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Original contribution

Chromosome 20q13.2 *ZNF217* locus amplification correlates with decreased E-cadherin expression in ovarian clear cell carcinoma with PI3K-Akt pathway alterations☆

Hsien-Neng Huang MD[a,b,1], Wen-Chih Huang MD[c,1], Ching-Hung Lin MD[d], Ying-Cheng Chiang MD[e], Hsin-Ying Huang PhD[f], Kuan-Ting Kuo MD[a,g,*]

[a]*Graduate Institute of Pathology, College of Medicine, National Taiwan University, Taipei, Taiwan, 10002*
[b]*Department of Pathology, National Taiwan University Hospital Hsin-Chu Branch, Hsinchu, Taiwan, 30059*
[c]*Department of Anatomic Pathology, Far Eastern Memorial Hospital, Taipei, Taiwan, 22060*
[d]*Department of Oncology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, 10002*
[e]*Department of Obstetrics and Gynecology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, 10002*
[f]*Department of Mathematics Education, National Chengchi University, Taipei, Taiwan, 11605*
[g]*Department of Pathology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan, 10002*

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Summary This study aims to evaluate the relationships between chromosome 20q13.2 *zinc finger protein 217* (*ZNF217*) locus amplification, ZNF217 expression, E-cadherin expression, and PI3K-Akt pathway alterations (activating *PIK3CA* mutations or loss of phosphatase and tensin homolog [PTEN] expression), and whether these molecular alterations can predict the clinical survival data in ovarian clear cell carcinoma (OCCC) patients. Samples and clinical data of 72 OCCC patients were collected. Chromosome 20q13.2 *ZNF217* locus amplification was detected by fluorescence *in situ* hybridization. ZNF217, E-cadherin and PTEN expression were assessed using immunohistochemical stain. *PIK3CA* mutation was identified by PCR-amplified gene sequencing. Cox proportional hazard regression model was used to estimate the adjusted hazard ratios of survival. Chromosome 20q13.2 *ZNF217* locus amplification was detected in 31% (22/72) of cases, and ZNF217 expression was increased in 40% (27/68) of cases. E-cadherin and PTEN expressions were decreased or lost in 44% (32/72) and 14% (10/72) of cases, respectively. Activating *PIK3CA* mutations were present in 35% (25/72) of cases. Thirty-three OCCC patients (46%) showed activating PI3K-Akt pathway alterations. Chromosome 20q13.2 *ZNF217* locus amplification was significantly associated with decreased E-cadherin expression ($P = .001$). In contrast, ZNF217 expression was not related to *ZNF217* amplification or E-cadherin expression. In OCCC patients with activating PI3k-Akt pathway, decreased E-cadherin expression ($P = .033$) and

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* Corresponding author. 3rd floor, No. 7, Chung Shan South Road, Taipei, Taiwan 10001.
[1] Hsien-Neng Huang and Wen-Chih Huang contributed equally to this article.
E-mail addresses: pathologykimo@gmail.com (K.-T. Kuo).

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advanced Federation of Gynecology and Obstetrics stage ($P = .014$) predicted shorter overall survival. Two conclusions were raised in our study. First, *ZNF217* plays a role in down-regulating E-cadherin expression and is a potential therapeutic target for OCCC patients. Second, E-cadherin expression is a prognostic marker for OCCC patients with activating PI3K-Akt pathway.

1. Introduction

Ovarian cancer is the sixth most commonly diagnosed cancer in women worldwide [1]. Compared to the most prevalent serous adenocarcinomas, ovarian clear cell carcinomas (OCCCs) are more likely to be diagnosed at a younger age, in Asians, and have a worse prognosis [2]. Despite treatment, including systematic debulking surgery and chemotherapy, the prognosis of advanced-stage OCCC patients is poorer than patients with serous adenocarcinoma due to the significantly less favorable response to platinum-based first-line chemotherapy [3]. The identification of prognostic factors is critical to predicting the clinical course and determining the treatment strategies.

