	2.759	1.121	6.790

Abbreviations: ADC, adenocarcinoma; ASC, adenosquamous carcinoma; CCT6A, chaperonin-containing TCP1 subunit 6A; CI, confidence interval; FIGO, federation international of gynecology and obstetrics; HPV, human papillomavirus; HR, hazards ratio; IHC, immunohistochemistry; OS, overall survival; SCC, squamous cell carcinoma.

Collectively, CCT6A is elevated in tumor tissues, and its high expression associates with larger tumor size, lymph node metastasis, higher FIGO stage and worse prognosis in cervical cancer patients. CCT6A may act as a potential prognostic biomarker to enhance the management of cervical cancer patients.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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How to cite this article: Ma J, Yang L, Feng H, Zheng L, Meng H, Li X. CCT6A may act as a potential biomarker reflecting tumor size, lymphatic metastasis, FIGO stage, and prognosis in cervical cancer patients. *J Clin Lab Anal*. 2021;35:e23793. https://doi.org/10.1002/jcla.23793