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Expression of CUE domain containing 2 protein in serous ovarian cancer tissue: predicting disease-free and overall survival of patients

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Abstract

Objective: The aim of this study was to predict disease-free (DFS) and overall (OS) survival of cancer patients through expression of CUE domain containing 2 (CUEDC2) protein.

Methods: In this retrospective study, we investigated CUEDC2 expression in 75 serous ovarian cancer tissues and 34 tubal fimbria tissues by immunohistochemistry. Chemoresistance was analyzed using clinical follow-up data.

Results: CUEDC2 expression scores were 1.35 ± 0.60, 1.54 ± 0.57, 1.78 ± 0.71, and 2.13 ± 0.27 for International Federation of Gynecology and Obstetrics (FIGO) stages I, II, III, and IV tissues, respectively, indicating that CUEDC2 expression increased with stage and that scores differed between patients with early and advanced cancers. We found no differences in CUEDC2 expression for tissues with low, medium, and high differentiation. CUEDC2 expression was unrelated to patient age, pathological grade, or presence or absence of lymph node metastasis, but was related to tumor stage. For CUEDC2-positive patients, median DFS and OS survival were 32.6 and 54.3 months, respectively. For CUEDC2-negative patients, median DFS and OS were 51.9 and 63.5 months, respectively. Expression of CUEDC2 was correlated with DFS but not OS.

Conclusion: CUEDC2 is highly expressed in ovarian cancer tissues and is related to tumor stage and DFS.

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Introduction

Ovarian cancer is one of the most common malignancies of the female reproductive system. About 239,000 patients are diagnosed with ovarian cancer every year, and 152,000 patients die from the disease annually.[1] Moreover, there are many types of malignant ovarian tumors, and serous tumors account for about 45% of all ovarian cancers.[2,3] Ovarian cancer is a multigenic gynecological malignancy with high mortality. In addition to early symptoms and family history of tumors,[4–7] resistance to chemotherapy is an important cause of high mortality related to ovarian cancer. In addition to surgery for ovarian cancer, chemotherapy is an important adjuvant treatment, and chemotherapy resistance hinders clinical treatment. Most patients die due to relapse or metastasis. At present, drug resistance of ovarian cancer is a worldwide research hotspot. The 5-year survival rate of this disease is low, and many patients develop chemotherapy resistance, but the mechanism of chemotherapy resistance of ovarian cancer remains unclear. CUE domain containing 2 (CUEDC2) is a drug resistance protein associated with breast cancer.[8]

The CUEDC2 protein includes the CUE domain, a tiny and highly conserved ubiquitin-binding sequence of approximately 40 amino acids. CUE is present in several eukaryotic proteins and plays crucial roles in several biological processes, including cell cycle, inflammation, and development of tumors. CUEDC2 is associated with IkB kinase α (IKKα) and IKKβ antagonists, and it controls and triggers phosphorylation of IKK. Its overexpression leads to earlier activation of anaphase-promoting complex/cyclostome (APC/C), which contributes to chromosome missegregation and aneuploidy, which in turn may result in tumor development.[9] In this study, we used immunohistochemistry to study the expression of CUEDC2 in serous ovarian cancer, and its relation to the International Federation of Gynecology and Obstetrics (FIGO) staging, disease-free survival (DFS), and overall survival (OS).

Materials and methods

Patient criteria and categorization

All patients enrolled at PLA General Hospital (Beijing) from January 2005 to July 2011 who had ovarian serous cystadenocarcinoma by pathological examination following surgery were contacted. For this retrospective cohort study, 175 patients were assessed through different means, including by phone, in person, and record review. Patients who were diagnosed with ovarian serous cystadenocarcinoma by pathological examination following surgery were included, irrespective of age. Patients were excluded if (1) they underwent neoadjuvant chemotherapy before surgery, (2) tumor tissue paraffin blocks were unavailable or inadequate, or (3) if they had other synchronous tumors or metastatic tumors to the ovary. In the final study, there were

109 participants; the remaining patients were excluded based on the above-mentioned excluded criteria. Tumor tissues were collected from 75 patients with serous ovarian carcinoma who underwent surgery in the obstetrics and gynecology department of the former PLA General Hospital. According to the dualistic model of ovarian cancer, the control group consisted of 34 oviduct sections from the same patients that were not invaded by tumor. Patients were divided into FIGO stage I, II, III, or IV. Because this was a retrospective study, ethical approval and informed consent were not required.

