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Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations and ZNF217 amplification in ovarian clear cell carcinoma

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AT-rich interactive domain 1A (ARID1A) is a subunit of switch/sucrose non-fermentable (SWI/SNF) complex. Recently, alterations of *ARID1A* gene, phosphatidylinositol 3-kinase-protein kinase B (PI3K-Akt) pathway and *zinc-finger protein 217* (*ZNF217*) gene have been identified as frequent molecular genetic changes in ovarian clear cell carcinoma. The relationships between these events have not been studied and integrated in the same cohort. This study was aimed at determining the correlation between these molecular events and other clinicopathological factors, including the prognostic impacts of these clinicopathological factors. A total of 68 ovarian clear cell carcinoma cases were collected and subjected to immunohistochemistry testing for ARID1A, SMARCA2, SMARCA4, SMARCB1 and phosphatase and tensin homolog (PTEN), mutation analysis for *phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha* (*PIK3CA*) gene and fluorescence *in situ* hybridization for *ZNF217* amplification. The correlations between ARID1A expression, PI3K-Akt pathway, *ZNF217* amplification and other clinicopathological factors were analyzed. Loss of ARID1A expression was present in 35 cases (52%) and loss of SMARCA2 expression occurred in 1 case. SMARCA4 and SMARCB1 expressions were preserved in all cases. *PIK3CA* mutations were present in 23 cases (34%) and loss of PTEN expression occurred in 8 cases (12%). Alterations in the PI3K-Akt pathway (*PIK3CA* mutations or loss of PTEN expression) were found in 42 cases (62%). *ZNF217* amplification was detected in 21 cases (31%). Loss of ARID1A expression was significantly related to younger patient age ($P=0.048$), PI3K-Akt pathway activation ($P=0.046$) and *ZNF217* amplification ($P=0.028$). All of the clinicopathological factors were not prognostic factors for ovarian clear cell carcinoma after multivariate analysis, except International Federation of Gynecology and Obstetrics staging ($P=0.001$). Our results showed that loss of ARID1A expression usually coexisted with PI3K-Akt pathway activation and/or *ZNF217* amplification. Synergic effects of loss of ARID1A and PI3K-Akt pathway activation as well as *ZNF217* amplification may be related to the development of ovarian clear cell carcinoma.

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The prevalence of ovarian clear cell carcinoma in North America and Europe is 1–12%.[1] Compared with other epithelial ovarian cancers, patients with clear cell carcinoma respond less favorably to platinum-based first-line chemotherapy.[2] Elucidation of common molecular genetic changes is crucial for understanding this disease and new treatment strategies.

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AT-rich interactive domain 1A (ARID1A) is one of the *ARID* family members with non-sequence-specific DNA-binding activity, and is the large subunit of switch/sucrose non-fermentable (SWI/SNF) complex.[3] Brahma (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2, SMARCA2), brahma-related gene 1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4, SMARCA4) and integrase interactor 1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1, SMARCB1) are other important components involved in SWI/SNF complexes.[4] In humans, *ARID1A* is a tumor-suppressor gene located at 1p36, which is frequently mutated in ovarian, endometrial, breast, urinary bladder and gastric cancers.[5–9] *ARID1A* mutations are present in 46% of ovarian clear cell carcinomas and 30% of endometrioid carcinomas.[5] ARID1A immunohistochemistry (IHC) is closely related to *ARID1A* mutational status,[10] and can be used as a surrogate marker for ARID1A mutation.

In addition to *ARID1A*, activating mutations in *phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha* (*PIK3CA*)[11] and *zinc-finger protein 217* (*ZNF217*) amplification[12] are common molecular genetic alterations in ovarian clear cell carcinoma. *PIK3CA* activating mutations and *ZNF217* amplification can be found in 40% of ovarian clear cell carcinoma patients.[11,12] Phosphatidylinositol 3-kinase-protein kinase B (PI3K-Akt) pathway activation can be caused by either *PIK3CA* mutations, *phosphatase and tensin homolog* (*PTEN*) mutations or a combination of these alterations.[13] *ZNF217* encodes a transcriptional repressor protein and forms the ZNF217 complex with CoREST, histone deacetylases HDAC1 and 2, and LSD-1.[14]

The biological roles of the SWI/SNF complex, PI3K-Akt pathway and *ZNF217* amplification in ovarian clear cell carcinoma have been separately investigated by several groups. However, data of relationships between these molecular genetic alterations are limited. Therefore, we analyzed the SWI/SNF complex (including ARID1A, SMARCA2, SMARCA4 and SMARCB1), PI3K-Akt pathway (including *PIK3CA* mutations and PTEN expression) and *ZNF217* amplification in 68 patients with ovarian clear cell carcinoma. We focused on the relationships between these molecular changes, their association to the clinicopathological factors and their prognostic impacts.

