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Up-regulation of HDAC6 Results in Poor Prognosis and Chemoresistance in Patients With Advanced Ovarian High-grade Serous Carcinoma

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Abstract. *Background: Ovarian high-grade serous carcinoma (HGSC) gradually acquires chemoresistance after recurrence. Our previous study on ovarian clear-cell carcinoma found histone deacetylase 6 (HDAC6) overexpression led to chemoresistance. This study aimed to evaluate HDAC6 as a predictor of chemoresistance and a therapeutic target for ovarian HGSC. Patients and Methods: The clinical significance of HDAC6 as a predictor of prognosis and chemoresistance in HGSC was immunohistochemically evaluated. In addition, expression of programmed cell death ligand-1 (PD-L1), and hypoxia-inducible factor-1α (HIF1α) were analyzed using clinical samples from 88 patients with ovarian HGSC, and their clinicopathological characteristics were reviewed. Results: Twenty-three patients had high HDAC6 expression, 10 positive PD-L1 expression, and 33 high HIF-1α expression. HDAC6 up-regulation was correlated with not undergoing interval debulking surgery (p<0.001), incomplete surgical resection (p=0.002), and frequent occurrence of stable disease/progressive disease according to the Response Evaluation Criteria in Solid Tumors (p=0.005) criteria. On Kaplan-Meier analysis, high HDAC6 expression was significantly associated with reduced progression-free (p=0.001) and overall (p=0.008) survival. On multivariate analysis, high HDAC6 expression (hazard ratio=1.65, 95% confidence interval 1.03-2.66; p=0.039) and surgery status were independent prognostic factors of progression-free survival. PD-L1 and HIF1α expression positively correlated with that of HDAC6. Conclusion: HDAC6 may become a potential therapeutic target in patients with ovarian HGSC since its up-regulation is considered to be associated with a poor prognosis in patients with this cancer.*

Ovarian cancer is the leading cause of death owing to cancer of the female genital tract, with high-grade serous carcinoma (HGSC) being the most frequent histological type (1). The most important prognostic factor for patients with HGSC is the tumor stage; approximately 75-80% of patients have advanced-stage disease when they start showing symptoms, and <25% of cases with stage III/IV HGSC are curable using current therapies (2). Typically, HGSC shows a good response to a combination of platinum and taxane agents, which is a standard chemotherapy regimen for epithelial ovarian cancer. However, HGSCs recur frequently and gradually acquire resistance to these standard chemotherapy regimens (2). The most common molecular changes in HGSCs are alterations of tumor protein 53 (*TP53*) and inactivation due to germline or somatic mutation or promoter methylation of breast cancer susceptibility gene (*BRCA*)*1* and *BRCA2* in approximately 50% of HGSCs (3). *TP53* activation was found to sensitize tumor cells to platinum-based chemotherapy, leading to cell-cycle arrest and apoptosis (4). Recently, new strategies have been devised for patients with ovarian cancer with *BRCA* mutations (5) or platinum-sensitive recurrence (6). However, treatments for patients with *TP53* mutations or those with platinum-resistant recurrence have yet to be developed.

Histone deacetylases (HDACs), which comprise 18 subtypes identified in humans, regulate tissue differentiation,

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Key Words: Ovarian cancer, high-grade serous carcinoma, histone deacetylase 6, programmed cell death ligand-1, hypoxia inducible factor-1α, prognosis.

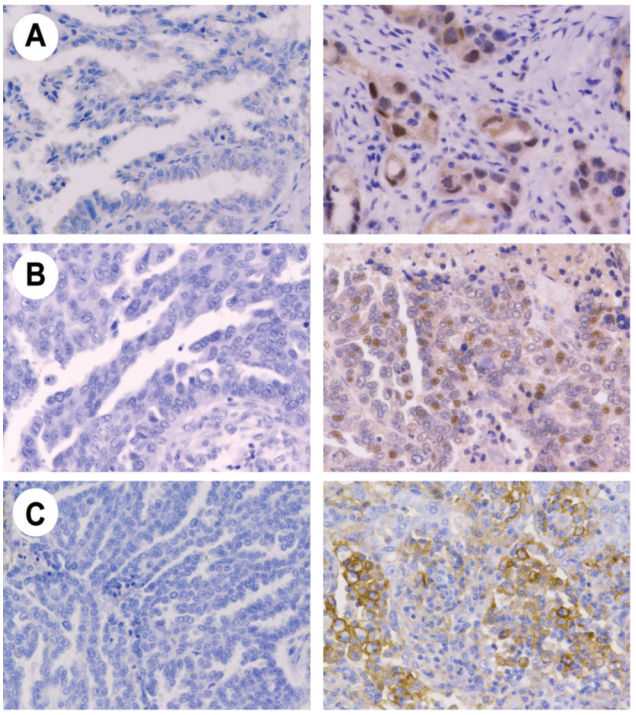

Figure 1. *Immunohistochemical expression of histone deacetylase 6 (A: left: low; right: high), hypoxia inducible factor-1α (B: left: low; right: high), and programmed cell death ligand-1 (C: left: negative; right: positive) in ovarian high-grade serous carcinoma. Original magnification, ×40.*

