RESEARCH ARTICLE

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Prognostic Significance of Chaperonin-Containing Tailless Complex Polypeptide 6A (CCT6A) in Ewing Sarcoma

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Abstract

Background: Chaperonin-Containing Tailless complex polypeptide 6A (*CCT6A*) is considered one of the biomarkers that play a role in cancer initiation, progression and resistance to body defenses and to anti-cancer drugs. Its exact prognostic role in Ewing sarcoma (ES) remains uncertain. The aim of this study is to assess immunohistochemical (IHC) expression of *CCT6A* in ES, and to assess the relation of its expression to different clinicopathological parameters and evaluate its prognostic significance in ES cases. **Methods:** This retrospective cohort study included 35 cases diagnosed as ES at the Oncology Center of Mansoura University (OCMU), Faculty of Medicine, Egypt. Clinicopathological and survival data were collected. IHC for *CCT6A* was performed and correlated with clinicopathological parameters and patients' prognosis. **Results:** High IHC expression of *CCT6A* was found in 28 cases out of the studied 35 ES cases (80%), while 7 cases only (20%) showed low *CCT6A* expression. High *CCT6A* expression showed significant association with tumor size ≥8 cm (P= 0.01), treatment with adjuvant radiotherapy either for local control, infiltrated surgical margins or poor histopathological response to chemotherapy (P= 0.01), poor histopathological response to chemotherapy (P= 0.02), and HUVOS grades I and II (P= 0.01). High *CCT6A* expression was found to be a predictor of shorter overall survival and was an independent poor predictive factor by multivariate analysis (P= 0.03). **Conclusion:** High *CCT6A* expression in ES associates with large tumor size, treatment with adjuvant radiotherapy for aforementioned indications, poor histopathological response to chemotherapy, and HUVOS grades I and II. Its high expression also independently predicts poor overall survival in ES patients. It may be used as a useful biomarker to predict prognosis of ES.

Keywords: Ewing sarcoma- survival- prognosis- *CCT6A*

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Introduction

With a peak incidence in the second decade of life, Ewing sarcoma (ES) is the second most prevalent malignant bone tumor in children and young adults [1]. Ewing sarcoma arises commonly in flat or long bones, and less frequently in soft tissues [2, 3]. The annual incidence rate in Europe is 7.5 cases per million children aged between 10 and 19 years old. Additionally, there are about 100 new ES cases in France every year [4]. A study at children cancer hospital of Egypt in a period from 2009 to 2018 reported 554 total number of ES cases [5].

The wide histological spectrum of ES and the existence of numerous histological mimics can make its diagnosis difficult [6]. Given that ES patients require particular treatment modalities, a precise diagnosis is crucial [7]. A combination of histological, immunohistochemical, and molecular findings to detect these genetic abnormalities on a routine basis and correlation with the clinical and radiological characteristics permits accurate diagnosis and guides clinical decision-making in most cases [8].

Ewing sarcoma is regarded as a tumor with a poor overall survival (OS) and prognosis, particularly when it recurs or metastasize [9]. Large tumors (volume ≥ 200 ml or largest diameter ≥ 8 cm), primary axial tumors, particularly pelvic ones, metastasis at diagnosis, and a poor histopathological response are all strongly linked to a lower survival rate in ES [10]. Metastasis at the time of diagnosis is the most significant established prognostic factor, and the most common sites are the lungs, bones, and bone marrow [11].

In European studies, local control rates are significantly impacted by the post-neoadjuvant histopathological response. Although there are various criteria, > 90% necrosis should be considered a good response to chemotherapy. Post-surigcal adjuvant radiotherapy (RT) is indicated in cases with infiltrated surgical margins, while European protocols also recommend RT for narrow

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surgical margins and/or poor histopathological response to chemotherapy (≥10% viable tumor cells in the specimen) [12]. The post-neoadjuvant histopathological response is also evaluated using the HUVOS grading system. It is assessed according to the percentage of tumor necrosis. Tumors with < 50% necrosis were assigned grade I, 50-89% as grade II, 90-99% as grade III and 100% necrosis as grade IV [13]. When multiagent chemotherapy was used in conjunction with surgery and radiotherapy, the 5-year OS improved to 65-75% in the localized stage, while rates in the metastatic stage barely exceeded 30% [12].