In recent reports, increased DNA copy number of chromosome 20q13.2 locus was identified as the most common copy number alteration in OCCCs, accounting for 36% of clinical cases [4]. Chromosome 20q13.2 locus contains *teashirt zinc finger homeobox 2* (*TSHZ2*), *BCAS1* (*breast carcinoma–amplified sequence 1*), and *zinc finger protein 217* (*ZNF217*), etc. [4]. Among these genes, *ZNF217* was identified as the most important gene for OCCCs [4]. *ZNF217* is a candidate oncogene first described in breast tumors in 1998 [5]. According to functional studies of *ZNF217*, aberrant expression of ZNF217 can cause cellular immortalization, telomerase repression, antiapoptosis, and increased metastatic potential [6,7]. *ZNF217* amplification was also reported to be crucial to cell growth and survival of OCCCs [8].

CDH1 gene is located at chromosome 16q22.1. Its product, epithelial cadherin (E-cadherin), is one of the cadherin family of calcium-dependent adhesion molecules [9]. E-cadherin is a critical component of cellular adhesion junctions for normal epithelial formation and integrity. It plays a crucial role in Wnt/β-catenin signalling pathway during epithelial-mesenchymal transition [10]. Decreased expression of E-cadherin is related to poor prognosis in colorectal and lung cancer [11,12]. Decreased E-cadherin expression has been reported as a poor prognostic factor of ovarian serous adenocarcinoma [13], but there were limited data about the prognostic significance of E-cadherin expression in OCCCs. To date, only one report demonstrated that E-cadherin was a useful prognostic marker in advanced (stage IIc-IV) OCCC patients undergoing chemotherapy [14].

In breast cancer cell line MCF7, ZNF217 directly binds to the *CDH1* gene promoter and represses E-cadherin expression [15]. Except for E-cadherin, another important molecule in the Wnt/β-catenin signalling pathway is β-catenin which is encoded by the *CTNNB1* gene. In OCCCs, the sequence mutation rate of the *CTNNB1* gene is only 3% [16]. To our knowledge, the relationship between *ZNF217* and E-cadherin in OCCCs has not been studied. Therefore, to elucidate the function of *ZNF217*, we investigated the relationship between *ZNF217* amplification and E-cadherin expression, and their prognostic effect for OCCC patients.

Somatic mutation of the *phosphoinositide-3-kinase catalytic subunit alpha* (*PIK3CA*) gene was reported as a common molecular genetic change in OCCCs [16]. Phosphatidylinositol 3-kinase-protein kinase B (PI3K-Akt) pathway activation can result from *PIK3CA* mutations, *phosphatase and tensin homolog* (*PTEN*) mutations or a combination of these alterations [17]. The above-mentioned study by Cowger et al was based upon the breast cancer cell line MCF7, which harbored *PIK3CA* mutation [15]. Therefore, we also investigated the relationships between the molecular alterations in OCCC patients with activating PI3K-Akt pathway (activating *PIK3CA* mutations or loss of PTEN expression).

2. Material and methods

2.1. Patients and tissue samples

Formalin-fixed, paraffin-embedded tissue samples of 72 cases of ovarian clear cell carcinoma dating from 1995 to 2010

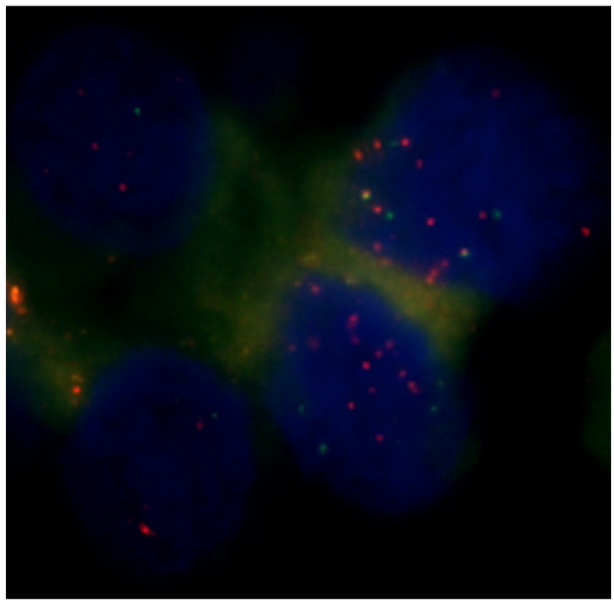

Fig. 1 Dual-color FISH chromosome 20q13.2 amplification in cancer cells of ovarian clear cell carcinoma: three or more red signals at the amplified chromosome 20q13.2 region and two green signals at the chromosome 20q11 control region.