Materials and methods

Patients and Tissue Materials

All specimens and clinical data were collected from patients who received debulking surgery at National Taiwan University Hospital. Formalin-fixed paraffin-embedded tissue specimens were obtained from the archives of 1996–2010, at the Department of Pathology in the same institution. All 68 patients were treated surgically and 61 of them also received chemotherapy. The median follow-up period was 46.5 months (11.8–163.6 months), except for the patients who died. The sections stained with hematoxylin and eosin were reviewed and diagnosed as ovarian clear cell carcinoma according to the World Health Organization classification.[15] To exclude high-grade serous adenocarcinoma, hepatocyte nuclear factor (HNF)-1beta, estrogen receptor (ER), progesterone receptor (PR), Wilms tumor 1 (WT1) and p53 immunostains were also performed in all cases.[16,17]

Table 1 Manufacturers, clones and dilution of antibodies

Antibody	*Manufacturer*	*Clone*	*Dilution*
ARID1A	Sigma-Aldrich (St Louis, MO, USA)	HPA005456	1:250
SMARCA2	BD (San Jose, CA, USA)	24/BRM	1:50
SMARCA4	Santa Cruz (Dallas, TX, USA)	H-88	1:50
SMARCB1	Sigma-Aldrich	2C2	1:50
PTEN	DAKO (Glostrup, Denmark)	6H2.1	1:100

Immunohistochemistry

Immunohistochemical staining of paraffin tissue sections was performed by the Leica BOND-III autostainer (Leica Microsystems, Buffalo Grove, IL, USA). Commercially available antibodies to ARID1A, SMARCA2, SMARCA4, SMARCB1 and PTEN were used. The manufacturers, clones and antibody dilutions are listed in Table 1. The ARID1A immunoreactivities were divided into undetectable or positive (weakly or strongly) for nuclear staining.[18] We used similar criteria for SMARCA2, SMARCA4 and SMARCB1. PTEN immunoreactivity was applied by two-tiered systems (undetectable or positive) and vascular endothelial cells were used as an internal positive control.[19]

DNA Extraction and Mutation Analysis of the PIK3CA Gene

DNA extraction, polymerase chain reaction (PCR) amplification for *PIK3CA* gene sequencing and mutation analysis were performed in all 68 samples using approaches previously described.[11,20]

Fluorescence *In Situ* Hybridization (FISH)

ZNF217 amplification was studied by FISH in all cases. The commercial probes (Kreatech, Amsterdam, The Netherlands) were used. The criterion for *ZNF217* amplification was an amplification ratio of ≥ 1.5, enumerated among 100 tumor cells.