Since prognostic biomarkers aid in risk management and stratification, they play a significant role in ES workup. They may be linked to either a favorable or unfavorable prognosis [9]. Recent research has demonstrated the predictive functions of biomarkers, particularly for tumor prognosis. Among these biomarkers is chaperonin containing TCP1 complex 6A (*CCT6A*) [14, 15]. The group II chaperonin complex known as Chaperonin-containing tailless complex polypeptide 1 (CCT) is made up of eight distinct subunits, referred to as CCT1 through CCT8, which can fold actins and tubulins, suggesting a potential involvement in the invasion, migration, and proliferation of cancer cells. CCT6 contains 2 subunits: 6A and 6B. Recently, *CCT6A* has received particular attention due to its various roles in cancer [16, 17].

It is possible that *CCT6A* contributes to the development and progression of different types of tumors. However, its level of expression and predictive function in ES are still unknown [18]. The purpose of this study was to investigate *CCT6A* expression in ES tissue samples and assess its relationship to various clinicopathological factors and the prognosis of the patient.

Materials and Methods

This retrospective study was performed on 35 cases diagnosed as Ewing sarcoma. These cases were diagnosed during the period from 2011 to 2019 at the pathology department laboratory, Oncology Centre Mansoura University (OCMU), Faculty of Medicine, Mansoura university, Egypt.

Hematoxylin and eosin (H & E) and immunohistochemically (IHC) stained slides were retrieved from the slides' archive and re-examined to verify the diagnosis of Ewing sarcoma, based on characteristic morphology and immunohistochemical staining. Tissue microarray blocks were constructed from formalin-fixed paraffin-embedded tissue blocks of these cases and then IHC for *CCT6A* is performed.

Our institute's database for the medical records of the examined cases at the Clinical Oncology and Nuclear Medicine department, patient's clinical sheets, and pathological reports provided the demographic and clinicopathological information of the included cases. They included patients' age, gender, presence of comorbidity, tumor origin, location, size (radiologically at 1st presentation), treatment modality and associated local recurrence or distant metastasis. In order to monitor tumor recurrence and patients' survival, all cases under study were monitored every three months. Recurrence or relapse of the tumor was verified by radiological or histopathological local appearance of the tumor at the same site or at a metastatic site. Overall survival (OS), the primary outcome of this study, was defined as the time between diagnosis and death or the end of the follow-up period (up to a maximum of 36 months). Progression-free survival (PFS) was calculated as the interval between the start of treatment and the date of death or the progression of the disease (metastasis or recurrence).

Tissue Microarray (TMA) Construction

Formalin-fixed paraffin-embedded tissue blocks of the included cases were used in this study. Using the mechanical tip pencil method, three tissue microarray blocks were created [19, 20]. Empty recipient paraffin blocks were prepared. Using a mechanical pencil tip that was 0.7 mm thick, wax cylinders were punched out of the recipient block, creating holes that were roughly 0.8 mm in diameter.

Hematoxylin and eosin (H&E) slides from each tumor were re-examined and labeled at the most representative areas before being applied to donor paraffin blocks. A mechanical pencil tip with a 0.9 mm diameter was used to identify and punch out the paraffin block sections that matched the designated areas. Tissue cores were transferred to recipient block holes after being carefully pressed out of the pencil tip using a tiny metal needle. According to a map created specifically for each block, these cores were placed into recipient blocks.

Three tissue cores were extracted from each donor block of ES cases at three distinct sites. In accordance with a pre-made map, several normal tissue cores—such as the placenta, pancreas, colonic mucosa, and liver—were added to the TMA blocks to serve as navigational and orientational markers. All fallen cores were repeated in a different block.

Immunohistochemistry for CCT6A

IHC was performed using Autostainer Link 48, utilizing its optimized reagents with pharmDx kits EnVision FLEX Visualization Systems (Link code K8000) and EnVision FLEX Hematoxylin (Link code K8008) in accordance with the standardized procedure included in the autostainer software's user manual.