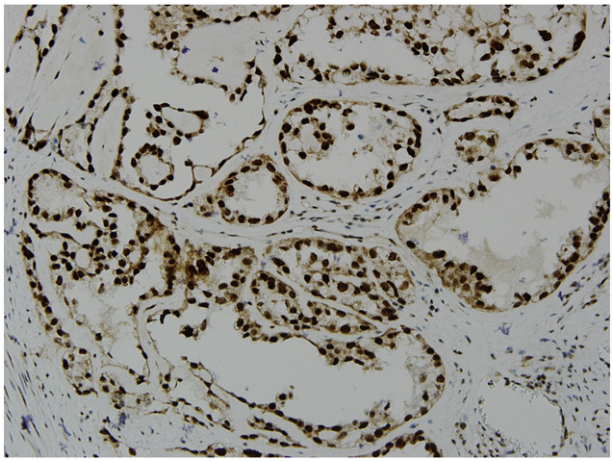

Fig. 2 Immunohistochemical stains of ZNF217 performed in an ovarian clear cell carcinoma (original magnification ×200).

were obtained from the archives of the Department of Pathology at the National Taiwan University Hospital. The sections stained with hematoxylin and eosin were reviewed and diagnosed based upon the World Health Organization classification [18]. All patients were treated with debulking surgery. Sixty-eight patients in the present study were recruited from our previous study [19]. This study was approved by the institutional review board of the National Taiwan University Hospital.

2.2. Fluorescence in situ hybridization

All cases were studied by fluorescence in situ hybridization (FISH) on paraffin tissue sections for the evaluation of chromosome 20q13.2 *ZNF217* locus amplification. The commercial probes (Kreatech, Amsterdam, The Netherlands) were used. The FISH probe used in our study detected the amplification region including *ZNF217* and *TSHZ2* genes. Hybridization signals were enumerated among one hundred tumor cells. The criterion for chromosome 20q13.2 amplification was an amplification ratio of ≥1.5 (Fig. 1).

2.3. DNA extraction and mutation analysis of the *PIK3CA* gene

DNA extraction, polymerase chain reaction amplification for *PIK3CA* gene sequencing, and mutation analysis were performed in all samples using approaches previously described [16,20].

2.4. Immunohistochemical stain

Immunohistochemical (IHC) stain of paraffin tissue sections was performed by the Leica BOND-III autostainer (Leica Microsystems, Buffalo Grove, IL). Commercially available anti-ZNF217 antibody (clone HPA051857, 1:100 dilution; Sigma-Aldrich, St Louis, MO), anti–E-cadherin antibody (clone NCH-38, 1:50 dilution; Dako, Glostrup, Denmark), and anti-PTEN antibody (clone 6H2.1, 1:50 dilution; Dako, Glostrup, Denmark) were used. ZNF217 IHC failed in 4 cases due to poor fixation.

ZNF217 expression (Fig. 2) was evaluated according to the criteria as previously described [8]. The fallopian tube epithelium was positive control, and ovarian surface epithelium was negative control. Decreased expression in E-cadherin was defined as a complete membranous staining in ≤10% of all tumor cells, similar to that of the study by Ho et al. [14]. The ovarian stromal cells and fallopian tube epithelium were negative and positive controls for E-cadherin. PTEN immunoreactivity was evaluated by two-tiered systems (undetectable or positive), and vascular endothelial cells were used as an internal positive control [21].