Table 2 Summary of clinicopathological factors of patients

Case No.	*Age*	*FIGO staging*	*ARID1A IHC*	*SMARCA2 IHC*	*SMARCA4 IHC*	*SMARCB1 IHC*	*SWI/SNF (-: altered)*	*PIK3CA mutation*	*PTEN IHC*	*ZNF217 amplification*	*PI3K-Akt pathway activation*
1	44	IC	−	+	+	+	−	Wild type	+	+	+
2	45	IC	+	+	+	+	+	Wild type	+	−	−
3	46	IIIC	+	+	+	+	+	Wild type	+	+	+
4	40	IC	+	−	+	+	−	Wild type	+	−	−
5	55	IC	−	+	+	+	−	E542K	+	−	+
6	50	IC	−	+	+	+	−	E545K	+	−	+
7	50	IA	−	+	+	+	−	H1047R	+	−	+
8	50	IC	+	+	+	+	+	Wild type	+	−	−
9	54	IIB	+	+	+	+	+	Wild type	+	−	−
10	36	IVC	−	+	+	+	−	G1049R	+	+	+
11	54	IA	−	+	+	+	−	Wild type	+	−	−
12	46	IC	+	+	+	+	+	H1047R	+	−	+
13	47	IC	+	+	+	+	+	H1047R	+	−	+
14	48	IC	+	+	+	+	+	Wild type	+	−	−
15	47	IA	+	+	+	+	+	Wild type	+	+	+
16	39	IC	−	+	+	+	−	E542K	+	−	+
17	44	IA	−	+	+	+	−	Wild type	+	+	+
18	55	IIIC	+	+	+	+	+	Wild type	+	−	−
19	55	IC	−	+	+	+	−	Wild type	+	−	−
20	58	IC	+	+	+	+	+	Y1021C	+	−	+
21	43	IC	+	+	+	+	+	Wild type	+	−	−
22	55	IIB	+	+	+	+	+	Wild type	+	−	−
23	53	IA	+	+	+	+	+	Wild type	+	−	−
24	50	IC	−	+	+	+	−	Wild type	+	−	−
25	52	IC	+	+	+	+	+	E545K	+	−	+
26	59	IIIC	+	+	+	+	+	Wild type	+	+	+
27	51	IC	−	+	+	+	−	Wild type	+	−	−
28	47	IC	+	+	+	+	+	E545K	+	−	+
29	41	IIIC	−	+	+	+	−	Wild type	−	−	+
30	44	IIIB	−	+	+	+	−	H1047R	+	+	+
31	62	IIIC	−	+	+	+	−	Wild type	+	+	+
32	44	IC	+	+	+	+	+	Wild type	−	+	+
33	57	IIIC	+	+	+	+	+	Wild type	+	+	+
34	63	IIIC	−	+	+	+	−	Wild type	+	−	−
35	47	IIIC	+	+	+	+	+	Wild type	+	−	−
36	39	IIIC	−	+	+	+	−	Wild type	+	+	+
37	52	IIIC	+	+	+	+	+	Wild type	+	−	−
38	58	IIA	+	+	+	+	+	Wild type	+	−	−
39	49	IC	−	+	+	+	−	Wild type	+	+	+
40	53	IIIC	+	+	+	+	+	Wild type	+	−	−
41	58	IVC	+	+	+	+	+	Wild type	+	−	−
42	44	IIIA	−	+	+	+	−	Wild type	−	−	+
43	47	IIIC	−	+	+	+	−	Wild type	+	−	−
44	62	IIIC	+	+	+	+	+	Wild type	−	−	+
45	45	IIIC	−	+	+	+	−	H1047R	+	+	+
46	70	IIA	−	+	+	+	−	Wild type	−	+	+
47	46	IA	−	+	+	+	−	Wild type	+	−	−
48	56	IC	+	+	+	+	+	Wild type	+	+	+
49	47	IC	−	+	+	+	−	H1047R	+	+	+
50	38	IC	−	+	+	+	−	Wild type	+	+	+
51	53	IC	−	+	+	+	−	E545K	+	−	+
52	33	IIIC	−	+	+	+	−	Wild type	−	−	+
53	36	IIIB	−	+	+	+	−	H1047L	+	−	+
54	52	IC	+	+	+	+	+	Wild type	+	−	−
55	33	IIC	−	+	+	+	−	E542K	+	−	+
56	55	IIIC	+	+	+	+	+	Wild type	+	−	−
57	47	IC	+	+	+	+	+	H1047L	+	−	+
58	37	IC	−	+	+	+	−	E545K	+	+	+
59	57	IIC	+	+	+	+	+	Wild type	+	−	−
60	48	IC	+	+	+	+	+	Wild type	+	−	−
61	59	IVC	−	+	+	+	−	E542K	−	−	+
62	45	IC	−	+	+	+	−	E542K	+	−	+
63	53	IVC	−	+	+	+	−	N1044K	+	+	+
64	48	IC	−	+	+	+	−	Wild type	+	+	+
65	41	IIIC	+	+	+	+	+	Q546K	+	−	+
66	53	IC	−	+	+	+	−	Wild type	+	−	−
67	54	IC	+	+	+	+	+	M1043I	−	−	+
68	50	IIIC	−	+	+	+	−	Wild type	+	+	+

Table 3 Association between ARID1A expression and clinicopathological factors in ovarian clear cell carcinoma patients