Table I. *Clinicopathological characteristics of study patients (N=88).*

Characteristic		Value
Age, years	Median (range)	61.1 (41-82)
Frequency, n (%)	≤60 Years	38
	>60 Years	50
Cancer antigen 125, U/ml	Median (range)	2,658 (37-24,200)
Frequency, n (%)	≤500 U/ml	19 (22%)
	>500 U/ml	69 (78%)
Treatment, n (%)	NAC+IDS	62 (70%)
	NAC only	26 (30%)
FIGO stage, n (%)	III	64 (73%)
	IV	24 (27%)
Surgery, n (%)	Complete resection	44 (50%)
	Incomplete resection	44 (50%)
RECIST status, n (%)	CR	14 (16%)
	PR	62 (71%)
	SD	5 (6%)
	PD	6 (7%)
CRS, n (%) (n=61)	1	9 (14%)
	2	16 (26%)
	3	36 (60%)
Recurrence, n (%)	Present	73 (83%)
	Absent	15 (17%)
Survival status, n (%)	Dead	46 (52%)
	Alive	42 (48%)

CR: Complete response; CRS: chemotherapy response score (1: resistant; 2: intermediate; 3: sensitive); FIGO: International Federation of Obstetrics and Gynecology; IDS: interval debulking surgery; NAC: neoadjuvant chemotherapy; PD: progressive disease; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease.

apoptosis, migration, mitosis and angiogenesis by chromatin-modification *via* the deacetylation of histone or non-histone proteins (7). The inhibitors that target multiple HDACs exhibit cytotoxic effects in various types of cancer, including ovarian (8), but they are limited in their application for cancer treatment because of various toxicities (9). Therefore, more selective and effective HDAC inhibitors are required. Our previous study showed that high HDAC6 expression was an independent poor prognostic factor in epithelial ovarian cancer (10). HDAC6 was shown to increase the level of deacetylated α-tubulin, which up-regulated cancer cell growth by enhancing microtubule dynamics (11, 12). HDAC6 down-regulation stabilizes p53 by increasing the levels of total p53 and p53 phosphorylation (13). In contrast, HDAC6 up-regulation leads to platinum resistance, whilst its down-regulation enhances platinum agent-induced DNA damage and apoptosis (13). Moreover, HDAC6 up-regulates several factors that cause chemoresistance, including hypoxia inducible factor-1α (HIF-1α) (14) and programmed death ligand-1 (PD-L1) (15). HDAC6-selective inhibitors were shown to be safe in clinical trials for multiple myeloma (16, 17). HDAC6-selective inhibitors also suppressed the proliferation of AT-rich interactive domain 1A (*ARID1A*)-mutated ovarian clear-cell carcinoma and improved the survival of tumor-bearing mice (18).

In the current study, using clinical samples of ovarian HGSC, we immunohistochemically analyzed the association between HDAC6 expression and HGSC prognosis, and aimed to evaluate HDAC6 as a predictor of chemoresistance and as a therapeutic target for ovarian HGSC.

Patients and Methods

Patients and samples. All the methods, including the review of the electronic medical charts and pathological analysis, were performed in accordance with the 1975 Declaration of Helsinki after obtaining the approval of the Institutional Review Board (IRB number, 16-257) and informed consent (or with a formal waiver of consent). Altogether, 88 patients with ovarian HGSC that was surgically resected and pathologically confirmed at the Saitama Medical University International Medical Centre between 2007 and 2017 were recruited. The clinicopathological characteristics of these patients were reviewed, including age, recurrence, progression-free survival (PFS), overall survival (OS), International Federation of Obstetrics and Gynecology stage, treatment methods, surgical status (complete or incomplete resection), Response Evaluation Criteria in Solid Tumors (RECIST) status (19), and chemotherapy response score (CRS) (20, 21). RECIST was used to stratify patients

Table II. *Clinicopathological characteristics and immunohistochemistry expressions in patients with high-grade serous carcinoma (N=88).*

		HDAC6 expression, n			HIF-1α expression, n			PD-L1 expression		
Characteristic	Subgroup	Low	High	*p*-Value	Low	High	*p*-Value	Negative	Positive	*p*-Value
Age, years	≤60 Years	28	10	0.582	26	12	0.219	33	5	0.446
	>60 Years	37	13		29	21		45	5	
Cancer antigen 125	≤500 U/ml	13	6	0.368	11	8	0.416	17	2	0.631
	>500 U/ml	52	17		44	25		61	8	
FIGO stage	III	48	16	0.443	43	21	0.109	55	9	0.180
	IV	17	7		12	12		23	1	
IDS	Performed	53	9	**<0.001**	13	13	0.093	21	5	0.129
	Not performed	12	14		42	20		57	5	
Surgical status	Complete resection	39	5	**0.002**	31	13	0.093	40	4	0.369
	Incomplete resection	26	18		24	20		38	6	
RECIST	CR/PR	61	15	**0.005**	50	26	0.063	69	7	0.110
	SD/PD	4	7		4	7		8	3	
CRS (n=61)	1	7	2	0.482	7	2	0.425	8	1	0.833
	2	15	1		9	7		15	1	
	3	32	4		26	10		34	2	
Recurrence	Present	53	20	0.406	42	31	**0.029**	64	9	0.460
	Absent	12	3		13	2		14	1	
Survival status	Dead	31	8	0.114	25	21	0.076	41	5	0.571
	Alive	34	8		30	12		37	5	

CR: Complete response; CRS: chemotherapy response score (1: resistant; 2: intermediate; 3: sensitive); FIGO: International Federation of Obstetrics and Gynecology; HDAC6: histone deacetylase 6; HIF-1α: hypoxia-inducible factor-1α; IDS: interval debulking surgery; PD: progressive disease; PD-L1: programmed death-1 ligand; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease. Statistically significant *p*-values (<0.05) are shown in bold.

according to the following responses: Complete response, partial response, stable disease (SD), and progressive disease (PD) using computed tomography before and after chemotherapy (19). Based on omental examination results, CRS was used to classify patients as follows: Patients with CRS of 3 had a complete/near complete response; those with CRS of 2 had a partial response; and those with CRS of 1 had minimal or no response (20, 21).