Semi-quantitative IHC interpretation was carried out with a standard light microscope. Anti-*CCT6A* rabbit monoclonal antibody (ABclonal, Catalog No. A3589, diluted 1:100) was used. Scoring was done using the staining intensity and density to assess *CCT6A* expression. Immunohistochemical staining has intensity scores ranging from 0 to 3 and density scores ranging from 0 to 4. The overall score, which is determined by multiplying the intensity and density scores, is 12. *CCT6A* low expression is defined as an IHC score of less than or equal to 3. Conversely, *CCT6A* high expression is defined as an IHC score greater than 3 [21]. For confirmation of the validity of *CCT6A* IHC analysis, its staining is assessed in both tumor tissue and non-tumor tissue specimens. *CCT6A* showed low expression in the non-tumor tissue specimens. On the other hand, it showed higher expression in ES tissue specimens [18]. Histological sections processed without

the addition of primary antibodies were used to create suitable negative controls. Areas of fibrosis, necrosis and tissue sections' edges weren't involved in scoring to avoid false positivity.

Statistical analysis

The SPSS software, version 25 (SPSS Inc., PASW statistics for Windows version 25 Chicago: SPSS Inc.), was used to analyze the data. Numbers and percentages were used to describe the qualitative data. Significance of the obtained results was judged at the (≤0.05) level. Qualitative data was compared between groups using Monte Carlo, Fisher exact, and Chi-Square tests as needed. Spearman correlation coefficient was used to correlate between continuous non-parametric data. Kaplan-Meier test was used to calculate overall survival and progression-free survival with using log rank $\chi 2$ to detect effect of risk factors affecting survival. In order to evaluate survival predictors and calculate the hazard ratio, Cox regression was utilized.

Ethical considerations

The investigation was conducted using archive material from paraffin tissue blocks that were kept in the pathology laboratory. The Institutional Research Board (IRB), code number: MDP.21.05.68) at Mansoura University's Faculty of Medicine has approved the task proposal. Patient confidentiality was maintained throughout the trial by using their code numbers instead of their names. Lastly, the donor paraffin blocks were restored in the archives for future patient or research use.

Results

Among the 35 studied ES cases, 30 cases showed classical morphology (small rounded monotonous cells with ill-defined cell borders, uniform round nuclei and scant cytoplasm), 4 cases showed neuroectodermal differentiation (Homer-Wright pseudorosettes) and only one case showed atypical morphology (large nuclei, vesicular chromatin, irregular nuclear contours, and prominent nucleoli). One of the diagnosed ES cases is illustrated in (Figure 1).

The demographic, clinical and histopathological data of studied ES cases are shown in (Table 1). The study population consisted of 35 ES patients with a mean age of 28.40±15.42 (age range 9-69years). The study consisted of 19 females and 16 males.

As regard treatment modality, all patients received chemotherapy with only 21 patients underwent surgery and 26 patients received radiotherapy either for local control, infiltrated surgical margins or for post-neoadjuvant poor histopathological response.

High *CCT6A* expression was detected in 28 cases (80%) (Figure 2 C and 2 D), while 7 cases (20%) showed low *CCT6A* expression (Figure 2 A and 2 B).

As demonstrated in (Table 2), *CCT6A* expression showed statistically significant association with tumor size (P= 0.01). There was detected high *CCT6A* expression in 95.2% of cases that are ≥8 cm in size compared to 57.1% of cases that are <8 cm in size. There were also

Table 1. Patient's Demographic, Clinical and Histopathological Data.

Clinicopathological parameters	N=35	%
Age/years		
<18	10	28.6
≥18	25	71.4
Age (years)		
Mean ±SD	28.40±15.42	
Median (MIN-MAX)	26 (9-69)	
Sex		
Male	16	45.7
Female	19	54.3
Co- morbidity		
Absent	31	88.6
Present	4	11.4
Primary tumor site		
Axial	18	51.4
Extremity	17	48.6
Tumor origin		
Bone	17	48.6
Soft tissue	18	51.4
Tumor size (Determined radiologically at 1st presentation)		
<8 cm	14	40
≥8 cm	21	60
Metastasis at diagnosis status		
No	28	80
Yes	7	20
Treatment		
Chemotherapy without surgery	14	40
Chemotherapy with surgery	21	60
Adjuvant radiotherapy		
No adjuvant radiotherapy	9	25.7
Adjuvant radiotherapy for local control	14	40.0
Adjuvant radiotherapy for infiltrated surgical margins	3	8.6
Adjuvant radiotherapy for poor histopathological response to chemotherapy	9	25.7
Relapse/progression		
No	15	42.9
Yes	20	57.1
Histopathological features		
Classical	30	85.7
Neuroectodermal differentiation	4	11.4
Atypical Ewing sarcoma	1	2.9
Patients underwent surgical intervention	N=21	%
Surgical margins		
Free	18	85.7
Infiltrated	3	14.3
Histological response to Chemotherapy		
Poor	18	85.7
Good	3	14.3
HUVOS grading		
I	12	57.1
II	6	28.6
III	1	4.8
IV	2	9.5

