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Original Article

SIAH2 expression predicts chemoresistance and poor clinical outcomes in patients with epithelial ovarian cancer

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Abstract: This study aims to investigate the protein expression of the seven in absentia homolog 2 (SIAH2), an E3 ubiquitin protein ligase, and its association with platinum resistance in epithelial ovarian cancer (EOC). The protein expression of SIAH2 was analyzed by immunohistochemistry in 167 patients with EOC. The association between SIAH2 expression and chemotherapy resistance in EOC was investigated. Furthermore, we analyzed the association between SIAH2 expression and clinicopathological features including prognosis in EOC samples. SIAH2 in platinum-resistant cases was overexpressed compared with that in platinum-sensitive cases (P<0.001). Platinum resistance was independently correlated with the International Federation of Gynecology and Obstetrics (FIGO) staging system and SIAH2 overexpression. In particular, high SIAH2 expression was correlated with platinum resistance in EOCs with optimal cytoreduction (P<0.001). High SIAH2 expression was positively correlated with age, FIGO stage, histological grade, residual tumor size, lymph node metastasis, and serum levels of CA125 but not histological type. Our findings indicate that SIAH2 overexpression is an independent predictor of platinum resistance, and that it may also be a potential biomarker for targeted therapy.

Keywords: SIAH2, seven in absentia homolog 2, epithelial ovarian cancer, platinum resistance

Introduction

Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer and the leading cause of mortality among gynecological malignancies. Despite the rapid advances of surgical treatments and new chemotherapeutic regimens, the five-year survival rate for patients with EOC remains less than 30% [1, 2]. At present, the standard therapy regimen for ovarian cancer is cytoreductive surgery combined with platinum-based chemotherapy. Chemotherapy plays an important role in the treatment of ovarian cancers. However, resistance to chemotherapeutic agents is a common occurrence that is often attributable to poor effect and prognosis [3-6]. Thus far, several studies have investigated biomarkers that can distinguish EOC patients on the basis of their response to platinum-based chemotherapy [7, 8]. However, the molecular basis for platinum resistance is largely undefined. Therefore, the reliable predictive biomarkers for the response to chemotherapy must be identified, and the issue of drug resistance must be addressed.

The seven in absentia homolog 2 (SIAH2) is one of the families of RING domain proteins that act as efficient ubiquitin ligases that target proteins for proteasomal degradation [9]. Recent findings suggested that SIAH2 plays a dominant role in the development and progression of diverse cancers [10]. Our previous study has demonstrated that SIAH2 overexpression is associated with histological grade, lymph node metastasis and poor prognosis in patients with EOC [11]. Moreover, SIAH2 downregulation is associated with resistance to endocrine therapy in breast cancer [12]. These findings suggested that SIAH2 may play a pivotal role in chemotherapy-associated drug resistance. However, the role of SIAH2 in platinum resistance remains unclear. The present study aims to investigate the role of SIAH2 and its association with platinum resistance in EOC.

Table 1. Association analyses between the expression levels of SIAH2 and the clinicopathological characteristics of EOCs

Variables	Patients n	SIAH2 expression		P^a
		Low	High	
All cases				
Age (years)				
≤55	84	34	50	$P = 0.031$
>55	83	20	63	
Histological type				
Serous	119	34	85	$P = 0.143$
Nonserous	48	20	28	
FIGO stage				
III	138	53	85	$P<0.001$
IV	29	1	28	
Histological grade				
G1	92	52	40	P, 0.001
G2/G3	75	2	73	
CA125 (Uml^{-1})				
≤35	56	32	24	$P<0.001$
>35	111	22	89	
Residual tumor (cm)				
≤1 cm	124	53	71	$P<0.001$
>1 cm	43	1	42	
Lymph node metastasis				
No	117	45	72	$P = 0.011$
Yes	50	9	41	

FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; SIAH2, Seven in Absentia Homolog 2; EOC, epithelial ovarian cancer; [a]Chi-square test.

Materials and methods

Patients and treatment

Paraffin-embedded tissue samples were collected from 167 patients with EOC diagnosed between September 2009 and July 2011 in the Affiliated Tumor Hospital of Harbin Medical University. All patients underwent maximal cytoreduction followed by platinum-based combination chemotherapy. The tumor stage was determined according to the International Federation of Gynecology and Obstetrics (FIGO) staging system [13]. The histological grade was classified based on the World Health Organization classification standards [14].