Immunohistochemical staining. Immunohistochemical expression of HDAC6 (polyclonal rabbit anti-HDAC6, 1:500, ab1440; Abcam, Cambridge, UK), PD-L1 (monoclonal rabbit anti-PD-L1, 1:100, 28-8 pharmDx; Dako North America, Carpinteria, CA, USA), and HIF-1α (polyclonal rabbit anti-HIF-1α, 1:100, NB100-479; Novus Biologicals, CO, USA) was analyzed by using 4-μm serial sections of formalin-fixed paraffin-embedded blocks. Dako Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, USA) was used per the manufacturer's protocol. Target Retrieval Solution was applied for antigen retrieval at 98˚C for 20 minutes. Sections were incubated with the primary antibodies at 25˚C for 60 minutes, followed by incubation with the secondary antibodies (EnVision FLEX/HRP; Agilent Technologies) at 25˚C for 30 minutes. The chromogen reaction was performed with diaminobenzidine plus H_2O_2.

Interpretation of immunohistochemical results. One gynecological oncologist (Mitsutake Yano) and one gynecological pathologist (Masanori Yasuda), both of who were blinded to the clinicopathological characteristics, evaluated the degree of immunohistochemical staining (Figure 1). The following scoring system was used: 0% stained cells indicated negative staining; 1-50% stained cells, mild staining; and 51-100% stained cells, marked staining. To optimize the differences in PFS and OS, the raw data were binarized for statistical analysis. For HDAC6 and HIF-1α, marked expression was considered to indicate high expression, while completely negative and mild expression was considered low expression. For PD-L1, mild and marked expression was defined as positive, while completely negative expression was considered negative.

Statistical analysis. Fisher's exact test or Pearson's chi-squared test was used to analyze the correlation between immunohistochemical expressions and the clinicopathological characteristics. Univariate survival analysis was performed by generating Kaplan-Meier curves, and differences between the groups were assessed using the log-rank statistic. The Cox proportional hazards model was used to perform univariate and multivariate survival analyses. All analyses were performed using SPSS v24.0 (SPSS Inc., IL, USA). *p*-Values of less than 0.05 were considered statistically significant.

Results

Patient characteristics and immunohistochemical expression. Table I shows the characteristics of the 88 patients with HGSC. All the patients included in the study were Japanese. All patients underwent platinum-based systemic chemotherapy as neoadjuvant treatment; however, interval debulking surgery was not performed in 26 patients (30%) because of unresectable lesions. Among the 88 patients, 23