2.5. Statistical analysis

The statistical analyses were conducted using PASW Statistics version 18.0.2 (IBM Corporation, Armonk, NY, USA). The association between chromosome 20q13.2 amplification status and other clinicopathologic parameters was

Table 1 Correlation of E-cadherin IHC and clinicopathological features

Characteristics	No.	E-cadherin IHC n (%)		*P*
		Decreased, n = 32 (44)	Positive, n = 40 (56)	
Age				.262
≤50	42	21 (66)	21 (53)	
>50	30	11 (34)	19 (48)	
FIGO stage				.007
I + II	46	15 (47)	31 (78)	
III + IV	26	17 (53)	9 (23)	
Lymph node metastasis [a]				.130
No	63	25 (83)	38 (95)	
Yes	7	5 (17)	2 (5)	
Chromosome 20q13.2				.001
Normal	50	16 (50)	34 (85)	
Amplification	22	16 (50)	6 (15)	
ZNF217 IHC [b]				.340
Negative	41	20 (67)	21 (55)	
Positive	27	10 (33)	17 (45)	
PIK3CA mutation				.121
Wild-type	47	24 (75)	23 (58)	
Mutated	25	8 (25)	17 (43)	
PTEN IHC				.019
Negative	10	8 (25)	2 (5)	
Positive	62	24 (75)	38 (95)	
Activating PI3K-Akt pathway				.874
Absent	39	17 (53)	22 (55)	
Present	33	15 (47)	18 (45)	

Abbreviation: IHC, immunohistochemical.
[a] Not assessed in 2 cases.
[b] Not assessed in 4 cases.

Table 2 Clinicopathological features in OCCC patients with or without activating PI3K-Akt pathway

Characteristics	No.	Activating PI3K-Akt pathway n (%)		*P*
		Present, n = 33 (46)	Absent, n = 39 (54)	
Age				.072
≤50	42	23 (70)	19 (49)	
>50	30	10 (30)	20 (51)	
FIGO stage				.967
I + II	46	21 (64)	25 (64)	
III + IV	26	12 (36)	14 (36)	
Lymph node metastasis [a]				.695
No	63	28 (88)	35 (92)	
Yes	7	4 (13)	3 (8)	
Chromosome 20q13.2 amplification				.578
No	50	24 (73)	26 (67)	
Yes	22	9 (27)	13 (33)	
ZNF217 IHC [b]				.649
Negative	41	19 (58)	22 (63)	
Positive	27	11 (42)	16 (37)	
E-cadherin IHC				.874
Decreased	32	15 (45)	17 (44)	
Positive	40	18 (55)	22 (56)	

Abbreviations: OCCC, ovarian clear cell carcinoma; IHC, immunohistochemical.
[a] Not assessed in 2 cases.
[b] Not assessed in 4 cases.

evaluated using the χ^2 test or Fisher's exact test. The influences of *ZNF217* amplification and other clinicopathological parameters on progression-free survival (PFS) and overall survival (OS) were analyzed by Cox proportional hazard model, and *P* values from Wald statistic were recorded. The Kaplan-Meier survival analysis with log-rank significance test was also used to estimate the probabilities of survival between OCCC patients with or without E-cadherin expression. The cutoff of significance level was .05. All of the *P* values were 2 sided.

3. Results

The mean age of patients was 48.9 years. About one-third (36%, 26/72) of the patients were at International Federation of Gynecology and Obstetrics (FIGO) stage III or IV. In addition to debulking surgery, most of them (89%, 64/72) also received chemotherapy. The median follow-up duration was 39 months (1-164 months).

Chromosome 20q13.2 amplification was present in 31% (22/72) of cases, and ZNF217 expression was increased in 40% (27/68) of cases. In immunostains, E-cadherin immunoreactivity was decreased in 44% (32/72) of all cases, and PTEN was negative in 14% (10/72) of all cases. Chromosome 20q13.2 amplification was significantly correlated with decreased E-cadherin expression ($P = .001$), but not related to age ($P = .100$), FIGO stage ($P = .104$), lymph node metastasis ($P = .097$), ZNF217 expression ($P = .210$), PTEN expression ($P = 1.000$), *PIK3CA* mutation ($P = .378$), or activating PI3K-Akt pathway ($P = .578$). Correlation between E-cadherin expression and clinicopathological features are listed in Table 1. In addition to chromosome 20q13.2 amplification, advanced FIGO stage ($P = .007$) and loss of PTEN expression ($P = .019$) were also related to decreased E-cadherin expression. ZNF217 expression was not associated with chromosome 20q13.2 amplification or E-cadherin expression.