The principles of chemotherapy were in accordance with the National Comprehensive Cancer Network guidelines published in 2009 [15]. Patients intravenously received six to eight cycles of platinum-based combination chemotherapy (at a 3-week interval) 3 weeks after the primary surgery. The chemotherapy regimen consisted of either cisplatin/carboplatin/nedaplatinplus paclitaxel or carboplatin/nedaplatin plus docetaxel or nedaplatinplus paclitaxel liposome.

The patients provided written informed consent, and ethical approval was obtained from the Ethical Committee of Affiliated Tumor Hospital of Harbin Medical University.

Chemotherapy-resistance evaluation

The response to chemotherapy was defined according to Gynecologic Oncology Group criteria [16]. Platinum-resistant disease was defined as a progression or recurrence after a platinum-free interval of less than 6 months. Platinum-sensitive disease was defined as the absence of the evidence of progression or recurrence after a platinum-free interval of greater than or equal to 6 months.

Follow-up evaluation

Several examinations were performed during the follow-up period, including pelvic magnetic resonance imaging, a color Doppler ultrasound of liver and kidney, X-rays, and serum levels of cancer antigen 125 (CA-125). The end points of the study were overall survival (OS) and disease-free survival (DFS).

Immunohistochemical staining

Paraffin-embedded slides were deparaffinized, rehydrated, subjected to epitope retrieval, and treated with H_2O_2 to block endogenous peroxidase activity. The slides were incubated with normal goat serum, followed by incubation with a polyclonal goat anti-SIAH2 antibody (sc-5508, Santa Cruz, USA) overnight at 4°C. After washing with phosphate-buffered saline (PBS), the sections were incubated with a biotin-labeled secondary antibody (Santa Cruz, USA). Finally, the sections were immersed in diaminobenzidine (Dako Company, Denmark). Then, they were counterstained with hematoxylin and dehydrated with alcohol and xylene. The negative control slides were stained with PBS instead of primary antibody.

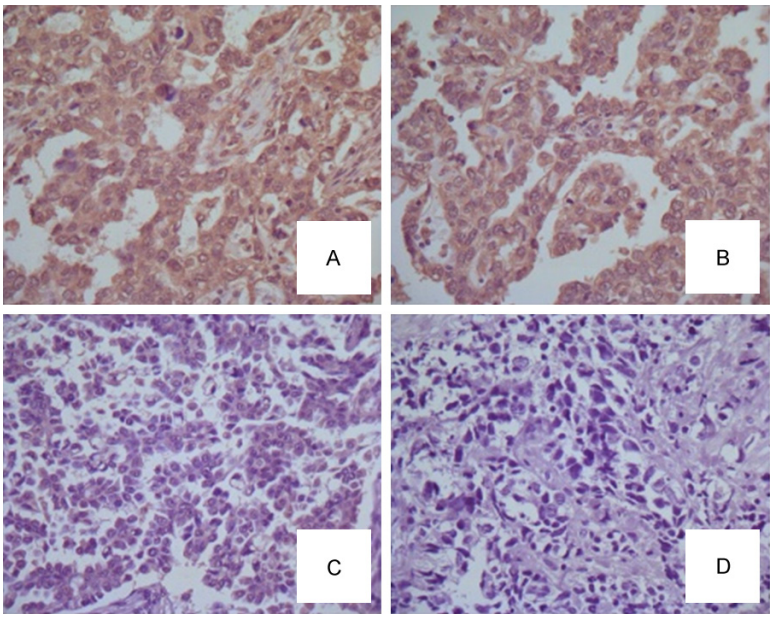

Figure 1. Immunohistochemical staining of SIAH2 in EOC specimens. A and B: High expression of SIAH2 in EOC; C and D: Low expression of SIAH2 in EOC.

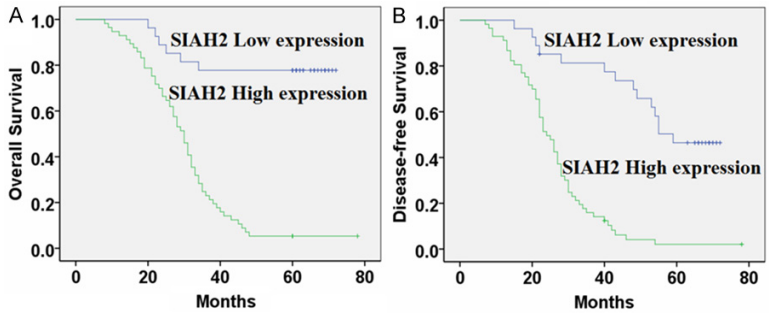

Figure 2. Kaplan-Meier analysis of overall survival and disease-free survival related to the expression of SIAH2. Patients with high expression of SIAH2 had a poorer prognosis than those of patients with low expression of SIAH2. A: Overall survival curves of the EOC according to their SIAH2 expression status, $P<0.001$; B: Disease-free survival curves of the EOC patients according to their SIAH2 expression status, $P<0.001$.