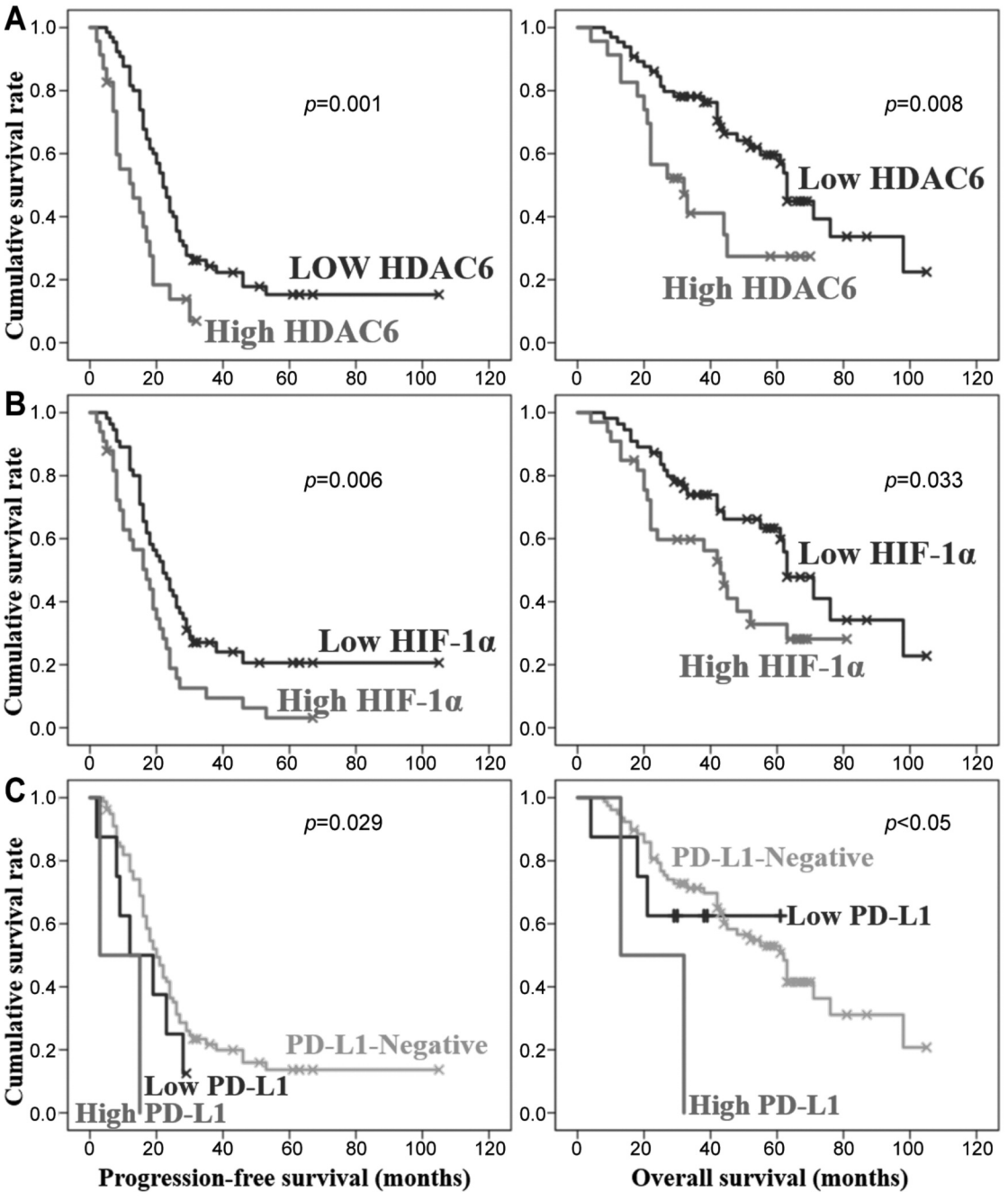

Figure 2. *Kaplan-Meier survival analysis of progression-free survival (left) and overall survival (right) according to expression of histone deacetylase 6 (HDAC6) (A), hypoxia-inducible factor-1α (HIF-1α) (B), and programmed cell death ligand-1 (PD-L1) (C). p-Values were obtained using the log-rank test.*

patients (26.1%) showed high HDAC6 expression, 10 patients (11.4%) showed positive PD-L1 expression, and 33 patients (37.5%) showed high HIF-1α expression. Table II shows the correlations between patient characteristics and immunohistochemical expression of HDAC6, HIF-1α, and PD-L1. High HDAC6 expression was significantly correlated with not undergoing interval debulking surgery ($p<0.001$), incomplete surgical resection ($p=0.002$), and frequently showing SD/PD according to RECIST criteria ($p=0.005$), but there was no significant correlation with CRS. A high expression of HIF-1α was correlated with recurrence ($p=0.029$). There was no significant correlation between PD-

Table III. *Univariate and multivariate analyses using the Cox proportional hazards model for patients with high-grade serous carcinoma.*

		PFS						OS					
		Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
Variable	Comparison	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Age	>60 *vs.* ≤60 Years	0.97	0.61-1.54	0.893				1.03	0.57-1.87	0.917			
FIGO stage	IV *vs.* III	1.48	0.90-2.43	0.120				1.49	0.81-2.74	0.199			
Surgery	Incomplete *vs.* complete	2.39	1.49-3.84	**<0.001**	2.43	1.31-4.52	**0.005**	2.50	1.37-4.59	**0.003**	2.45	1.33-4.51	**0.004**
RECIST status*	SD/PD *vs.* CR/PR	2.69	1.41-5.13	**0.003**				2.39	1.06-5.43	**0.037**			
CRS	3 *vs.* 1/2	1.43	1.07-1.90	**0.017**				1.47	1.01-2.15	**0.042**			
HDAC6 expression	High *vs.* low	1.53	1.17-1.99	**0.002**	1.65	1.03-2.66	**0.039**	1.51	1.10-2.08	**0.011**			

CI: Confidence interval; CR: complete response; CRS: chemotherapy response score; FIGO: International Federation of Obstetrics and Gynecology; HDAC6: histone deacetylase 6; HR: hazard ratio; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: stable disease. *Excluded from the multivariate analysis due to confounding with other variables (surgery and HDAC6 expression). Statistically significant *p*-values (<0.05) are shown in bold.

L1 expression and the clinicopathological characteristics. HDAC6 expression was significantly positive correlated with PD-L1 (p=0.002) and HIF-1α (p=0.008) expression.

Correlation of HDAC6, PD-L1, and HIF-1α expression with survival. On Kaplan-Meier survival curves, high HDAC6 expression was significantly associated with a decrease in both PFS (p=0.001) and OS (p=0.008) (Figure 2A). High HIF-1α expression was significantly associated with a decrease in PFS (p=0.006) and OS (p=0.033) (Figure 2B). Positivity for PD-L1 expression was significantly associated with a decrease in PFS (p=0.029) and OS (p<0.05) (Figure 2C). On univariate analysis using the Cox proportional hazards model, high HDAC6 expression, SD/PD RECIST status, CRS of 1 or 2, and incomplete resection were prognostic factors for PFS and OS (Table III). On multivariate analysis, high HDAC6 expression [hazard ratio (HR)=1.65, 95% confidence interval (CI)=1.03-2.66; p=0.039] and incomplete resection (HR=2.18, 95% CI=1.35-3.52; p=0.002) were independent prognostic factors for poor PFS. Incomplete resection (HR=2.45, 95% CI=1.33-4.51; p=0.004) was the only independent prognostic factor for poor OS on multivariate analysis (Table III).