		No. (%)		
Characteristics	*No.*	*ARID1A negative,* n = *35*	*ARID1A positive,* n = *33*	P-*value*
Age	68			0.048
Mean		47.5	51.1	
FIGO staging				0.572
I + II	43	21 (60)	22 (67)	
III + IV	25	14 (40)	11 (33)	
PIK3CA *mutation*				0.105
Wild-type	45	20 (57)	25 (76)	
Mutated	23	15 (43)	8 (24)	
PTEN IHC				0.710
Negative	8	5 (14)	3 (9)	
Positive	60	30 (86)	30 (91)	
PI3K-Akt pathway				0.046
Non-activated	39	16 (46)	23 (70)	
Activated	29	19 (54)	10 (30)	
Akt pathway				0.007
Non-activated	26	8 (23)	18 (55)	
Activated	42	27 (77)	15 (45)	
ZNF217 amplification				0.028
No	47	20 (57)	27 (82)	
Yes	21	15 (43)	6 (18)	

Statistical Analysis

The statistical analyses were conducted using PASW Statistics (IBM Corporation, Armonk, NY, USA). The association between ARID1A expression and age was evaluated using the *t*-test. The association between ARID1A expression and other clinicopathologic parameters was evaluated using the χ^2 test or Fisher's exact test. The influences of clinicopathological parameters on progression-free survival (PFS) and overall survival (OS) were analyzed by Cox proportional hazard model. *P*-values from Wald's statistic were recorded. The cutoff of significance level was 0.05.

Results

The clinical data and results of IHC, mutation analysis and FISH are listed in Table 2, and association between ARID1A expression and other clinicopathological factors are summarized in Table 3. Loss of ARID1A expression (35/68, 52%; Figures 1a and b), *PIK3CA* mutations (23/68, 34%) and *ZNF217* amplification (21/68, 31%) were common in ovarian clear cell carcinoma. SMARCA4 and SMARCB1 expressions were preserved in all cases, but only one case showed loss of SMARCA2 expression (Figures 1c and d). Loss of PTEN expression was present in 12% of all cases (Figures 1e and f).

The frequencies of PI3K-Akt pathway (PTEN expression/*PIK3CA* mutations) activation and *ZNF217* amplification in the ARID1A-negative carcinomas were higher than those in the ARID1A-positive carcinomas ($P = 0.046$ and 0.028, respectively). Loss of ARID1A expression was also correlated with younger patient age ($P = 0.048$). *PIK3CA* mutations and loss of PTEN expression, respectively, were not associated with ARID1A expression ($P = 0.105$ and 0.710, respectively).

The prognostic effects of clinicopathological factors are summarized in Table 4. International Federation of Gynecology and Obstetrics (FIGO) staging was the most important prognostic predictor for ovarian clear cell carcinoma ($P = 0.001$). Loss of PTEN expression also had impact on PFS and OS in univariate analysis ($P = 0.050$ for PFS and 0.026 for OS), but its significance disappeared after multivariate analysis ($P = 0.144$ for PFS and 0.360 for OS). Other clinicopathological factors including age, ARID1A expression, *PIK3CA* mutations, PI3K-Akt pathway and *ZNF217* amplification were not significant prognostic factors.

Discussion

Compared with other epithelial ovarian cancers, clear cell carcinoma patients are more likely to be younger, of Asian descent, present at an early stage and suffer a poorer survival after adjusting other prognostic factors such as age and grade of disease.[21] Owing to less favorable responses to platinum-based first-line chemotherapy in the patients of ovarian clear cell carcinoma,[2] effort for discovering molecular genetic changes are ongoing. The major differential diagnosis of ovarian clear cell carcinoma in the adult is high-grade serous adenocarcinoma. Combined with morphological features, a panel of immunostains including HNF-1beta, ER, PR, WT-1 and p53 is helpful for the differential diagnosis.[16,17] To date, the mutations of chromatin-remodeling gene *ARID1A*, activating mutations in *PIK3CA*[11] and *ZNF217* amplification[12] are the most common molecular genetic alterations in ovarian clear cell carcinoma. Loss of PTEN protein expression is also found in one-third of the ovarian clear cell carcinoma patients.[22] To our knowledge, no study has reported the combination of all these common molecular genetic alterations in the same patient cohort.