The intensity of staining was evaluated as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining. The percentage of stained cells was categorized as follows: 0 = 0% positive cells, 1 = 1%-10% positive cells, 2 = 11%-50% positive cells, 3 = 51%-80% positive cells, 4 = 81%-100% positive cells. The total score of the stained cells was calculated with the sum of the aforementioned two scores, where grade 0 = 0, grade 1 = 2-3, grade 2 = 4, grade 3 = 5, grade 4 = 6-7. Tumor tissues with grade 2-4 were defined as positive expression, and those with grade 0-1 were defined as negative expression. The total score was obtained by adding the scores on staining reaction and staining intensity. Scores of 5 or less were categorized as SIAH2 low expression, whereas scores greater than 5 were categorized as SIAH2 high expression.

Statistical analysis

The χ^2 test or Fisher's exact test was used to test the differences of categorical variables. The relationship between SIAH2 overexpression and chemotherapy resistance was evaluated through univariate and multivariate logistic regression with covariate adjustment. OS or DFS was measured with Kaplan-Meier method with log-rank test. A Cox proportional-hazard regression was performed for multivariate analysis of prognostic predictors. All statistical analysis was performed with SPSS 21.0 software (SPSS, Chicago, IL, USA). *P* values were considered statistically significant if <0.05.

Results

Patient characteristics

The demographic and clinical characteristics of the patients with EOC are summarized in **Table 1**. Of the 167 patients, 119 (71.3%) cases were serous ovarian carcinoma, and 48 (28.7%) cases were nonserous ovarian carcinoma. A total of 138 patients had EOC classified as FIGO stage III, and 29 had FIGO stage IV. The tumor size after cytoreductive surgery was smaller than 1 cm in 124 patients (74.3%). The serum CA-125 concentration was more than 35 in 111 patients (66.5%). Lymph node metastases were observed in 50 patients (29.9%).

SIAH2 expression

Figure 1 shows that SIAH2 was localized in the cytoplasm of tumor cells. Of the patients with

Table 2. Univariate survival analysis of OS and DFS in 167 patients with EOC

Variables	n	OS		P^a	DFS		P^a
		Mean ± SE (month)	95% CI		Mean ± SE (month)	95% CI	
Age (years)							
≤55	84	38±2	34-42	$P = 0.166$	33±2	29-37	$P = 0.115$
>55	83	45±3	39-50		39±3	34-44	
Histological cell type							
Serous	119	41±2	38-45	$P = 0.072$	35±2	32-38	$P = 0.730$
Nonserous	48	39±4	32-46		38±4	30-46	
FIGO stage							
III	138	48±2	44-51	$P<0.001$	41±2	37-45	$P<0.001$
IV	29	17±1	14-19		14±1	12-16	
Histological grade							
G1	92	53±3	47-58	$P<0.001$	45±2	41-50	$P<0.001$
G2/G3	75	29±2	26-32		25±1	22-28	
CA125 (Uml^{-1})							
≤35	56	49±4	41-56	$P = 0.013$	43±4	36-50	$P = 0.007$
>35	111	37±2	34-41		32±2	29-35	
Residual tumor (cm)							
≤1 cm	124	49±2	45-53	$P<0.001$	42±2	38-46	$P<0.001$
>1 cm	43	23±2	20-26		20±1	17-23	
Lymph node metastasis							
No	117	46±2	42-50	$P = 0.001$	40±2	36-44	$P<0.001$
Yes	50	33±3	27-40		27±3	22-32	
SIAH2 expression							
Low expression	54	62±3	56-67	$P<0.001$	55±3	50-60	$P<0.001$
High expression	113	31±1	28-34		26±1	24-28	

FIGO, International Federation of Gynecology and Obstetrics; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; SIAH2, Seven in Absentia Homolog 2; OS, overall survival; DFS, disease-free survival; EOC, epithelial ovarian cancer; [a]Log-rank test.