Discussion

HDAC6 up-regulation has been reported to be a poor prognostic factor in various cancer types, such as breast (22), esophageal (23), renal cell (24), and ovarian clear-cell (14). Our present study, however, is the first to report that HDAC6 expression is an independent prognostic factor for advanced ovarian HGSC. In this study, it was shown that high HDAC6 expression was an independent poor prognostic factor in patients with advanced ovarian HGSC. HDAC6 up-regulation was demonstrated to be significantly correlated with indicators of chemoresistance, such as surgical residual tumor and SD/PD according to RECIST criteria. Our study findingis consistent with the results of Wang *et al.* (13), who showed that HDAC6 up-regulation led to resistance to platinum agents, but its down-regulation enhanced platinum agent-induced DNA damage and apoptosis. *TP53* activation, the most common mutation in HGSC, sensitized cells to platinum agents and led to cell-cycle arrest and apoptosis (4). HDAC6 down-regulation stabilizes p53 by increasing the total p53 level and p53 phosphorylation (13). Moreover, the deacetylation of alpha-tubulin induced by HDAC6, reduces the effect of taxane microtubule-stabilizing agent (Figure 3) (25). When HDAC6 was blocked, resistance to taxane agents was reversed in human epithelial ovarian cancer (25, 26). These results suggest that HDAC6 up-regulation is among the factors of recalcitrancy to standard platinum-based chemotherapy for HGSC.

Optional treatment agents for ovarian cancer include bevacizumab [anti-vascular endothelial growth factor (VEGF)] and olaparib [poly(ADP-ribose) polymerase inhibitor] for *BRCA*-mutant or platinum-sensitive recurrent tumors (4, 5), as well as pembrolizumab (PD-1/PD-L1 blockage) for microsatellite instability-high tumors (27). The current study showed that there was a positive correlation between HDAC6 and PD-L1 expression in ovarian HGSC, which has also been demonstrated in ovarian clear-cell carcinoma (14). HDAC6 inhibition has been reported to enhance the response to immunotherapy *via* PD1/PD-L1 (28, 29). The anticancer effect

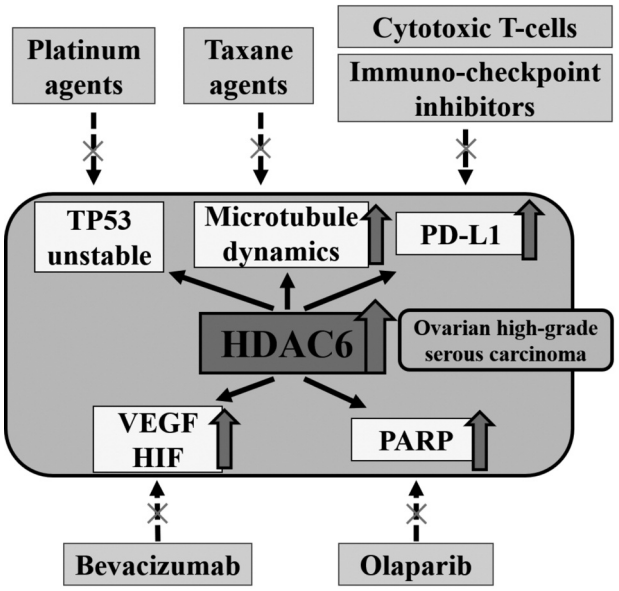

Figure 3. *Scheme of histone deacetylase 6 (HDAC6) functions: HDAC6 destabilizes tumor protein 53 (TP53) by deacetylation and suppresses apoptosis. These effects of HDAC6 are responsible for resistance to platinum agents. HDAC6 also leads to enhancement of microtubule dynamics, tolerance to hypoxia and immunotherapy, and DNA repair dysfunction via tubulin, hypoxia-inducible factor-1α (HIF-1α), programmed cell death ligand-1 (PD-L1), and poly ADP-ribose polymerase (PARP). HDAC6 results in tolerance to taxane agents, cytotoxic T-cells, immune-checkpoint inhibitors, bevacizumab, and olaparib. VEGF: Vascular endothelial growth factor.*

of HDAC6 inhibitors was triggered by the G_2/M cell-cycle arrest, apoptosis, and loss of mitochondrial membrane potential *via* the reduction of VEGF and poly (ADP-ribose) polymerase (30). In the present study, the expression of HDAC6 and HIF-1α, a factor upstream of VEGF, were significantly positively correlated (Figure 3). Therefore, HDAC6 might be a potential therapeutic target for HGSC that is resistant to standard or optional chemotherapy.

HDAC6-selective inhibitors have been shown to exhibit an antitumor effect in several cancer cell lines (16, 30-32), and are well tolerated with minimal toxicity from observations in clinical trials (16). The incidence of kidney failure (33) and peripheral neuropathy (34), which are common adverse effects caused by platinum and taxane agents, respectively, were reduced after using HDAC6-selective inhibitors. A phase Ib clinical trial of ricolinostat, an HDAC6-specific inhibitor, in combination with paclitaxel, demonstrated its safety in recurrent ovarian cancer (35). HDAC6 inhibition has potentially demonstrated antitumor effect (16, 30-32), synergistic antitumor activity with various chemotherapies (13, 25, 26, 28, 29), its safety (16, 17, 35), and furthermore efficacy in preventing or reversing chemotherapy-induced peripheral neuropathy (34, 35) and kidney failure (33). Therefore, we suggest that HDAC6 is a potentially important and safe therapeutic target for ovarian HGSC.