Reagents

CUEDC2 antibody was provided by the Instrument Center of The Academy of Military Medical Sciences (Beijing, China). The secondary antibody was purchased from Zhongshan Jinqiao Company (Zhongshan, China).

CA-125 measurement

CA-125 was detected as described previously,[10] using an immunoassay with the two monoclonal antibodies, M 11 and OC 125 (Fujirebio Diagnostics Inc., Malvern, PA, USA).

Expression of estrogen and progesterone receptors

The expression of estrogen receptor (ER) and progesterone receptor (PR) in ovarian tissue was measured by immunohistochemistry, as described previously.[11] In brief, antigen extraction was conducted for 30 minutes via citric acid (pH 6.0). Incubation with 10% fetal calf serum for 30 minutes prevented non-specific antibody binding. Anti-human mouse ER (1:200) or monoclonal PR antibody (1:1000, Dako, Shanghai, China) was added at room temperature for 1 hour, washed with phosphate-buffered saline (PH 7.2), and incubated for 30 minutes using biotinylated anti-mouse IgG (Dako). The antigen–antibody complexes were visualized using 3,3-diaminobenzidine (DAB) and counterstained with hematoxylin. Positive ER or PR cells were numbered serially around the tumor tissue. Ovarian cancer cells that undergo a nuclear reaction is a good sign, and positive staining for ER and PR are associated with good prognosis.

Immunohistochemistry scoring

Immunohistochemistry was used for staining. Sections from tissues were used for immunohistochemical staining according to a standard method as described previously.[9] Briefly, each 4-μm tissue section was deparaffinized with xylene and rehydrated. After rehydration, the sections were autoclaved in 10 m*M* citrate buffer (pH 6.0) at 120°C for 2.5 minutes for antigen retrieval, cooled to 30°C, and washed with phosphate-buffered saline (PBS, pH 7.3). After endogenous peroxidase had been quenched with aqueous 3% H_2O_2 for 10 minutes and washed with PBS, the sections were incubated at 4°C overnight with CUEDC2 antibody (1:200 dilution; Abcam, Cambridge, UK) in antibody diluent solution and then washed with PBS. Next, the sections were incubated with secondary antibody (Fuzhou Maixin Biotechnology Development Co., Fujian, China) for 30 minutes at room temperature. Color development was performed with Polink-2 HRP DAB detection kit. Nuclei were lightly counterstained with hematoxylin and the sections were observed under the microscope. The scoring method was as follows. At 4× magnification, the total area of the tumor tissue and the total area of the normal tissue under a whole section were evaluated. The grading system was adopted from a previous study.[9] Briefly, staining intensity was divided into

4 scores: brown = 3, clay black = 2, yellow- = 1, 0 = no coloring. Then the total score was calculated as follows: score 3 × percentage of corresponding tumor area + score 2 × percentage of corresponding tumor area + score 1 × percentage of corresponding tumor area + score 0 × percentage of the corresponding tumor area. Tissues were independently scored by two pathologists to avoid bias error.

Statistical analysis

The statistical software SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A P-value <0.05 was considered significant. One-way ANOVA (Kruskal–Wallis test) was used to determine differences between FIGO stages. We defined DFS as the period from the date of diagnosis to the first (local or distant) recurrence, and OS as the time from the date of diagnosis to the death of the patient from serous ovarian carcinoma. At the last follow-up date, patients who remained living were censored and patients who suffered from factors other than ovarian serous carcinoma were censored at death. Survival curves were developed using the Kaplan–Meier product-limit form, and the log-rank test was used to compare them.

Results

General data

There were 75 patients in the primary ovarian cancer group (age 52.7 ± 11.1 years) and 34 patients in the control group (age 50.9 ± 11.4 years); the two groups did not differ in age. No patients received adjuvant treatment before surgery, and the surgical methods were tumor cell reduction surgery or staging surgery for ovarian cancer. After surgery, the diagnosis of ovarian serous cystadenocarcinoma was confirmed by pathology. Of these, 61 cases were poorly differentiated, 10 cases were moderately differentiated, and 4 cases were well differentiated. Fifteen cases were FIGO stage I, 11 cases were stage II, 42 cases were stage III, and 8 cases were stage IV. Next, we measured the amount of cancer antigen 125 protein (CA-125), which is an important biomarker for the detection, follow-up, and staging of ovarian cancer.[12] Serum levels of CA-125 are shown in Table 1. No differences in serum CA-125 were found between groups.