Table 2. Associations between CCT6A Expression and Different Clinicopathological Parameters

Clinicopathological parameter	CCT6A Expression		Test of significance
	Low	High	
	N=7(%)	N=28(%)	
Age/years			
<18	2 (20)	8 (80)	FET
≥18	5 (20)	20 (80)	P=1.0
Sex			
Male	4 (25)	12 (75)	FET
Female	3 (15.8)	16 (84.2)	P=0.7
Co-morbidity			
Absent	7 (22.6)	24 (77.4)	FET
Present	0	4 (100)	P=0.6
Primary tumor site			
Axial	3 (16.7)	15 (83.3)	FET
Extremity	4 (23.5)	13 (76.5)	P=0.7
Tumor origin			
Bone	4 (23.5)	13 (76.5)	FET
Soft tissue	3 (16.7)	15 (83.3)	P=0.7
Tumor size			
<8 cm	6 (42.9)	8 (57.1)	FET
≥8 cm	1 (4.8)	20 (95.2)	P=0.01
Treatment			
Chemotherapy without surgery	1 (7.1)	13 (92.9)	FET
Chemotherapy with surgery	6 (28.6)	15 (71.4)	P=0.2
Adjuvant radiotherapy			
No adjuvant radiotherapy	5 (55.6)	4 (44.4)	MC
Adjuvant radiotherapy for local control	1 (7.1)	13 (92.9)	P=0.01
Adjuvant radiotherapy for infiltrated surgical margins	1 (33.3)	2 (66.7)	
Adjuvant radiotherapy for poor histopathological response to chemotherapy	0	9 (100)	
Surgical margins*			
Free	5 (27.8)	13 (72.7)	FET
Infiltrated	1 (33.3)	2 (66.7)	P=0.9
Histological response to chemotherapy*			
Poor	3 (16.7)	15 (83.3)	FET
Good	3 (100)	0	P=0.02
HUVOS grading*			
I	3 (25)	9 (75)	MC
II	0	6 (100)	P=0.01
III	1 (100)	0	
IV	2 (100)	0	
Relapse/progression			
No	3 (20)	12 (80)	FET
Yes	4 (20)	16 (80)	P=0.9
Metastasis at diagnosis			
No	7 (25)	21 (75)	FET
Yes	0	7 (100)	P=0.3
Histopathological features			
Classical	6 (20)	24 (80)	Mc
Neuroectodermal differentiation	1 (25)	3 (75)	P=0.9
Atypical Ewing sarcoma	0	1 (100)	

FET, Fisher exact test; MC, Monte Carlo test; P, Probability value (statistically significant when ≤ 0.05); *, means patients underwent surgical intervention =21.