PIK3CA mutation was present in 35% (25/72) of all OCCC patients. PI3K-Akt pathway alteration was present in 46% (33/72) of all OCCC patients. Clinicopathological features including age, FIGO stage, lymph node metastasis, ZNF217 expression, E-cadherin expression and chromosome 20q13.2 amplifications were not statistically different

Table 3 Correlation of chromosome 20q13.2 amplification and clinicopathological features in OCCC patients with or without activating PI3K-Akt pathway

Characteristics	OCCC patients with activating PI3K-Akt pathway			OCCC patients without activating PI3K-Akt pathway		
	Chromosome 20q13.2, normal, n = 24	Chromosome 20q13.2, amplification, n = 9	*P*	Chromosome 20q13.2, normal, n = 26	Chromosome 20q13.2, amplification, n = 13	*P*
Age >50	8/24 (33%)	2/9 (22%)	.536	16/26 (62%)	4/13 (31%)	.070
Advanced FIGO stage (III + IV)	7/24 (29%)	5/9 (56%)	.230	8/26 (31%)	6/13 (46%)	.345
Positive lymph node metastasis [a]	2/24 (8%)	2/8 (25%)	.254	1/26 (4%)	2/12 (17%)	.229
Positive ZNF217 IHC [b]	9/22 (41%)	2/8 (25%)	.672	12/25 (48%)	4/13 (31%)	.307
Decreased E-cadherin IHC	7/24 (29%)	8/9 (89%)	.004	9/26 (35%)	8/13 (62%)	.110

Abbreviations: OCCC, ovarian clear cell carcinoma; IHC, immunohistochemical.
[a] Not assessed in 2 cases.
[b] Not assessed in 4 cases.

Table 4 Univariate and multivariate analysis of risk factors for progression-free survival and overall survival in OCCC patients

	No.	Progression-free survival, HR (95% CI)[a]	*P*	Overall survival, HR (95% CI)	*P*
Univariate analysis in OCCC patients (n = 72)					
Age			.622		.453
≤50	42	1.0		1.0	
>50	30	1.20 (0.58-2.46)		1.33 (0.64-2.76)	
FIGO stage			<.001		<.001
I + II	46	1.0		1.0	
III + IV	26	4.82 (2.26-10.31)		4.65 (2.16-9.99)	
Lymph node metastasis [a]			.001		.001
No	63	1.0		1.0	
Yes	7	6.09 (2.13-17.45)		5.04 (1.86-13.65)	
Chromosome 20q13.2 amplification			.756		.832
No	50	1.0		1.0	
Yes	22	0.88 (0.39-1.98)		0.92 (0.41-2.07)	
ZNF217 IHC [b]			.376		.306
Negative	41	1.0		1.0	
Positive	27	0.70 (0.31-1.55)		0.66 (0.30-1.47)	
E-cadherin IHC			.045		.024
Decreased	32	1.0		1.0	
Positive	40	0.47 (0.23-0.99)		0.42 (0.20-0.89)	
PIK3CA mutation			.177		.261
Wild-type	47	1.0		1.0	
Mutated	25	0.57 (0.25-1.29)		0.63 (0.28-1.41)	
PTEN IHC			.103		.067
Negative	10	1.0		1.0	
Positive	62	0.47 (0.19-1.17)		0.43 (0.17-1.06)	
Activating PI3K-Akt pathway			.648		.810
Absent	39	1.0		1.0	
Present	33	0.85 (0.41-1.74)		0.92 (0.44-1.89)	
Multivariate analysis in OCCC patients (n = 72)					
FIGO stage			.005		.011
I + II	46	1.0		1.0	
III + IV	26	3.45 (1.46-8.13)		3.15 (1.30-7.66)	
Lymph node metastasis [a]			.110		.172
No	63	1.0		1.0	
Yes	7	2.51 (0.81-7.74)		2.13 (0.72-6.35)	
E-cadherin IHC			.387		.384
Decreased	32	1.0		1.0	
Positive	40	0.71 (0.32-1.56)		0.70 (0.31-1.58)	
Univariate analysis in OCCC patients with activating PI3K-Akt pathway (n = 33)					
Age			.549		.483
≤50	23	1.0		1.0	
>50	10	1.41 (0.46-4.31)		1.53 (0.47-5.01)	
FIGO stage			<.001		.001
I + II	21	1.0		1.0	
III + IV	12	11.95 (3.03-47.07)		9.23 (2.38-35.80)	
Lymph node metastasis [c]			.001		.001
No	28	1.0		1.0	
Yes	4	21.42 (3.31-135.01)		22.78 (3.57-145.51)	
Chromosome 20q13.2 amplification			.147		.090
No	24	1.0		1.0	
Yes	9	2.32 (0.74-7.21)		2.81 (0.85-9.25)	
ZNF217 IHC [d]			.103		.120
Negative	19	1.0		1.0	
Positive	11	0.28 (0.06-1.30)		0.30 (0.06-1.38)	