Table 1. Serum CA-125 levels in ovarian cancer tissues and corresponding control tissues.

	Serum CA-125 (U/mL)	
Stage	Ovarian cancer	Control
I	183.1	109.7
II	407.9	514.8
III	1160.9	1124.7
IV	988.0	405.3
Low differentiation	814.4	853.4
Middle differentiation	1189.8	637.7
High differentiation	596.1	596.1

Expression of CUEDC2 in serous ovarian cancer tissues

CUEDC2 was scored in serous ovarian cancer tissues after immunohistochemical staining. First, we compared the two groups: the case group comprised primary serous ovarian cancer tissues, and the control group comprised ovarian tissues that were not invaded by tumor. There were 75 cases in the case group, with a CUEDC2 score of 1.69 ± 0.67, and 34 cases in the control group, with a score of 0.99 ± 0.44. The score in the ovarian cancer group was significantly higher than that of the control group ($P = 0.0005$), as shown in Figure 1a.

Second, we assessed CUEDC2 scores within FIGO groups. The intra-group comparison results were as follows: CUEDC2

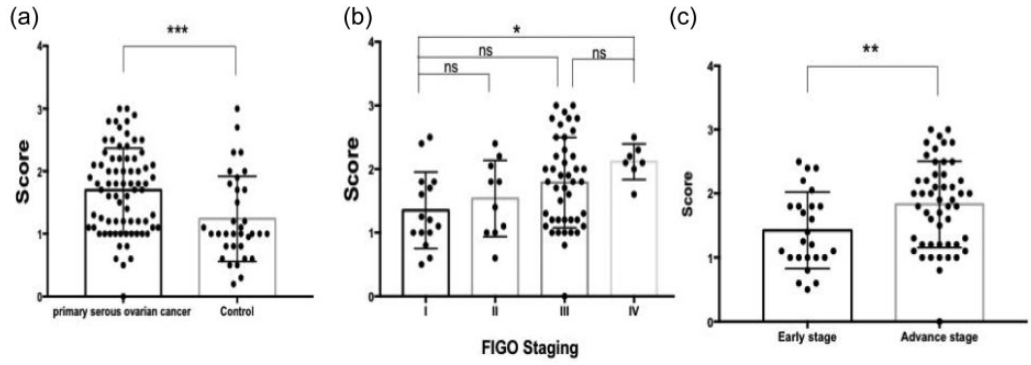

Figure 1. (a) Scores of CUE domain containing 2 (CUEDC2) protein expression in the ovarian cancer group and control group (noncancerous ovarian tissues). The scores in the ovarian cancer group were significantly higher than those of the control group ($P = 0.0005$). (b) Scores of CUEDC2 expression in primary serous cystadenocarcinoma at different International Federation of Gynecology and Obstetrics (FIGO) stages. One-way ANOVA (Kruskal–Wallis test) was used to determine differences between FIGO stages. The only significant comparison was between tissues of FIGO stage I and stage IV ($P = 0.0386$); all others were nonsignificant (ns). (c) Stage I and II tissues were then combined into an early-stage group, and stage III and IV into an advanced-stage group. Scores of CUEDC2 expression were significantly lower in the early-stage group than in the advanced-stage group ($P = 0.0098$). The mean 95% confidence interval (CI) was 1.695 in the ovarian cancer group and 1.24 in the control group. All data are shown as mean ± SEM, with individual case tissues shown as a single dot.

Table 2. Expression score of CUE domain containing 2 protein in primary serous cystadenocarcinoma.

	FIGO staging				Pathological grading		
	I	II	III	IV	Low differentiation	Middle differentiation	High differentiation
No. of cases	15	12	42	7	61	10	4
Score	1.35 ± 0.60	1.54 ± 0.57	1.78 ± 0.71	2.13 ± 0.27	1.66 ± 0.66	1.78 ± 0.79	1.87 ± 0.78

FIGO International Federation of Gynecology and Obstetrics.

score 1.35 ± 0.60, 1.54 ± 0.57, 1.78 ± 0.71, and 2.13 ± 0.27 in FIGO stage I, II, III, and IV, respectively. One-way ANOVA (Kruskal–Wallis test) was used to determine the significance of the group comparisons. The only significant comparison was between tissues of FIGO stage I and stage IV ($P = 0.0386$) (Figure 1b). According to these results, CUEDC2 score increased with increasing ovarian cancer stage (Table 2, Figure 2). We then combined stages I and II into an early ovarian cancer group, and stages III and IV into an advanced ovarian cancer group, and found that the score for the early stage group was significantly lower than that of the advanced group ($P = 0.0098$) (Figure 1c and Table 3). Thus, CUEDC2 expression increased with advancing cancer stage.