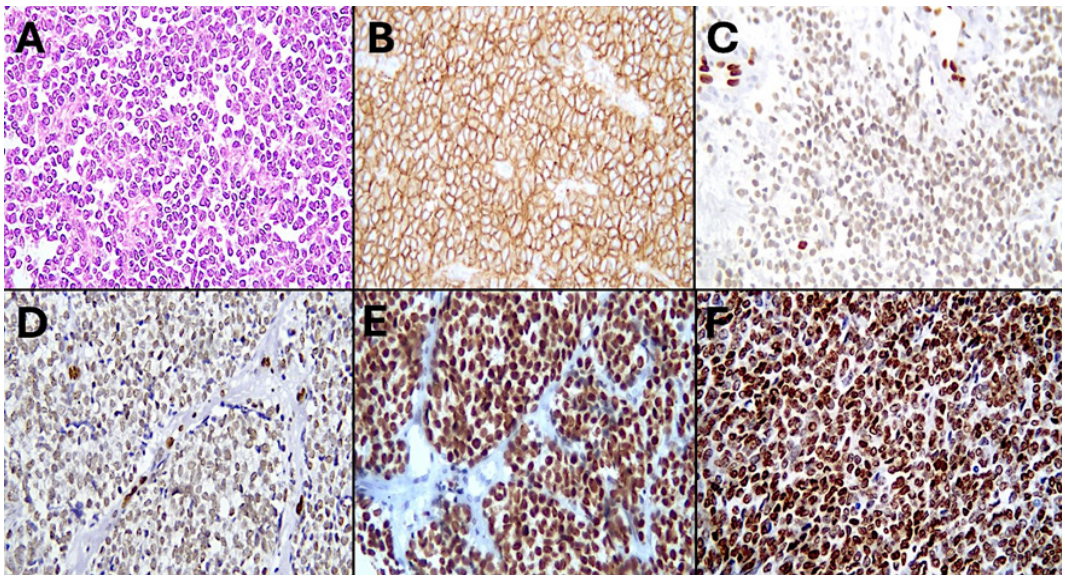

Figure 1. (A) A case of Ewing sarcoma showing classical morphology (H&E, ×400), (B) CD99 diffuse strong positive membranous reaction (IHC, x400), (C) ERG moderate positive nuclear reaction with strong positive nuclear reaction in endothelial cells as positive internal control (IHC, x400), (D) FLI1 moderate positive nuclear reaction with strong positive nuclear reaction in endothelial cells as positive internal control (IHC, x400), (E) NKX2.2 diffuse strong nuclear reaction (IHC, x400), (F) PAX7 diffuse strong nuclear reaction (IHC, x400).

statistically significant associations between *CCT6A* expression and treatment with adjuvant radiotherapy either for local control, infiltrated surgical margins or poor histopathological response to chemotherapy (P= 0.01), histopathological response to chemotherapy (P= 0.02), and HUVOS grading (P= 0.01). There were no detected associations between *CCT6A* expression and other clinicopathological variables.

During the follow up period, 20 patients (57.1%) underwent relapse/tumor progression, and 28 patients (80%) died due to disease related factors. The median OS of all included ES patients was 26 months (95% confidence interval (CI): 17.3-34.7) and the median PFS for them was 33 months (95% CI: 11.3-54.7). Univariate analyses of clinicopathologic variables affecting the 3-year PFS and OS rates are shown in (Table 3) and (Table 4) respectively. As regard PFS, univariate analysis showed significant association between shorter PFS and soft tissue tumor origin (P= 0.04), and existence of co-morbidity (P= 0.04). A multivariate Cox regression analysis revealed that soft tissue tumor origin was an independent prognostic predictor for lower PFS (P= 0.04). No significant association was found between PFS and *CCT6A* expression.

Regarding OS, univariate cox regression analysis revealed significant association between shorter OS and high expression of *CCT6A* (P= 0.02), treatment with chemotherapy without surgery (P= 0.02), treatment with radiotherapy for local control (P= 0.04), and presence of metastasis at diagnosis (P= 0.05). Multivariate analysis reported that high *CCT6A* expression (P= 0.03) and treatment with chemotherapy without surgery (P= 0.049) are independent prognostic predictors for shorter OS in ES cases as shown in (Table 5) and (Figure 3).