As far as we are aware, the current study is the first to verify the clinicopathological correlation between HDAC6 and indicators of chemoresistance in clinical samples. But further confirmation is warranted *via* a large-scale population study. Secondly, the present study consisted solely of semi-qualitative immunohistochemical analysis. Therefore, following a previous project of an experimental anticancer therapeutic model using ovarian clear-cell carcinoma cell lines (36), a quantitative analysis of HDAC6 protein and mRNA expression is planned to investigate the anticancer effect of HDAC6 inhibitors in serous carcinoma cell lines.

Conclusion

HDAC6 up-regulation resulted in a poor prognosis for patients with advanced ovarian HGSC because of chemoresistance. Therefore, HDAC6-selective inhibitors might become a promising therapeutic agent for ovarian HGSC that is resistant to the current standard/optional chemotherapy regimens.

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Conflicts of Interest

The Authors declare no competing interests in regard to this study.

Authors' Contributions

Conception/design: Mitsutake Yano, Hisashi Narahara and Masanori Yasuda. Provision of study material or patients: Mitsutake Yano, Aiko Ogasawara, Kosei Hasegawa and Masanori Yasuda. Collection and/or assembly of data: Mitsutake Yano, Aiko Ogasawara and Kosei Hasegawa. Data analysis and interpretation: Mitsutake Yano, Mariko Miyazawa, Naoki Ogane, Aiko Ogasawara, Kosei Hasegawa, Hisashi Narahara and Masanori Yasuda. Manuscript writing: Mitsutake Yano, Masanori Yasuda. Final approval of article: Mitsutake Yano, Mariko Miyazawa, Naoki Ogane, Aiko Ogasawara, Kosei Hasegawa, Hisashi Narahara and Masanori Yasuda.

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References

1 Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, Ngan HY, Pecorelli S and Beller U: Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet *95(Suppl 1)*: S161-192, 2006. PMID: 17161157. DOI: 10.1016/S0020-7292(06)60033-7

2 Bookman MA: First-line chemotherapy in epithelial ovarian cancer. Clin Obstet Gynecol *55(1)*: 96-113, 2012. PMID: 22343232. DOI: 10.1097/GRF.0b013e31824b45da

3 Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. Nature *474(7353)*: 609-15, 2011. PMID: 21720365. DOI: 10.1038/nature10166

4 Kigawa J, Sato S, Shimada M, Kanamori Y, Itamochi H and Terakawa N: Effect of p53 gene transfer and cisplatin in a peritonitis carcinomatosa model with p53-deficient ovarian cancer cells. Gynecol Oncol *84(2)*: 210-215, 2002. PMID: 11812076. DOI: 10.1006/gyno.2001.6488

5 Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, Lisyanskaya A, Floquet A, Leary A, Sonke GS, Gourley C, Banerjee S, Oza A, González-Martín A, Aghajanian C, Bradley W, Mathews C, Liu J, Lowe ES, Bloomfield R and DiSilvestro P: Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med *379(26)*: 2495-2505, 2018. PMID: 30345884. DOI: 10.1056/NEJMoa1810858

6 Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, Korach J, Huzarski T, Poveda A, Pignata S, Friedlander M, Colombo N, Harter P, Fujiwara K, Ray-Coquard I, Banerjee S, Liu J, Lowe ES, Bloomfield R, Pautier P and SOLO2/ENGOT-Ov21 investigators: Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol *18(9)*: 1274-1284, 2017. PMID: 28754483. DOI: 10.1016/S1470-2045(17)30469-2

7 Falkenberg KJ and Johnstone RW: Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov *13(9)*: 673-691, 2014. PMID: 25131830. DOI: 10.1038/nrd4360

8 Sonnemann J, Gänge J, Pilz S, Stötzer C, Ohlinger R, Belau A, Lorenz G and Beck JF: Comparative evaluation of the treatment efficacy of suberoylanilide hydroxamic acid (SAHA) and paclitaxel in ovarian cancer cell lines and primary ovarian cancer cells from patients. BMC Cancer *6*: 183, 2006. PMID: 16834771. DOI: 10.1186/1471-2407-6-183

9 Modesitt SC, Sill M, Hoffman JS, Bender DP and Gynecologic Oncology Group: A phase II study of vorinostat in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol *109(2)*: 182-186, 2008. PMID: 18295319. DOI: 10.1016/j.ygyno.2008.01.009

10 Yano M, Yasuda M, Sakaki M, Nagata K, Fujino T, Arai E, Hasebe T, Miyazawa M, Miyazawa M, Ogane N, Hasegawa K and Narahara H: Association of histone deacetylase expression with histology and prognosis of ovarian cancer. Oncol Lett *15(3)*: 3524-3531, 2018. PMID: 29456726. DOI: 10.3892/ol.2018.7726