(*continued on next page*)

Table 4 (*continued*)

	No.	Progression-free survival, HR (95% CI)[a]	*P*	Overall survival, HR (95% CI)	*P*
E-cadherin IHC			.004		.007
Decreased	15	1.0		1.0	
Positive	18	0.17 (0.05-0.57)		0.12 (0.03-0.55)	
Multivariate analysis in OCCC patients with activating PI3K-Akt pathway (n = 33)					
FIGO stage			.027		.014
I + II	21	1.0		1.0	
III + IV	12	5.81 (1.22-27.55)		19.23 (1.81-204.95)	
Lymph node metastasis[c]			.121		.180
No	28	1.0		1.0	
Yes	4	3.94 (0.70-22.34)		2.94 (0.61-14.21)	
E-cadherin IHC			.056		.033
Decreased	15	1.0		1.0	
Positive	18	0.27 (0.07-1.04)		0.15 (0.03-0.86)	

Abbreviations: OCCC, ovarian clear cell carcinoma; IHC, immunohistochemical; CI, confidence interval; HR, hazard ratio.

[a] Not assessed in 2 cases.
[b] Not assessed in 4 cases.
[c] Not assessed in 1 case.
[d] Not assessed in 3 cases.

between OCCC patients with or without activating PI3K-Akt pathway (Table 2). The associations of chromosome 20q13.2 amplification and other clinicopathological factors in OCCC patients with or without activating PI3K-Akt pathway are listed in Table 3. The relationship between chromosome 20q13.2 amplification and E-cadherin expression was present in OCCC patients with activating PI3K-Akt pathway (P = .004), but absent in OCCC patients without activating PI3K-Akt pathway (P = .110).

Univariate and multivariate analyses of risk factors for progression-free and overall survival in OCCC patients are summarized in Table 4. OCCC patients with advanced FIGO stage, positive lymph node metastasis, and decreased E-cadherin expression were inclined to shorter PFS and OS in univariate analysis. FIGO stage was the only prognostic factor after multivariate analysis (P = .005 for PFS and 0.011 for OS, respectively). For OCCC patients with activating PI3K-Akt pathway, both FIGO stage and E-cadherin expression were prognostic factors for OS after multivariate analysis (P = .014 and .033, respectively). Chromosome 20q13.2 was not a statistically significant prognostic factor of OS and PFS (P = .147 and .090, respectively). Kaplan–Meier analysis also revealed that decreased E-cadherin expression predicted a shorter OS for the OCCC patients with activating PI3K-Akt pathway (P = .001) (Fig. 3A). E-cadherin expression had no prognostic impact in OCCC patients without activating PI3K-Akt pathway (Fig. 3B).