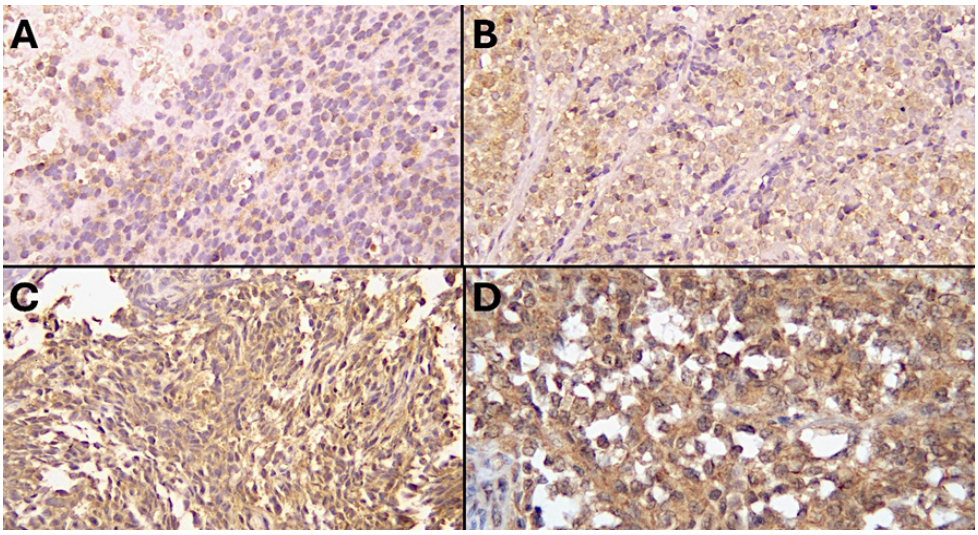

Figure 2. (A and B): CCT6A low expression (IHC, x400), (C and D): CCT6A high expression (IHC, x400).

Table 3. Univariate Survival Analysis of the Progression-Free Survival in Ewing Sarcoma

Clinicopathological parameters	Univariate Survival Analysis of PFS			
	Median PFS survival (95% CI)	3-year PFS (%)	HR (95% CI)	P value
Total	33 (11.3-54.7)		-	-
Age /years				
<18	44.9(27.1-62.6)	71.40%	Ref	
≥18	51.97 (25.9-78)	39.50%	1.6 (0.4-7.5)	0.5
Sex				
Male	85 (45.1-124.9)	64%	Ref	
Female	30.4 (21.2-39.6)	31.50%	2.7 (0.7-10.5)	0.1
Co-morbidity				
Absent	50 (25.8-74.2)	53.50%	Ref.	
Present	22 (0.001-45.9)	0	4.5 (1.1-18.9)	0.04*
Primary tumor site				
Axial	26 (16.6-35.4)	30%	1.5 (0.5-4.6)	
Extremity	50 (24.6-75.4)	56%	Ref.	0.5
Tumor origin				
Bone	111 (82.07-139.9)	90%	Ref.	
Soft tissue	29.3 (19.9-38.6)	26.10%	8.2 (1.1-63.5)	0.04*
Tumor size				
<8 cm	50 (15.1-84.9)	60%	Ref.	
≥8 cm	26 (16.9-35)	32.80%	1.4 (0.4-4.5)	0.6
Treatment				
Chemotherapy without surgery	26 (0.001-53.2)	41.70%	Ref.	
Chemotherapy with surgery	33 (21.3-44.7)	45%	1.2 (0.3-5.4)	0.8
Adjuvant radiotherapy				
No adjuvant radiotherapy	32 (13.1-50.9)	43.20%	Ref.	
Adjuvant radiotherapy for local control	26 (0.001-53.2)	41.70%	0.9 (0.2-5.1)	0.9
Adjuvant radiotherapy for infiltrated surgical margins	50 (50-50)	66.70%	1.1 (0.2-6.2)	0.9
Adjuvant radiotherapy for poor histopathological response to chemotherapy	33 (0.001-66.9)	43.20%	1.2 (0.3-4.8)	0.8
Surgical margins				
Free	63.2 (32.9-93.4)	42.20%	Ref.	
Infiltrated	41.7 (22.8-60.5)	66.70%	1.02 (0.2-4.9)	0.9
Histological response to Chemotherapy				
Poor	52.3 (24.5-80.8)	41%	1.5 (0.2-11.8)	
Good	32 (15.9-48)	75%	Ref.	0.7
CCT6A expression				
Low	50 (0.001-107.7)	68.60%	Ref.	
High	26 (16.9-35)	30.80%	2.4 (0.6-9.3)	0.2

PFS, Progression free survival; CI, Confidence interval; HR, Hazard ratio; Ref, Reference; P, Probability; value (statistically significant when ≤ 0.05)

Discussion

Recent studies show an important role for *CCT6A* in tumor pathology [16, 22-24]. Ying et al. (2017) demonstrated that *CCT6A* enhanced non-small cell lung cancer cell survival and metastasis [25]. According to some studies, hepatocellular carcinoma tumor tissues have higher expression levels of proteins encoded by *CCT6A*, and these patients' overall survival is shorter [16]. Other studies revealed that *CCT6A* was negatively correlated with survival and was both expression and amplification inducible in glioblastomas [26]. Also, it has been reported that *CCT6A* significantly contributes to the development of breast carcinoma [27]. Moreover, *CCT6A* expression revealed to be linked to poor prognosis in colonic adenocarcinoma patients [28]. However, little information was known about expression level and prognostic significance of *CCT6A* in Ewing sarcoma [18].