Table 3. Multivariate survival analysis of OS and DFS in patients with EOC

Variables	OS		P^a	DFS		P^a
	Exp (B)	95% CI		Exp (B)	95% CI	
FIGO stage	11.348	6.595-19.526	$P<0.001$	10.104	5.928-17.222	$P<0.001$
SIAH2	8.487	4.438-16.231	$P<0.001$	5.256	3.226-8.561	$P<0.001$
Residual tumor (cm)	2.668	1.743-4.083	$P<0.001$	2.504	1.650-3.801	$P<0.001$

FIGO, International Federation of Gynecology and Obstetrics; SIAH2, Seven in Absentia Homolog 2; OS overall survival; DFS disease-free survival; CI confidence interval; EOC, epithelial ovarian cancer; [a]Cox regression test.

EOC, 54 patients (32.3%) had SIAH2 low expression, whereas 113 patients (67.7%) had SIAH2 high expression. **Table 1** summarizes the association between SIAH2 expression and clinicopathological parameters in EOC. SIAH2 expression in EOC specimens was significantly correlated with age, FIGO stage, histological grade, residual tumor size, lymph node metastasis, and serum levels of CA125 (**Table 1**; $P<0.05$) but not with histological type (**Table 1**; $P>0.05$).

Effect of SIAH2 overexpression on prognosis in patients with EOC

High SIAH2 expression was significantly correlated with OS and DFS for patients with EOC through univariate Kaplan-Meier survival curves (**Figure 2A** and **2B**; **Table 2**; $P<0.001$).

Multivariate analysis via Cox regression models revealed that SIAH2 expression, FIGO stage,

Table 4. The association between chemotherapy resistance and clinicopathological characteristics in patients with EOC

Variables	Total	Platinum resistance		*P*
		Present (%)	Absent (%)	
Age (years)				*P* = 0.308
≤55	84	26 (31.7)	56 (68.3)	
>55	83	31 (38.8)	49 (61.3)	
Histological cell type				*P* = 0.772
Serous	119	45 (37.8)	74 (62.2)	
Nonserous	48	17 (35.4)	31 (64.6)	
FIGO stage				*P*<0.001
III	138	35 (25.4)	103 (74.6)	
IV	29	27 (93.1)	2 (6.9)	
Histological grade				*P*<0.001
G1	92	23 (25.0)	69 (75.0)	
G2/ G3	75	39 (52.0)	36 (48.0)	
CA125 (Uml^{-1})				*P* = 0.155
≤35	56	25 (44.6)	31 (55.4)	
>35	111	37 (33.3)	74 (66.7)	
Residual tumor (cm)				*P* = 0.004
≤1 cm	124	38 (30.6)	86 (69.4)	
>1 cm	43	24 (55.8)	19 (44.2)	
Lymph node metastasis				*P* = 0.997
No	117	12 (10.3)	105 (89.7)	
Yes	50	50 (100.0)	0 (0.0)	
SIAH2 expression				
Low expression	54	9 (16.7)	45 (83.3)	*P*<0.001
High expression	113	53 (46.9)	60 (53.1)	

FIGO, International Federation of Gynecology and Obstetrics; AEG-1, SIAH2, Seven in Absentia Homolog 2; EOC, epithelial ovarian cancer; [a]univariate logistic regression.

and large residual tumor size were independent prognostic factors for both OS and DFS (**Table 3**; *P*<0.001).

Effect of SIAH2 overexpression on chemotherapy resistance in patients with EOC

Among the 167 patients, 62 patients exhibited chemotherapy resistance, whereas 105 patients presented chemotherapy sensitivity. Among the 62 patients with chemotherapy resistance, 53 patients (85.5%) had SIAH2 overexpression. The drug resistance of chemotherapy was significantly associated with SIAH2 expression, histological grade, FIGO stage, lymph node metastasis, and residual tumor size (**Table 4**; *P*<0.05).

The multivariate analysis showed through binary logistic regression results that SIAH2 overexpression was independently associated with chemotherapy resistance (**Table 5**; odds ratio [OR]: 2.540, 95% confidence interval [CI]: 1.069-6.032; *P* = 0.035).

Discussion

The associations between SIAH2 overexpression and chemotherapy resistance in EOC have not been reported thus far. This study is the first to investigate the association between SIAH2 overexpression on prognosis and chemotherapy resistance through EOC samples. In this study, we analyze SIAH2 expression in 167 patients with EOC via immunohistochemistry and determine that SIAH2 expression is an independent factor for both prognosis and chemotherapy resistance. Our results suggest that high SIAH2 expression plays a critical role in EOC progression and may serve as an important biomarker in predicting platinum chemosensitivity.