We compared CUEDC2 score across pathological grades of primary serous ovarian cancer (61 poorly differentiated cases, 10 moderately differentiated cases, and 4 well-differentiated cases. The CUEDC2

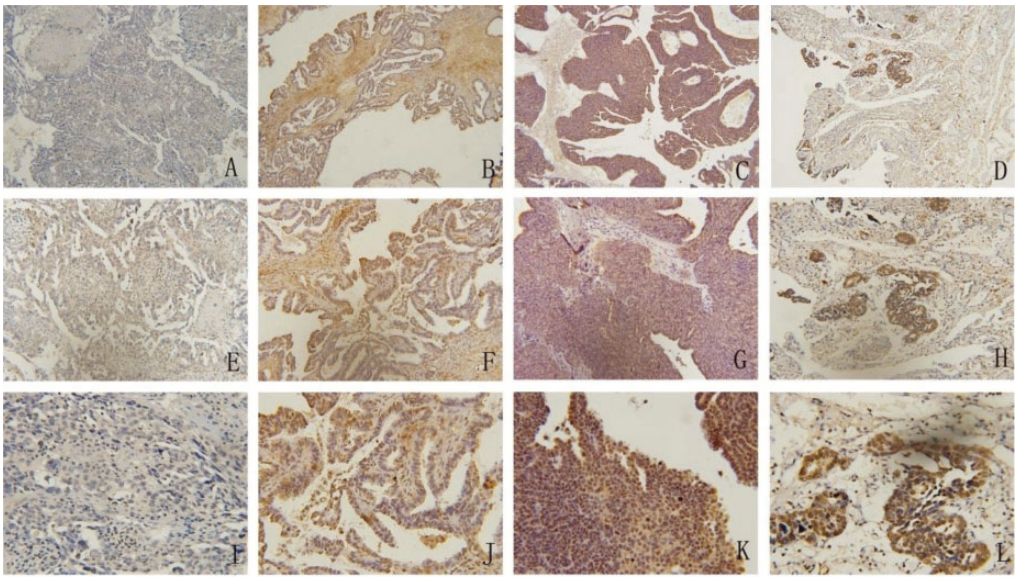

Figure 2. Expression of CUE domain containing 2 (CUEDC2) protein in primary ovarian serous cystadenocarcinoma tissues at 100× magnification (a–d), 200× magnification (e–h), and 400× magnification (i–l). Panels a, e, and i show staining of stage I tissues; panels b, f, and j show staining of stage II tissues; panels c, g, and k show staining of stage III issues; and panels d, h, and l show staining of stage IV tissues. CUEDC2 expression score increased with increasing ovarian cancer stage.

Table 3. Expression score of CUE domain containing 2 protein in early- and advanced-stage tissues of primary ovarian serous cystadenocarcinoma.

	Early stage	Advanced stage
No. of cases	26	49
Score	1.43 ± 0.58	1.83 ± 0.68

The early-stage score was significantly lower than the late-stage score ($P = 0.0098$).

scores were 1.66 ± 0.66, 1.78 ± 0.79, and 1.87 ± 0.78, respectively, and did not differ significantly.

Expression of CUEDC2, ER, and PR

Expression of ER is significantly higher in ovarian serous carcinoma than in benign serous ovarian tumors.[13] We evaluated the expression of ER and PR in 53 primary serous ovarian cancer tissues with positive and negative CUEDC2 expression (CUEDC2 scores ≤ 1 were defined as negative, and those with >1 as positive) (Table 4). We used rank-related statistical methods for data analysis. First, we analyzed CUEDC2 expression in ER-positive (38) and ER-negative (29) tissues ($r = 0.2982$). Next, we analyzed CUEDC2 expression in PR-positive (28) and PR-negative (39) tissues ($r = 0.2917$). Neither correlation was significant, indicating that

Table 4. Expression of ER and PR in primary serous ovarian cancer tissues with positive and negative expression of CUEDC2.