In our study, multivariate analysis reported that treatment with chemotherapy without surgical intervention,

Table 4. Univariate Survival Analysis of the Overall Survival in Ewing sarcoma.

Clinicopathological parameters	Univariate Survival Analysis of OS			
	Median OS survival (95% CI)	3-year OS (%)	HR (95%CI)	P value
Total	26 (17.3-34.7)		-	-
Age /years				
<18	17 (5.4-28.6)	10%	1.5 (0.6-3.3)	
≥18	26 (16.2-35.8)	32%	Ref.	0.3
Sex				
Male	17 (13.1-20.9)	31.30%	Ref.	
Female	27 (22.9-31.1)	21.10%	1 (0.5-2.04)	0.9
Co-morbidity				
Absent	26 (16.2-35.8)	29%	Ref.	
Present	12 (0.001-31.6)	0%	1.6 (0.6-4.7)	0.4
Primary tumor site				
Axial	17 (10.1-23.9)	22.20%	1.1 (0.5-2.3)	
Extremity	27 (21.7-32.3)	29.40%	Ref.	0.8
Tumor origin				
Bone	23 (12.9-33.1)	17.60%	1.4 (0.7-3)	
Soft tissue	26(7.3-44.7)	33.30%	Ref.	0.4
Tumor size				
<8 cm	21 (0.8-41.2)	35.70%	Ref.	
≥8 cm	26 (15.9-36.1)	19%	1.3 (0.6-2.9)	0.5
Treatment				
Chemotherapy without surgery	16 (9.9-22.1)	7.10%	2.4(1.1-5.1)	
Chemotherapy with surgery	27 (22.5-31.5)	38.10%	Ref.	0.02*

OS, Overall survival; CI, Confidence interval; HR, Hazard ratio; Ref, Reference; P, Probability; value (statistically significant when ≤ 0.05)

Table 5. Multivariate Survival Analysis of the Overall Survival in Ewing Sarcoma

Clinicopathological parameters	HR (95%CI)	P value
CCT6A expression		
Low	Ref.	0.03*
High	3.9 (1.1-13.1)	
Treatment		
Chemotherapy without surgery	2.2 (1.003-4.8)	0.049*
Chemotherapy with surgery	Ref.	

CI, Confidence interval; HR, Hazard ratio; Ref, Reference; P, Probability value; (statistically significant when ≤ 0.05)

and high IHC expression of *CCT6A* are independent predictors of prognosis indicating shorter OS in ES patients. The significant association between shorter OS and high IHC expression of *CCT6A* in our study agrees with one previous study that also stated this significant association [18]. That study performed *CCT6A* gene expression analysis of 32 Ewing sarcoma cases. Their multivariate analysis revealed that age was also an independent poor prognostic factor [18]. This difference may be due to dissimilar age groups classification and different techniques in both studies. That previous study didn't include treatment modality in their variables.

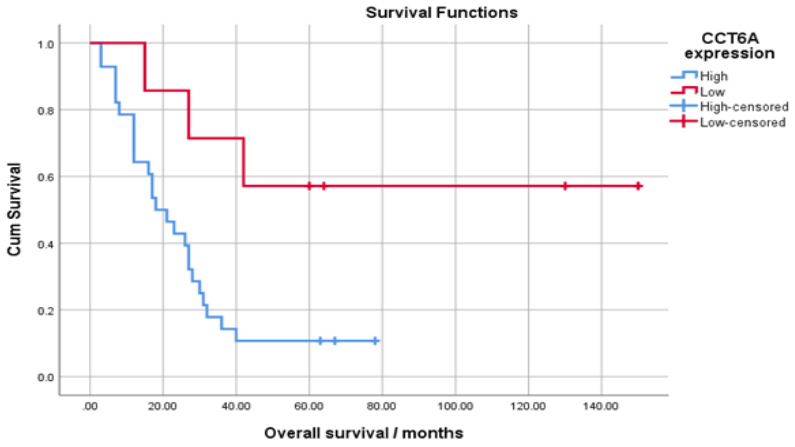

Figure 3. Overall Survival of the Entire Cohort Studied according to *CCT6A* Expression.