The SWI/SNF complex in mammals is an ATP-dependent chromatin-remodeling complex crucial for cell proliferation, development and differentiation.[23] ARID1A is one of the ARID family members with non-sequence-specific DNA-binding activity, and is the large subunit of SWI/SNF complex.[3] Among the related transcription factors and transcriptional complexes, SMARCA4, SMARCA2 and SMARCB1 are important.[4] *ARID1A* is a tumor-suppressor gene located at 1p36, which is frequently mutated in ovarian, endometrial, breast, urinary bladder and gastric cancers.[5–9] In gynecologic cancers, *ARID1A* promotes formation of SWI/SNF-mediated chromatin remodeling and is present in 46% of ovarian clear cell carcinomas and 30% of endometrioid carcinomas.[5,24] In our study, we confirmed that loss of ARID1A expression was

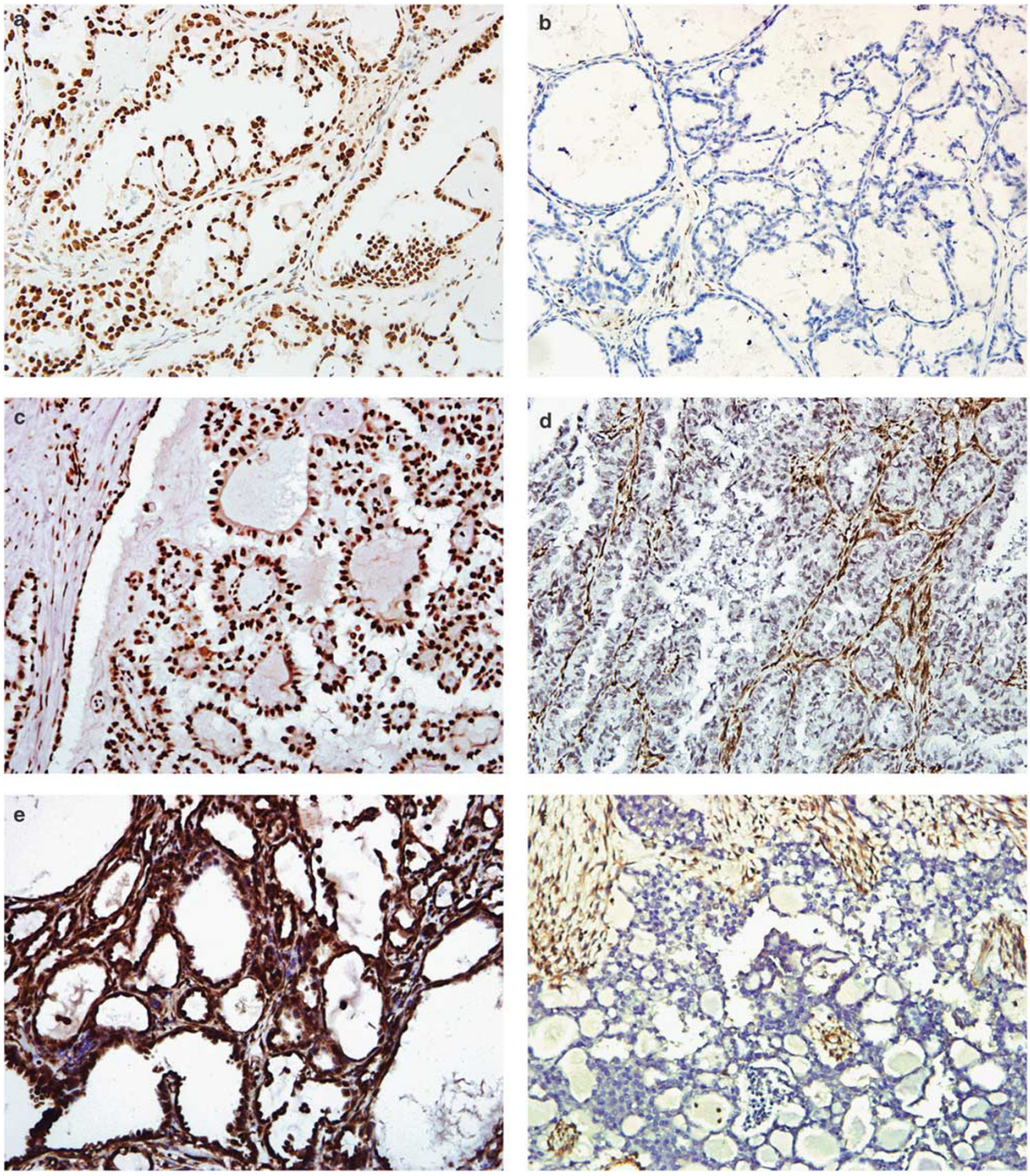

Figure 1 ARID1A, SMARCA2 and PTEN expressions in representative ovarian clear cell carcinoma cases. (**a**) A clear cell carcinoma with diffuse ARID1A immunoreactivity. (**b**) A clear cell carcinoma without ARID1A immunoreactivity. (**c**) Positive SMARCA2 immunostain. (**d**) Negative SMARCA2 immunostain. (**e**) Intact PTEN immunoreactivity. (**f**) Loss of PTEN immunoreactivity. Immunoperoxidase immunohistochemical stain, original magnification $\times 200$.

present in 52% of ovarian clear cell carcinomas. The result was close to those of previous studies by Maeda *et al* (59.1%) and Xiao *et al* (57.7%).[10,25] Compared with ARID1A, loss of expression of other components related to SWI/SNF complex such as SMARCA2, SMARCA4 and SMARCB1 were rare events in ovarian clear cell carcinoma. Similar findings were reported by Bosse *et al* in endometrial cancer.[26] PI3K-Akt pathway activation is a novel molecular genetic change in ovarian clear