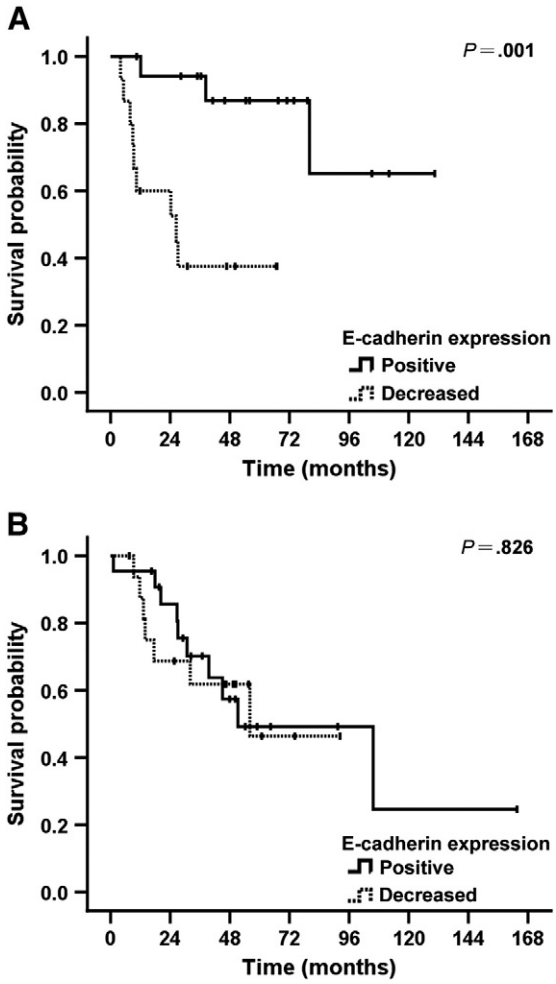

Fig. 3 Kaplan-Meier curves of OS for the OCCC patients classified by E-cadherin expression. A, E-cadherin expression in OCCC patients with activating PI3K-Akt pathway. B, E-cadherin expression in OCCC patients without activating PI3K-Akt pathway.

4. Discussion

OCCCs account for 5% to 25% of all malignant epithelial ovarian tumors [22]. Compared with the most common serous adenocarcinoma, patients with OCCC have a lower

response rate to chemotherapy and a poorer prognosis [23]. Amongst several identified molecular events, *ARID1A*, *PIK3CA*, *ZNF217*, and *TERT* promoter were recently identified as common molecular genetic changes in OCCCs [4,16,24]. *ZNF217* encodes a transcriptional repressor protein and forms the ZNF217 complex with CoREST, histone deacetylases HDAC1 and 2, and LSD-1 [25]. A profound effect of ZNF217 on cancer formation and progression were also implied in previous studies [6,7]. In several of the potential oncogenes proposed, the ZNF217 locus at chromosome 20q13.2 has been recently identified as the most commonly amplified region in OCCCs [4]. Rahman et al also indicated the adverse outcomes for OCCC patients with *ZNF217* amplification [8].

In this study, the frequency of chromosome 20q13.2 amplification in OCCCs was 31% by FISH (22/72); this lies between the results detailed in two previous reports (36% and 20%, respectively) [4,8]. *ZNF217* and *TSHZ2* genes were included in the amplification region detected by the FISH probe used in our study. However, a previous study suggested that *ZNF217* but not *TSHZ2* was the candidate oncogene [4].

The proportion of decreased E-cadherin expression in OCCC ranged from 6.25% to 63% in different studies [14,26,27]. Different criteria applied in these studies (threshold from ≤10% to complete loss) may have contributed to the significant variation. In our study, we used a cutoff of ≤10%, and 44% of all cases showed decreased expression of E-cadherin.

