

Associations of sirtuins with clinicopathological variables and prognosis in human ovarian cancer

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Abstract. Ovarian cancer (OC) is the fifth most frequent cause of cancer-associated mortality worldwide, and is accompanied by asymptomatic progression. Sirtuins (SIRT) are a family of nicotinamide adenine dinucleotide-dependent protein deacetylases, comprising seven members (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7). Accumulating evidence has demonstrated that SIRT act as prognostic estimators in certain types of cancer such as lung cancer, prostate cancer, gastric cancer, breast cancer and colorectal cancer. However, it remains unknown whether individual SIRTs can serve as independent prognostic factors in OC. In the present study, the Kaplan-Meier plotter online database was utilized to examine the prognostic values of SIRT mRNA expression in patients with OC. The results demonstrated that the overexpression of SIRT3, SIRT5, SIRT6 and SIRT7 mRNAs was associated with a good prognosis in patients, whereas elevated mRNA levels of SIRT1 and SIRT4 indicated poor survival in patients with OC. In addition, among the favorable predictors, SIRT3, SIRT5, SIRT6 and SIRT7 overexpression were associated with overall survival (OS), according to clinical characteristics, such as histological classification, clinical stage, pathology grade, drug therapy and tumor protein p53 mutation status in patients with OC. Similarly, SIRT4 mRNA overexpression was

associated with poor OS in pathological grade III cancer. High SIRT1 and SIRT4 expression were associated with unfavorable OS at all clinical stages. Furthermore, SIRT1 and SIRT4 were negatively associated with OS in drug-treated patients. In summary, the present study demonstrated that the SIRT family is associated with the prognosis of human OC, suggesting that individual SIRTs may also act as prognostic predictors in patients.

Introduction

Ovarian cancer (OC) is one of the most frequent causes of mortality associated with gynecologic malignancy, and the fifth leading cause of health issues among women and cancer-associated deaths worldwide during the past 2 decades (1,2). A large number of patients (>50%) are diagnosed at an advanced stage, mainly due to the asymptomatic development of OC (3). Currently, the therapeutic strategies for OC consist of radical surgical resection, chemotherapy based on taxanes and platinum, and targeted therapeutic management (4). Despite the aforementioned treatments, the overall survival (OS) rate remains at only ~30% (5), partially due to drug resistance and a lack of specific biomarkers that can be used to detect the disease. Therefore, it is urgent to identify favorable prognostic factors of OC to improve the clinical outcomes of patients.

Sirtuins (SIRT) are a family of deacetylases that comprises seven types in mammals (SIRT1-7), with different subcellular localization patterns and enzymatic activities (6). Since the discovery of SIRT, the seven members, activated by nicotinamide adenine dinucleotide, have been closely associated with an extended life span by counteracting oxidative damage (7). Therefore, SIRT could contribute greatly to aging (8). Among the seven identified SIRTs, SIRT1 is located in the nucleus; SIRT2 is located in the cytoplasm; SIRT3, SIRT4 and SIRT5 are localized in the mitochondria; and SIRT6 and SIRT7 are present in the nucleus. Notably, due to the unique ability of SIRTs to control the redox environment, accumulating evidence has demonstrated that SIRTs are involved in the pathology of various cancer types such as lung cancer, prostate cancer, gastric cancer and breast cancer (9-13). More specifically,

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Abbreviations: SIRT, sirtuin; OC, ovarian cancer; KM plotter, Kaplan-Meier plotter; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio; CI, confidence interval; TP53, tumor protein p53; CRC, colorectal cancer

Key words: sirtuins, Kaplan-Meier plotter, ovarian cancer, prognosis, predictor

previous studies have reported that SIRT1s act as independent prognostic factors of several carcinomas, including colorectal cancer and non-small cell lung cancer (14,15). To date, few individual SIRT1s have been reported to be associated with OC. Shuang *et al* (16) found that SIRT1 could contribute to chemoresistance and the invasive capacity of OC cells, thereby boosting the proliferation of OC. Additionally, silencing of SIRT1 increases the protein expression of estrogen receptor β , which is regarded as an effective inhibitor of OC cells (17). On the other hand, SIRT3 exerts an antitumor effect on the induction of mitochondrial-dependent apoptosis via 5' AMP-activated protein kinase activation in OC cells (18). Regarding SIRT6, its dual roles as a tumor oncogene and suppressor in OC remain ambiguous (19,20). Furthermore, the prognostic values of the SIRT family in OC remain to be elucidated. In the present study, using the Kaplan-Meier (KM) plotter, the prognostic significance of the SIRT transcription family was comprehensively investigated in patients with OC.