Table 4 Univariate (a) and multivariate (b) analysis of survival in ovarian clear cell carcinoma patients

		Progression-free survival		*Overall survival*	
Covariate	*No.*	*HR (95% CI)*	P-*value*	*HR (95% CI)*	P-*value*
(*a*)					
Age			0.300		0.305
≤ 50 years	39	1.0		1.0	
> 50 years	29	1.49 (0.70–3.18)		1.49 (0.70–3.20)	
FIGO staging			<0.001		<0.001
I	37	1.0		1.0	
II	6	2.67 (0.70–10.23)		2.01 (0.50–8.01)	
III	21	4.99 (1.99–12.52)		4.03 (1.63–9.92)	
IV	4	19.70 (5.42–71.56)		60.06 (13.64–264.51)	
ARID1A IHC			0.989		0.862
Negative	35	1.0		1.0	
Positive	33	0.99 (0.47–2.12)		0.94 (0.44–1.99)	
SWI/SNF			0.763		0.966
Altered	36	1.0		1.0	
Normal	32	1.12 (0.53–2.40)		1.02 (0.48–2.16)	
PIK3CA *mutation*			0.265		0.432
Wild-type	45	1.0		1.0	
Mutated	23	0.61 (0.26–1.45)		0.71 (0.30–1.68)	
PTEN IHC			0.050		0.026
Negative	8	1.0		1.0	
Positive	60	0.37 (0.14–1.00)		0.32 (0.12–0.87)	
PI3K-Akt pathway			0.893		0.856
Non-activated	39	1.0		1.0	
Activated	29	0.95 (0.44–2.07)		1.08 (0.49–2.34)	
AKT pathway			0.282		0.377
Non-activated	26	1.0		1.0	
Activated	42	0.66 (0.31–1.41)		0.71 (0.33–1.52)	
ZNF217 amplification			0.581		0.621
No	47	1.0		1.0	
Yes	21	0.79 (0.33–1.86)		0.80 (0.34–1.91)	
(*b*)					
FIGO staging			0.001		0.001
I	37	1.0		1.0	
II	6	1.98 (0.41–9.64)		1.73 (0.35–8.42)	
III	21	6.46 (2.55–16.37)		4.99 (1.99–12.54)	
IV	4	8.22 (1.41–48.05)		26.29 (3.75–184.41)	
PTEN IHC			0.144		0.360
Negative	8	1.0		1.0	
Positive	60	0.43 (0.14–1.33)		0.59 (0.19–1.82)	

Abbreviations: CI, confidence interval; HR, hazard ratio.