11 Miyake Y, Keusch JJ, Wang L, Saito M, Hess D, Wang X, Melancon BJ, Helquist P, Gut H and Matthias P: Structural insights into HDAC6 tubulin deacetylation and its selective inhibition. Nat Chem Biol *12(9)*: 748-754, 2016. PMID: 27454931. DOI: 10.1038/nchembio.2140

12 Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF and Yao TP: HDAC6 is a microtubule-associated deacetylase. Nature *417(6887)*: 455-458, 2002. PMID: 12024216. DOI: 10.1038/417455a

13 Wang L, Xiang S, Williams KA, Dong H, Bai W, Nicosia SV, Khochbin S, Bepler G and Zhang X: Depletion of HDAC6 enhances cisplatin-induced DNA damage and apoptosis in non-small cell lung cancer cells. PLoS One *7(9)*: e44265, 2012. PMID: 22957056. DOI: 10.1371/journal.pone.0044265

14 Yano M, Katoh T, Miyazawa M, Miyazawa M, Ogane N, Miwa M, Hasegawa K, Narahara H and Yasuda M: Clinicopathological correlation of ARID1A status with HDAC6 and its related factors in ovarian clear cell carcinoma. Sci Rep *9(1)*: 2397, 2019. PMID: 30787326. DOI: 10.1038/s41598-019-38653-0

15 Lienlaf M, Perez-Villarroel P, Knox T, Pabon M, Sahakian E, Powers J, Woan KV, Lee C, Cheng F, Deng S, Smalley KSM, Montecino M, Kozikowski A, Pinilla-Ibarz J, Sarnaik A, Seto E, Weber J, Sotomayor EM and Villagra A: Essential role of HDAC6 in the regulation of PD-L1 in melanoma. Mol Oncol 10(5): 735-750, 2016.PMID: 26775640. DOI: 10.1016/j.molonc.2015.12.012

16 Yee AJ, Bensinger WI, Supko JG, Voorhees PM, Berdeja JG, Richardson PG, Libby EN, Wallace EE, Birrer NE, Burke JN, Tamang DL, Yang M, Jones SS, Wheeler CA, Markelewicz RJ and Raje NS: Ricolinostat plus lenalidomide, and dexamethasone in relapsed or refractory multiple myeloma: a multicentre phase 1b trial. Lancet Oncol *17(11)*: 1569-1578, 2016. PMID: 27646843. DOI: 10.1016/S1470-2045(16)30375-8

17 Santo L, Hideshima T, Kung AL, Tseng JC, Tamang D, Yang M, Jarpe M, van Duzer JH, Mazitschek R, Ogier WC, Cirstea D, Rodig S, Eda H, Scullen T, Canavese M, Bradner J, Anderson KC, Jones SS and Raje N: Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. Blood *119(11)*: 2579-2589, 2012. PMID: 22262760. DOI: 10.1182/blood-2011-10-387365

18 Bitler BG, Wu S, Park PH, Hai Y, Aird KM, Wang Y, Zhai Y, Kossenkov AV, Vara-Ailor A, Rauscher FJ III, Zou W, Speicher DW, Huntsman DG, Conejo-Garcia JR, Cho KR, Christianson DW and Zhang R. ARID1A-mutated ovarian cancers depend on HDAC6 activity. Nat Cell Biol *19(8)*: 962-973,2017. PMID: 28737768. DOI:10.1038/ncb3582

19 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45(2)*: 228-247, 2009. PMID: 19097774. DOI: 10.1016/j.ejca.2008.10.026

20 Böhm S, Faruqi A, Said I, Lockley M, Brockbank E, Jeyarajah A, Fitzpatrick A, Ennis D, Dowe T, Santos JL, Cook LS, Tinker AV, Le ND, Gilks CB and Singh N: Chemotherapy Response Score: Development and Validation of a System to Quantify Histopathologic Response to Neoadjuvant Chemotherapy in Tubo-Ovarian High-Grade Serous Carcinoma. J Clin Oncol *33(22)*: 2457-2463, 2015. PMID: 26124480. DOI: 10.1200/JCO.2014.60.5212

21 Cohen PA, Powell A, Böhm S, Gilks CB, Stewart CJR, Meniawy TM, Bulsara M, Avril S, Brockbank EC, Bosse T, de Azevedo Focchi GR, Ganesan R, Glasspool RM, Howitt BE, Kim HS, Lee JY, Le ND, Lockley M, Manchanda R, Mandalia T, McCluggage WG, McNeish I, Midha D, Srinivasan R, Tan YY, van der Griend R, Yunokawa M, Zannoni GF, HGSC CRS Collaborative Network (Supplementary 1) and Singh N: Pathological chemotherapy response score is prognostic in tubo-ovarian high-grade serous carcinoma: A systematic review and meta-analysis of individual patient data. Gynecol Oncol *154(2)*: 441-448, 2019. PMID: 31118141. DOI: 10.1016/j.ygyno.2019.04.679

22 Li C, Cao L, Xu C, Liu F, Xiang G, Liu X, Jiao J and Niu Y: The immunohistochemical expression and potential prognostic value of HDAC6 and AR in invasive breast cancer. Hum Pathol *75*: 16-25 ,2018. PMID: 29180246. DOI: 10.1016/j.humpath.2017.11.010

23 Xie X, Luo K, Li Y, Ling Y, Zhang S, Xie X and Wen J: Histone deacetylase 6 expression in metastatic lymph nodes is a valuable prognostic marker for resected node-positive esophageal squamous cell cancer. Cancer Manag Res *10*: 5451-5460, 2018. PMID: 30519093. DOI: 10.2147/CMAR.S178575