Materials and methods

Acquisition of data and statistical analysis. The prognostic values of individual SIRT mRNA levels from 1,657 patients with OC were investigated using the online KM plotter (<http://kmplot.com/analysis>) database. Until now, 54,675 genes are included in the database and thus can be examined to analyze the survival of patients with breast cancer (21), lung cancer (22), OC (23) and gastric cancer. In the present study, OS, progression-free survival (PFS) and post-progression survival (PPS) of patients with primary epithelial OC were assessed using the KM survival plot. Furthermore, clinical characteristics, including two main primary epithelial OC histologies, stage, grade, tumor protein p53 (TP53) mutation status and treatment choice were analyzed. Generally, seven SIRT subtypes (SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7) were input into the database (<http://kmplot.com/analysis/index.php?p=service&cancer=ovar>) to generate KM survival plots. Individuals were divided into two groups (high expression group and low expression group), according to the median expression of the SIRT gene. The hazard ratios (HRs) with 95% CIs and log-rank P values were illustrated with the Cox proportional hazard model in the database. $P < 0.05$ was considered to indicate a statistically significant difference.

Tumor xenograft model. A2780 OC cells were purchased from American type culture collection and cultured to establish a nude mice tumor model. For culture, Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Inc.) containing streptomycin (100 $\mu\text{g}/\text{ml}$), penicillin (100 U/ml) and 10% FBS (Thermo Fisher Scientific, Inc.) were used. The medium was replaced every 2 days, and the cells were incubated in a moist atmosphere containing 5% CO_2 at 37°C. Once adherent cells had grown to ~90% confluence, the cells were digested with 0.25% trypsin-0.02% EDTA for subculture and subsequent experimental treatment.

The athymic nude mice (BALB/C-nu/nu; age, 6 weeks; male; weight, 18-22 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and bred in pathogen-free conditions under 22°C, 12 h light/12 h dark, with free access to

sterile water and food, in the Wenzhou Medical University Laboratory Animal Center (Wenzhou, China). A total of 15 randomized nude mice were used for the tumor xenograft model. The mice were anesthetized with, and 1×10^7 A2780 OC cells (in 100 μl PBS) were injected subcutaneously into the armpit of each nude mouse. After 3 weeks, the mice were euthanized with 2% isoflurane excess carbon dioxide with the flow rate of 3l/min. The animal study was approved by the Wenzhou Medical University Ethics Committee (approval no. wydw2019-0214).

Immunohistochemical staining. The tumor tissues were harvested for immunohistochemistry. Tumor tissues were fixed in 4% paraformaldehyde at 25°C overnight, dehydrated with different concentrations of ethanol (75, 85, 95 and 100%) and 100% dimethylbenzene separately, and embedded in paraffin. The specimens were subsequently cut into 4- μm thick sections. The sections were rehydrated by placing them in a descending alcohol series (100, 90, 85 and 75%) and ddH₂O for 5 min. Subsequently, the sections were washed with PBS for 5 min. The washing step was repeated twice more. Following that, slides were placed in 0.01 M sodium citrate buffer (Merck KGaA) at 95°C for 5 min and then cooled to room temperature. The slides were blocked with 10% bovine serum albumin (BSA; Merck KGaA) for 1 h at 37°C. Tissue sections were incubated with SIRT1 (1:200; cat. no. 13161-1-AP; Proteintech Group, Inc.), SIRT3 (1:200; cat. no. ab217319; Abcam) and SIRT6 (1:200; cat. no. 13572-1-AP; ProteinTech Group, Inc.) primary antibodies overnight at 4°C. Secondary antibody conjugated to horseradish peroxidase (1:200; cat. no. PV-6001; Origene Technologies, Inc.) was then added to the slides for 1 h at 37°C and 3,3'-diaminobenzidine were subsequently added to the slices for 2 min. The sections were washed with PBS 3 times. The sections were dried and stained with hematoxylin for 5 min followed by staining with differentiation solution for 1 min. The sections were washed once with running water for 10 min and covered with a coverslip of neutral resin. An inverted light microscope was used to observe the expression of SIRT1, SIRT3 and SIRT6 in the tumor tissues at a magnification of $\times 200$.