	ER		PR	
CUEDC2	Negative	Positive	Negative	Positive
Positive	5	9	6	8
Negative	24	29	22	31
Total	29	38	28	39

ER, estrogen receptor; PR, progesterone receptor; CUEDC2, CUE domain containing 2 protein.

expression of CUEDC2 was not significantly correlated with expression of ER or PR.

Expression of CUEDC2 and clinicopathological features

The chi-square test was used to compare relationships between expression of *CUEDC2* and age, pathological grade, and the presence or absence of lymph node metastasis of ovarian cancer. Expression of CUEDC2 has been highly correlated in various histopathological classification subgroups. Expression of CUEDC2 did not differ for samples in the medium-high pathological grade subgroup (78.57%) compared with those in the low-grade subgroup (78.69%). More CUEDC2-positive samples were found in patients with advanced FIGO stages (85.71%) than in patients with early FIGO stages (57.69%) ($P = 0.01$). Positive expression of CUEDC2 was numerically but not significantly greater in cases with lymph node metastasis (83.33%) than in those without lymph node metastasis (75.51%), as shown in Table 5.

Expression of CUEDC2 and prognosis

We followed up patients to calculate DFS and OS. Three patients were lost to follow-up, for a dropout rate of 4.05%. The median DFS of CUEDC2-positive patients was 32.6 months and the median OS was 54.3 months, whereas the median DFS of CUEDC2-negative patients was 51.9 months and the median OS was 63.5 months. Positive expression of CUEDC2 was not correlated with OS, but was correlated with DFS ($P < 0.05$) (Figures 3 to 5).

Discussion

The *CUEDC2* gene is located on chromosome 10 at 10q24.32; it has a total length of 9.42 kb and encodes 287 amino acids, including one CUE domain. CUE is a moderately conserved structure of about 40 amino acids, and it is found in a variety of eukaryotic cell proteins. The *CUEDC2* gene encodes a protein whose function is not yet clear. The abnormally high expression of CUEDC2 proteins in many tumors, such as hepatocellular carcinoma and cholangiocarcinoma,[14] leads to premature

Table 5. Correlation between positive expression of CUE domain containing 2 protein and clinical indicators.

		CUEDC2 positive			
Clinico-pathological features	Total no. of cases	No. of cases	Percentage	χ^2 value	*P*-value
Age (years)					
≤50	30	21	70.00%	2.24	0.13
>50	45	38	84.44%		
Pathological grade					
Medium-high	14	11	78.57%	0.12	0.72
Low	61	48	78.69%		
FIGO stage					
I–II	26	15	57.69%	5.86	0.01
III–IV	49	42	85.71%		
Lymph node metastasis					
Absent	49	37	75.51%	0.12	0.73
Present	18	15	83.33%		

FIGO, International Federation of Gynecology and Obstetrics.

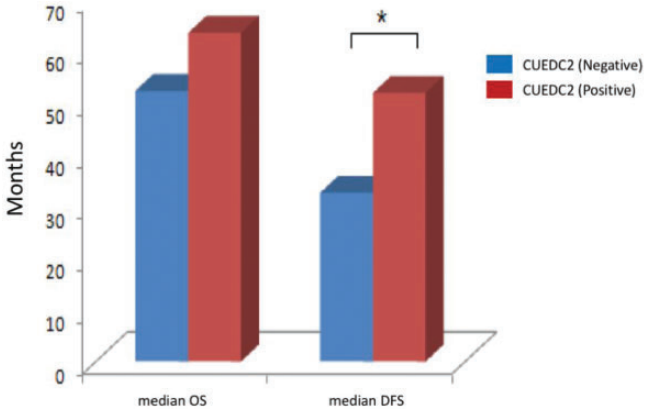

Figure 3. Median overall survival (OS) and median disease-free survival (DFS) in CUE domain containing 2 (CUEDC2)-negative and CUEDC2-positive groups. Three cases were excluded because they were lost to follow-up.