In our study, *ZNF217* amplification was significantly correlated to decreased expression of E-cadherin. This is the first report detailing the *ZNF217* amplification and decreased E-cadherin expression in OCCC patients. Cowger et al suggested that ZNF217 may serve as an important targeting factor for E-cadherin gene repression by directly binding to the *CDH1* gene promoter [15]. Rahman et al reported that *ZNF217* amplification was associated with lymph node metastasis in OCCCs [28]. Our results implied that profound effects of *ZNF217* amplification, including lymph node metastasis, may be achieved by down-regulating E-cadherin expression.

ZNF217 expression was not related to ZNF217 amplification in OCCCs in our study. Compared with another study by Rahman et al, the prevalences of increased ZNF217 expression in OCCCs were similar (40% *vs* 33%) [8]. However, previous studies revealed a correlation between *ZNF217* amplification and ZNF217 expression [8,29]. Different antibody and methodology may account for the discrepancies between studies. Besides, mechanisms other than gene amplification may also be involved in the ZNF217 expression in OCCCs. Whether or not ZNF217 can also regulate the E-cadherin expression by binding the *CDH1* promoter in OCCCs requires more studies to clarify.

PI3K-Akt pathway activation is a common molecular genetic change in OCCC, and can be caused by either *PIK3CA* mutations, PTEN loss or a combination of these alterations [17]. OCCC was viewed as a priority for subtype-specific clinical trials of novel therapeutic agents at a National Cancer Institute State of the Science meeting on ovarian cancer [30]. Clinical trials of PI3K-Akt pathway inhibitors in different cancers (including ovarian cancer) are also ongoing [31,32]. In this study, the relationship of *ZNF217* amplification and decreased E-cadherin expression was attributed to the OCCC patients with PI3K-Akt pathway alterations. *ZNF217* amplification was not correlated to E-cadherin expression in OCCC patients without the activating PI3K-Akt pathway. In comparison, *ZNF217* amplification may have more effect on OCCC patients with PI3K-Akt pathway alterations. ZNF217 is a therapeutic target during breast cancer progression, and triciribine was designed for patients overexpressing ZNF217 [33]. In OCCC, *ZNF217* amplification also had therapeutic implications [8]. The present study is the first report on the relationship between *ZNF217* amplification and decreased E-cadherin expression in OCCC patients with PI3K-Akt alterations. Our study implies that by down-regulating E-cadherin expression, ZNF217 inhibitors may be more effective in OCCC patients with PI3K-Akt alterations, making this a therapeutic goal in cancer treatment [34].

The prognostic impact of *ZNF217* amplification has been discussed in breast, gastric, colorectal, prostate, and ovarian serous cancers [25]. To date, only one article published in English has evaluated the prognostic effect of *ZNF217* amplification on OCCCs (60 cases) [8]. In our study, *ZNF217* amplification did not relate to shorter PFS or OS, and was not an independent prognostic factor. In contrast with the study by Rahman et al, we used different probes and a different cutoff level for amplification evaluation [8]. In advanced (stage IIc-IV) OCCC patients undergoing chemotherapy, decreased E-cadherin expression was reported as an adverse prognostic factor of OS by Ho et al [14]. Only 25 patients in our study were stage IIc to IV with chemotherapy, and the prognostic impact of E-cadherin expression was statistically significant in these patients. In this study, E-cadherin was a poor prognostic factor in the cohort with activating PI3K-Akt pathway, but not an independent prognostic factor in the whole cohort or the cohort without activating PI3K-Akt pathway. Our study provided a potential marker for evaluating the prognosis of OCCC patients that received PI3K-Akt pathway inhibitor regimens. Except for E-cadherin expression in our study, so far there has been no other validated prognostic factor for patient stratification in OCCC patients with PI3K-Akt pathway alterations.

In summary, we confirmed that chromosome 20q13.2 *ZNF217* locus amplification was a common genetic alteration in OCCCs. In the forthcoming era of PI3K-Akt pathway inhibitors, it is helpful to identify the specific mechanisms and prognostic factors in OCCC patients with PI3K-Akt pathway alterations. This study is the first report to suggest that *ZNF217* amplification is correlated to decreased E-cadherin expression, and E-cadherin expression is an adverse prognostic factor in OCCC patients with activating PI3K-Akt pathway alterations.

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