Results

Multivariate analysis and survival outcomes of patients with OC based on the expression of SIRT1s. KM survival data on all seven SIRT members examined in the present study can be acquired from www.kmplot.com. Firstly, the prognostic value of SIRT1 (Affymetrix ID, 218878_s_at) was evaluated. OS, PFS and PPS curves were generated for all patients with OC (Fig. 1A). High SIRT1 expression was significantly associated with worse OS ($P = 0.0029$; HR, 1.22; 95% CI, 1.07-1.39) and PFS ($P = 0.016$; HR, 1.17; 95% CI, 1.03-1.33). However, there was no association identified between SIRT1 and PPS ($P = 0.2$; HR, 1.12; 95% CI, 0.94-1.34). In the present study, the two common histological subtypes of ovarian cancer (endometrioid and serous cancer) were used for subsequent analyses. Furthermore, the OS curves of patients with different OC subtypes were plotted (Fig. 1B). The results demonstrated that the OS of patients with endometrioid cancer ($P = 0.053$; HR, 4.94; 95% CI, 0.82-29.69) or serous cancer ($P = 0.074$;

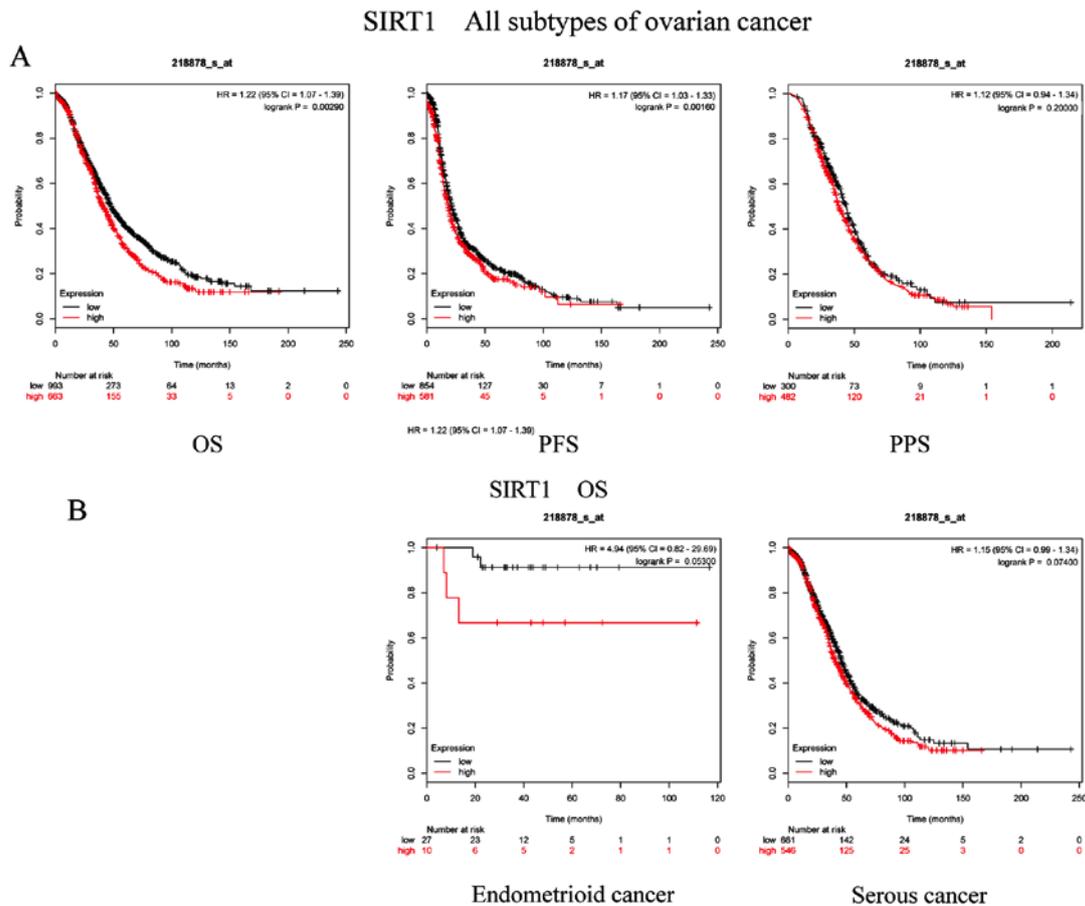


Figure 1. Prognostic significance of SIRT1 mRNA expression in OC patients. Prognostic significance of SIRT1 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