Multivariate analysis in a study carried out by Morsy et al. [12] revealed similar finding where treatment without surgery is an independent prognostic predictor of shorter OS. On the other hand, that study showed that the disease stage at time of diagnosis either localized or metastatic, response to neoadjuvant chemotherapy and primary tumor size were significantly associated with OS. They also reported that radiotherapy when used for local control came next in significance with only slightly better OS in the multivariable analysis [12]. These differences could be attributed to variations in sample size and follow up periods in both studies.

The present study didn't report any statistically significant association between age and PFS or OS. On the contrary, Sirikul et al. [29] found that adult patients had significantly poorer PFS and OS. That study used different cut-off value for age groups classification (< or ≥ 25 years). They also used 5-year period instead of 3-year for investigating their survival outcomes [29].

Both univariate and multivariate Cox regression analyses in the present study didn't find a significant correlation between 3-year PFS and *CCT6A* expression. Additionally, we found no evidence of a significant correlation between *CCT6A* expression and relapse/progression. On the contrary, *CCT6A* high gene expression was significantly correlated with equal or less than 5-year PFS in a previous study. That study also showed that *CCT6A* gene expression's median value in this subgroup of patients was highest in the group with metastasis, followed by those that showed relapse, and finally, the primary group patients. Consequently, this proposes a relationship between the high *CCT6A* gene expression and tumor metastasis [18]. This variation may be attributed to different follow up periods for progression free survival.

In our study, univariate analysis showed that soft tissue tumor origin and presence of co-morbidity had significant association with 3-year PFS. The multivariate analysis revealed that soft tissue tumor origin was an independent prognostic predictor for shorter PFS. However, a study by Morsy et al. [12] exhibited that stage of disease at diagnosis either localized or metastatic, surgical intervention used as a local modality, treatment protocol adequacy, histopathologic subtype, primary tumor site and primary tumor size had significant association with PFS in the univariate analysis. In their multivariate analysis, both treatment protocol adequacy and primary tumor site lost their statistical significance as predictors of PFS while other variables stood as significant predictors. Tumor origin didn't show any significant association with PFS in that study [12]. These discrepancies could be due to different sample sizes, follow up periods for PFS and some different variables in both studies.

This current study showed that *CCT6A* high IHC expression had significant association with large tumor size, treatment with adjuvant radiotherapy as a local control or for infiltrated surgical margins or for post neoadjuvant poor histopathological response. Additionally, there has been significant association with poor histopathological response to chemotherapy, and HUVOS grades I and II. Jiang et al. [18] reported significant association between *CCT6A* gene expression and age. This difference be due to different age groups classification and different techniques in both studies. Moreover, they didn't include our significantly associated variables in their study.

There were some limitations in our study: (1) It was a single-center study; thus, there could be some selective biases; (2) Limited sample size, further studies with larger sample size would be better; (3) Diagnosis of our cases wasn't confirmed by molecular testing due to limited resources, so some of them may be one of the Ewing-like sarcoma group of tumors which may affect treatment response; (4) Our study was done using IHC method for evaluation of *CCT6A* expression, further studies using both gene analysis and IHC methods are needed; (4) This study didn't investigate the precise mechanism by which *CCT6A* contributes to the pathophysiology of Ewing sarcoma, which is needed to be further investigated.

In conclusion, high *CCT6A* IHC expression correlates with large tumor size, treatment with adjuvant radiotherapy as a local control or for infiltrated surgical margins or for post neoadjuvant poor histopathological, HUVOS grades I and II and poor histopathological response to chemotherapy. High *CCT6A* expression may serve as independent poor prognostic indicator of OS in Ewing sarcoma.

Author Contribution Statement

All authors contribute equally, all authors reviewed the results and approved the final version of the manuscript.

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Compliance with Ethical Standards

This study was conducted upon approval of the committed Institutional Research Board (IRB) at Faculty of Medicine, Mansoura University, Egypt (Code Number: MDP.21.05.68). The study was processed under the ethical standards of the Helsinki declaration.

This study wasn't approved by any scientific body and isn't part of an approved student thesis.

Availability of data

The datasets are available from the corresponding author upon request.

Conflict of interest statement

The authors declare no relevant financial affiliations or conflicts of interest.

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