SIAH2 is a new gene domain-containing ubiquitin ligase that has an evolutionarily conserved E3RING finger with a catalytic RING domain on its N-terminus followed by 2 zinc fingers and a C-terminal substrate-binding domain [17]. Elevated expression of SIAH2 is reported in diverse human cancers; including lung [18] and prostate tumors [19], breast carcinoma [20], and oral cancer [21]. Evidence suggests that SIAH2 contributes to tumor development and tumorigenesis progression. SIAH2 proteins are also implicated in multiple cellular processes, including hypoxia response, survival, and mitochondrial biogenesis [22-24]. The protein expression of SIAH2, which is correlated with clinicopathologic factors and poor prognosis in various cancers, such as oral squamous cell carcinoma [21] and prostate cancer [19], has been explored. Our study reveals that SIAH2 high expression is related to poor prognosis in patients with EOC. Our results are consistent with the previous findings on the roles of SIAH2 in tumor progression in various cancers. All of these findings suggest the important biological role of SIAH2 in carcinogenesis and tumor progression.

Table 5. Multivariate analysis of the association between SIAH2 expression and chemotherapy resistance

Variable	B	SE	*P*	OR	95% CI
FIGO stage					
III					
IV	3.423	0.766	*P*<0.001	30.655	6.831-137.568
SIAH2					
Low expression					
High expression	0.932	0.441	*P* = 0.035	2.540	1.069-6.032

FIGO, International Federation of Gynecology and Obstetrics; SIAH2, Seven in Absentia Homolog 2; EOC, epithelial ovarian cancer; CI confidence interval; [a]multivariate logistic regression.

SIAH2 has been recently identified as a biomarker involved in the sensitivity of tumor cells to chemotherapy. Jansen et al. determined that the tumor with low SIAH2 levels is associated with resistance to endocrine therapy in primary breast tumor specimens as well as in vitro [25]. SIAH2 is also apparently an important modulator of tamoxifen sensitivity in ER-positive MCF-7 cells through the regulation of ER-α expression [12]. A recent study performed on the SIAH2-/- mice demonstrated that vascular normalization via loss of SIAH2 results in increased sensitivity to doxorubicin-based chemotherapy [26]. Chemotherapy resistance is one of the most important prognostic factors in EOCs [27]. Approximately 30% of patients show platinum resistance and aggressive disease progression [28]. However, intrinsic or acquired chemotherapy resistance remains a dominating clinical challenge and a pivotal factor affecting the survival outcome of these patients. The present study also analyzes the association between SIAH2 expression and chemotherapy resistance. Our analysis indicates that SIAH2 expression is an independent factor for platinum chemotherapy resistance in EOC, which suggests that SIAH2 overexpression plays an important role in chemotherapy resistance. The results of this study are consistent with previous reported results.

At present, some clues that can help explain the mechanisms through which SIAH2 mediates chemotherapy resistance exist. Another study reported that SIAH2 inhibition can increase HIPK2 and DYRK2 levels, thereby increasing cell sensibility to DNA damage-induced apoptosis; this finding suggested a molecular mechanism underlying chemotherapy resistance in solid tumors [29]. New findings also offer a preclinical proof of concept indicating that targeting SIAH2 can sufficiently attenuate Hif-1α-mediated angiogenesis and hypoxia signaling, thereby improving responses to chemotherapy. SIAH2 may cause drug resistance in cancer through the opposite regulation by E2 and EGF [25]. Finally, SIAH2 may confer sensitivity to anticancer drugs by regulating miR-335/SIAH2/HDAC3 axis [30].

The important role of SIAH2 in drug resistance implies that it is a possible target for anticancer therapy. At present, reports on some therapies targeting SIAH2 exist. Garcia et al. indicated that interfere on signaling pathway activation and targeting Siah-2 signals via intravesical immunotherapy may provide novel therapeutic opportunities for non-muscle invasive bladder cancer [31]. Shah et al. revealed that SIAH2 inhibitor can be used as an effective treatment for melanoma via attenuating hypoxia and MAPK signaling [32]. These data indicate that SIAH2 is a potential novel target for EOC therapy.

In conclusion, this study indicates that SIAH2 can be used as an important biomarker to predict patients with EOC, who are highly susceptible to platinum resistance. Taken together, these observations may guide clinical therapy, that is, patients with SIAH2 overexpression may be frequently monitored to detect platinum resistance early enough and to help them benefit from second-line chemotherapy or molecular targeted therapy. Therefore, the immunohistochemical analysis of SIAH2 may have significant clinical implications.

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Disclosure of conflict of interest

None.

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