cell carcinoma, and can be caused by either *PIK3CA* mutations, PTEN loss or a combination of these alterations.[13] In this study, the ARID1A expression did not significantly correlate with *PIK3CA* mutations or PTEN expression alone. Although the frequency of *PIK3CA* mutations in the ARID1A-deficient carcinomas was higher than that in the ARID1A-intact carcinomas, it was not significantly different. Similar results have been reported in several studies, with or without statistical significance.[27,28] We also found that loss of ARID1A expression was related to younger patient age and activated PI3K-Akt pathway (activating *PIK3CA* mutations or loss of PTEN expression). A similar correlation between the ARID1A expression and PI3K-Akt pathway was noted in endometrial cancer but was not statistically significant.[26]

ZNF217 encodes a transcriptional repressor protein and forms the ZNF217 complex with CoREST, histone deacetylases HDAC1 and 2, and LSD-1.[14] ZNF217 overexpression can cause cellular immortalization, telomerase repression, antiapoptosis and increased metastatic potential.[29,30] Recently, Krig *et al*[31] reported that *ZNF217* could activate the PI3K-Akt pathway by regulating ErbB3 expression in breast cancer cells. *ZNF217* amplification and *PIK3CA* mutations were almost mutually exclusive in the study by Rahman *et al.*[32] In our study, the correlation between *PIK3CA* mutations and *ZNF217* amplification was not statistically significant (data not shown, $P=0.541$). *PIK3CA* mutations and *ZNF217* amplification affected 38 cases, and 6 cases (16%) harbored both *PIK3CA* mutations and *ZNF217* amplification. The results were not

consistent with Rahman's study.[32] Compared with Rahman's study, we used different FISH probes and different criteria for amplification.

If we combined activating *PIK3CA* mutations, *ZNF217* amplification and loss of PTEN expression as indicators of activating Akt pathway, the relationship between ARID1A expression and the activating Akt pathway was more statistically significant ($P=0.007$) than the PI3K-Akt pathway ($P=0.048$). The results clearly pointed out that cases with activating Akt pathway (*PIK3CA* mutations, *ZNF217* amplification or loss of PTEN expression) had a higher frequency of loss of ARID1A expression (64%) than those without PI3K-Akt pathway alteration (31%). Our study also revealed that *ZNF217* amplification occurred significantly more frequently in the group with loss of ARID1A expression. This was the first report on the correlation between ARID1A expression and *ZNF217* amplification. Further investigation is needed to find the mechanism.

Except for the FIGO staging, all of the other clinicopathological factors were not associated with PFS and OS after multivariate analysis. Loss of ARID1A expression was found to be an adverse prognostic factor in gastric cancer,[9] cervical cancer[33] and endometrial clear cell carcinoma.[6] In ovarian clear cell carcinoma, the prognostic effects of ARID1A expression were inconsistent in different studies by Shih *et al*[10] and Katagiri *et al*.[34] Both our study and Shih's study revealed that ARID1A expression was not a prognostic factor for ovarian clear cell carcinoma. Absence of prognostic significance of *PIK3CA* mutations and PI3K-Akt pathway was confirmed in our study as in the previous studies.[10,27,35] In this study, we demonstrated that loss of PTEN expression was not an independent prognostic factor after multivariate analysis; this finding is similar to that of Ho *et al*.[36] *ZNF217* amplification was reported to be a poor prognostic factor in the study by Rahman *et al*,[32] while in this study, we did not find the prognostic effect of *ZNF217* amplification. In brief, FIGO staging was the most important and the only prognostic factor in our study.

Considering the complexities of the PI3K-Akt pathway, a combination of different therapeutic agents related to this pathway may be considered in order to get the greatest effect. One recent example involved combining two inhibitors targeting PI3K/AKT/mTOR and RAF/MEK/ERK pathways in ovarian cancer patients.[37] Our study suggested that additional factors such as *ZNF217* may also activate the PI3K-Akt pathway, providing a potential target for this dual-targeting strategy.

In conclusion, this is the first report demonstrating the correlation between *ARID1A*, PI3K-Akt pathway and *ZNF217* in ovarian clear cell carcinoma, by integration of expression of ARID1A, SMARCA2, SMARCA4, SMARCB1 and PTEN, *PIK3CA* mutations, as well as *ZNF217* amplification. More studies have to be conducted to clarify the truncated relationship between these common molecular genetic changes for a better understanding of the disease pathogenesis and subsequent therapeutic strategy.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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