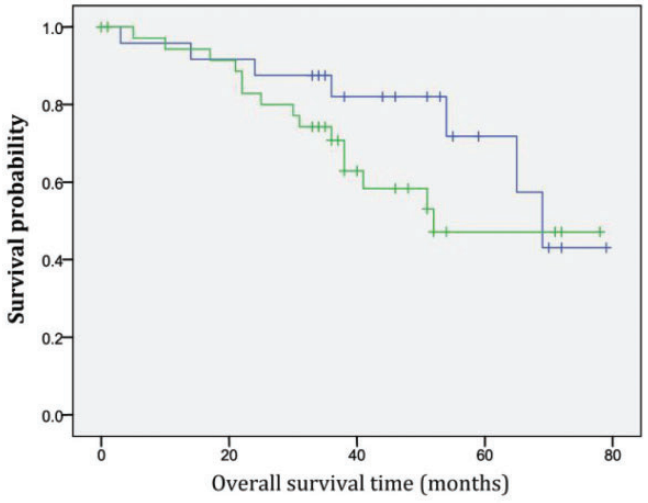

Figure 4. Correlation between CUE domain containing 2 (CUEDC2) protein and overall survival (OS), where blue represents the CUEDC2-negative group and green represents the CUEDC2-positive group. For comparison of the two groups: 95% confidence interval: 0.261–1.113; $P > 0.05$.

closure of spindle checkpoints and premature activation of APC/C, which in turn results in polyploid genomic instability and tumor formation.

Through the analysis of biological information, it was found that CUEDC2 protein could combine with ubiquitin, which allowed identification of monoubiquitin and polyubiquitin. Zhang et al. found that CUEDC2 and PR could interact both in vivo and in vitro, in an immunoprecipitation experiment of glutathione-S-transferase (GST). Through a ubiquitination experiment, the same researchers found that CUEDC2 may promote progesterone-induced PR degradation through the ubiquitin-proteasome pathway and inhibit the growth of breast

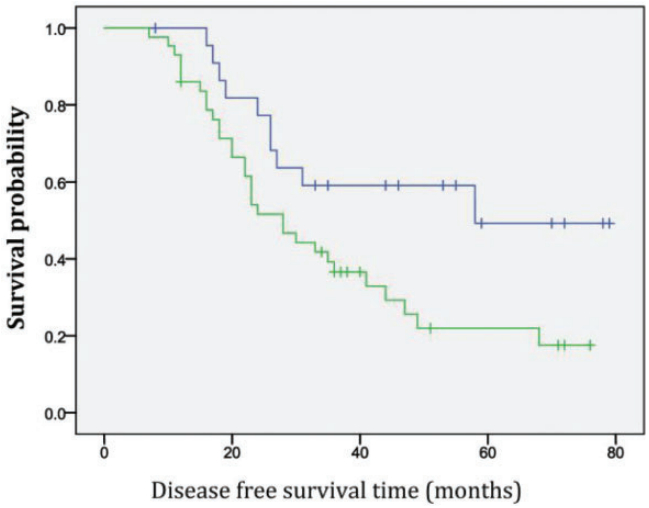

Figure 5. Correlation between CUE domain containing 2 (CUEDC2) protein and disease-free survival (DFS), where blue represents the CUEDC2-negative group and green represents the CUEDC2-positive group. For comparison of the two groups: 95% confidence interval: 2.4–3.3; $P < 0.05$.

cancer cells.[15] In 2011, another study revealed that CUEDC2 can downregulate the expression of ER-α, causing chemotherapy resistance. CUEDC2 protein expression was assessed in 228 tissue sections of breast cancer patients and showed that high expression of CUEDC2 protein could inhibit the response of cancer cells to tamoxifen. Therefore, *CUEDC2* may be a new drug resistance gene for breast cancer.[8] Most breast cancers are ER-positive malignant tumors, and the rate of positive expression of ER is significantly higher in ovarian serous cancer than in benign serous ovarian cancer. Therefore, in this study, we detected and evaluated the expression of CUEDC2 in serous ovarian cancer.

Through statistical analysis, we found that expression of CUEDC2 was increased in primary serous ovarian cancer tissues, and that expression increased in advanced stages of disease; expression in early-stage cancer tissues was significantly lower than that in advanced-stage tissues. Therefore, we believe that CUEDC2 is related to the occurrence and progression of ovarian cancer. In addition, we found that patients with low expression of CUEDC2 had longer DFS, suggesting that expression of this gene is correlated with tumor prognosis. According to epidemiological investigations, the etiology, pathogenesis, and progression of ovarian cancer are largely dependent on estrogen activity.[16,17] From the perspective of its pathogenesis, although CUEDC2 may promote progesterone-induced PR degradation and inhibit the growth of cancer cells through the ubiquitin-proteasome pathway, our results suggest that expression of CUEDC2 is not correlated with expression of ER and PR.