24 Zhang Z, Cao Y, Zhao W, Guo L and Liu W: HDAC6 serves as a biomarker for the prognosis of patients with renal cell carcinoma. Cancer Biomark *19(2)*: 169-175, 2017. PMID: 28128740. DOI: 10.3233/CBM-160298

25 Angelucci A, Mari M, Millimaggi D, Giusti I, Carta G, Bologna M and Dolo V: Suberoylanilide hydroxamic acid partly reverses resistance to paclitaxel in human ovarian cancer cell lines. Gynecol Oncol *119(3)*: 557-563,2010. PMID: 20825984. DOI: 10.1016/j.ygyno.2010.07.036

26 Marcus AI, O'Brate AM, Buey RM, Zhou J, Thomas S, Khuri FR, Andreu JM, Díaz F and Giannakakou P: Farnesyltransferase inhibitors reverse taxane resistance. Cancer Res *66(17)*: 8838-8846, 2006. PMID: 16951201. DOI: 10.1158/0008-5472.CAN-06-0699

27 Marcus L, Lemery SJ, Keegan P and Pazdur R: FDA approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. Clin Cancer Res *25(13)*: 3753-3758, 2019. PMID: 30787022. DOI: 10.1158/1078-0432.CCR-18-4070

28 Bae J, Hideshima T, Tai YT, Song Y, Richardson P, Raje N, Munshi NC and Anderson KC: Histone deacetylase (HDAC) inhibitor ACY241 enhances anti-tumor activities of antigen-specific central memory cytotoxic T lymphocytes against multiple myeloma and solid tumors. Leukemia *32(9)*: 1932-1947, 2018. PMID: 29487385. DOI: 10.1038/s41375-018-0062-8

29 Fukumoto T, Fatkhutdinov N, Zundell JA, Tcyganov EN, Nacarelli T, Karakashev S, Wu S, Liu Q, Gabrilovich DI and Zhang R: HDAC6 Inhibition synergizes with anti-pd-l1 therapy in arid1a-inactivated ovarian cancer. Cancer Res *79(21)*: 5482-5489, 2019. PMID: 31311810. DOI: 10.1158/0008-5472.CAN-19-1302

30 Dong J, Zheng N, Wang X, Tang C, Yan P, Zhou HB and Huang J: A novel HDAC6 inhibitor exerts an anti-cancer effect by triggering cell cycle arrest and apoptosis in gastric cancer. Eur J Pharmacol *828*: 67-79, 2018. PMID: 29563065. DOI: 10.1016/j.ejphar.2018.03.026

31 Azuma K, Urano T, Horie-Inoue K, Hayashi S, Sakai R, Ouchi Y and Inoue S: Association of estrogen receptor alpha and histone deacetylase 6 causes rapid deacetylation of tubulin in breast cancer cells. Cancer Res *69(7)*: 2935-2940, 2009. PMID: 19318565. DOI: 10.1158/0008-5472.CAN-08-3458

32 Amengual JE, Johannet P, Lombardo M, Zullo K, Hoehn D, Bhagat G, Scotto L, Jirau-Serrano X, Radeski D, Heinen J, Jiang H, Cremers S, Zhang Y, Jones S and O'Connor OA: Dual targeting of protein degradation pathways with the selective hdac6 inhibitor acy-1215 and bortezomib is synergistic in lymphoma. Clin Cancer Res *21(20)*: 4663-4675, 2015. PMID: 26116270. DOI: 10.1158/1078-0432.CCR-14-3068

33 Tang J, Shi Y, Liu N, Xu L, Zang X, Li P, Zhang J, Zheng X, Qiu A and Zhuang S: Blockade of histone deacetylase 6 protects against cisplatin-induced acute kidney injury. Clin Sci (Lond) *132(3)*: 339-359, 2018. PMID: 29358506. DOI: 10.1042/CS20171417

34 Krukowski K, Ma J, Golonzhka O, Laumet GO, Gutti T, van Duzer JH, Mazitschek R, Jarpe MB, Heijnen CJ and Kavelaars A: HDAC6 inhibition effectively reverses chemotherapy-induced peripheral neuropathy. Pain *158(6)*: 1126-1137, 2017. PMID: 28267067. DOI: 10.1097/j.pain.0000000000000893

35 Lee EK, Tan-Wasielewski Z, Matulonis UA, Birrer MJ, Wright AA, Horowitz N, Konstantinopoulos PA, Curtis J and Liu JF: Results of an abbreviated Phase Ib study of the HDAC6 inhibitor ricolinostat and paclitaxel in recurrent ovarian, fallopian tube, or primary peritoneal cancer. Gynecol Oncol Rep *29*: 118-122, 2019. PMID: 31467965. DOI: 10.1016/j.gore.2019.07.010

36 Miyazawa M, Yasuda M, Miyazawa M, Ogane N, Katoh T, Yano M, Hirasawa T, Mikami M and Ishimoto H: Hypoxia-inducible factor-1α suppression in ovarian clear-cell carcinoma cells by silibinin administration. Anticancer Res *40(12)*: 6791-6798, 2020. PMID: 33288572. DOI: 10.21873/anticanres.14702

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