The findings of this study may be limited by the fact that this was a single-center study, and selection bias may have occurred. Evaluation of multicenter sources of tissue with a greater number of samples may yield more reliable results. The basic function and mechanism of CUEDC2 in penetrating and metastasizing ovarian cancer need to be elucidated. Improving our understanding of the processes of

invasion and metastasis in ovarian cancer has important therapeutic significance to enhance the prognosis of patients with ovarian cancer. Considering the different pathogenesis of cancers, whether CUEDC2 is a new target for the treatment of ovarian cancer requires further study.

Conclusions

The current study represents a pilot assessment but our results show that CUEDC2 may be highly expressed in ovarian cancer tissues and is related to tumor stage and DFS. High expression was observed in tissues from patients with advanced stages of serous ovarian cancer, and expression of CUEDC2 was positively correlated with DFS but not OS. Further studies are needed to validate CUEDC2 as a possible target for the treatment of ovarian cancer.

Declaration of conflicting interest

The authors declare that there is no conflict of interest

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References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: E359–E386.
2. Arik D and Kulacoglu S. p53, bcl-2, and nm23 expressions in serous ovarian tumors: correlation with the clinical and histopathological parameters. *Turk Patoloji Derg* 2011; 27: 38–45.
3. Sung PL, Chang YH, Chao KC, et al. Global distribution pattern of histological subtypes of epithelial ovarian cancer: a database analysis and systematic review. *Gynecol Oncol* 2014; 133: 147–154.
4. Tognon G, Carnazza M, Ragnoli M, et al. Prognostic factors in early-stage ovarian cancer. *Ecancermedicalscience* 2013; 7: 325.
5. Su Z, Graybill WS and Zhu Y. Detection and monitoring of ovarian cancer. *Clin Chim Acta* 2013; 415: 341–345.
6. Simon AE, Wardle J, Grimmett C, et al. Ovarian and cervical cancer awareness: development of two validated measurement tools. *J Fam Plann Reprod Health Care* 2012; 38: 167–174.
7. Liu XH, Man YN and Wu XZ. Recurrence season impacts the survival of epithelial ovarian cancer patients. *Asian Pac J Cancer Prev* 2014; 15: 1627–1632.
8. Pan X, Zhou T, Tai YH, et al. Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer. *Nat Med* 2011; 17: 708–714.
9. Wang A, Guo C, Sun Y, et al. Overexpression of CUEDC2 predicts poor prognosis in ovarian serous carcinomas. *J Cancer* 2015; 6: 542–547.
10. Anderson KS, Wong J, Vitonis A, et al. p53 autoantibodies as potential detection and prognostic biomarkers in serous ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 859–868. DOI: 10.1158/1055-9965.EPI-09-0880
11. Shen F, Zhang X, Zhang Y, et al. Hormone receptors expression in ovarian cancer taking into account menopausal status: a retrospective study in Chinese population. *Oncotarget* 2017; 8: 84019–84027. DOI: 10.18632/oncotarget.20251
12. Schilthuis MS, Aalders JG, Bouma J, et al. Serum CA 125 levels in epithelial ovarian cancer: relation with findings at second-look operations and their role in the detection of tumour recurrence. *BJOG* 1987; 94: 202–207.
13. Kanaya N, Somlo G, Wu J, et al. Characterization of patient-derived tumor

xenografts (PDXs) as models for estrogen receptor positive (ER+ HER2− and ER+ HER2+) breast cancers. *J Steroid Biochem Mol Biol* 2017; 170: 65–74.
14. Xiao J, Leng A, Zhang Y, et al. CUEDC2: multifunctional roles in carcinogenesis. *Front Biosci (Landmark Ed)* 2019; 24: 935–946.
15. Zhang PJ, Zhao J, Li HY, et al. CUE domain containing 2 regulates degradation of progesterone receptor by ubiquitin–proteasome. *EMBO J* 2007; 26: 1831–1842.
16. Matsuo K, Sheridan TB, Mabuchi S, et al. Estrogen receptor expression and increased risk of lymphovascular space invasion in high-grade serous ovarian carcinoma. *Gynecol Oncol* 2014; 133: 473–479.
17. Gourley C. Hormone receptors and ovarian cancer survival. *Lancet Oncol* 2013; 9: 794–795.