HR, 1.15; 95% CI, 0.99-1.34) was not associated with SIRT1 mRNA expression.

Furthermore, the prognostic significance of SIRT2 mRNA expression (Affymetrix ID, 220605_s_at) was analyzed. Elevated SIRT2 levels were significantly associated with PFS (P=0.025; HR, 0.85; 95% CI, 0.74-0.98) and PPS (P=0.011; HR, 1.27; 95% CI, 1.06-1.53) in patients with OC (Fig. 2A). By contrast, mRNA expression levels of SIRT2 in patients with OC did not exhibit any association with OS (P=0.4; HR, 0.95; 95% CI, 0.83-1.08). Histological subtype outcomes indicated that SIRT2 expression had no effect on the OS of patients with endometrioid cancer (P=0.19; HR, 3.84; 95% CI, 0.43-34.41) or serous cancer (P=0.17; HR, 1.13; 95% CI, 0.95-1.33; Fig. 2B).

Additionally, the prognostic role of SIRT3 mRNA expression (Affymetrix ID, 221913_at) was examined (Fig. 3A and B). High expression of SIRT3 was associated with favorable OS (P=0.00093; HR, 0.8; 95% CI, 0.7-0.91) and PPS (P=0.045; HR, 0.84; 95% CI, 0.71-1.00) in patients with OC. However, there was no significant association between PFS and SIRT3 mRNA levels in patients with OC (P=0.083; HR, 0.89; 95% CI, 0.77-1.02). With regard to serous cancer, high expression of SIRT3 exhibited an evident effect on OS among patients with OC (P=0.00962; HR, 0.82; 95% CI, 0.7-0.95). Furthermore, there was no significant association between the SIRT3 mRNA levels and the OS of patients with endometrioid cancer (P=0.38; HR, 0.46; 95% CI, 0.08-2.75).

Subsequently, the prognostic implications of SIRT4 mRNA expression (Affymetrix ID, 220047_at) were explored. High mRNA expression levels of SIRT4 were significantly associated with unfavorable OS (P=0.0012; HR, 1.24; 95% CI, 1.09-1.41), PFS (P=0.00017; HR, 1.27; 95% CI, 1.12-1.44) and PPS (P=0.013; HR, 1.24; 95% CI, 1.05-1.47) in patients with OC (Fig. 4A). Additionally, increased SIRT4 mRNA expression also indicated poor OS in patients with both endometrioid cancer (P=0.016; HR, 9.36; CI, 1.04-84.6) and serous cancer (P=0.011; HR, 1.22; 95% CI, 1.05-1.42; Fig. 4B).

The prognostic importance of SIRT5 mRNA expression (Affymetrix ID, 229112_at) was subsequently examined. Interestingly, enhanced SIRT5 mRNA levels were notably associated with improved OS (P=0.048; HR, 0.81; 95% CI, 0.66-1.00) and PPS (P=0.0011; HR, 0.66; 95% CI, 0.52-0.85), but with poor PFS (P=0.0018; HR, 1.36; 95% CI, 1.12-1.65; Fig. 5A). Additionally, Fig. 5B reveals the association between SIRT5 and the OC subtypes. It was clear that the upregulation of SIRT5 mRNA expression was notably associated with favorable OS in serous cancer (P=0.036; HR, 0.78; 95% CI, 0.62-0.98). However, SIRT5 mRNA expression was not associated with OS in patients with endometrioid cancer (P=0.32; HR, 3.01; 95% CI, 0.31-29).

Subsequently, the prognostic significance of SIRT6 mRNA expression levels (Affymetrix ID, 219613_s_at) was investigated. High SIRT6 expression was significantly associated

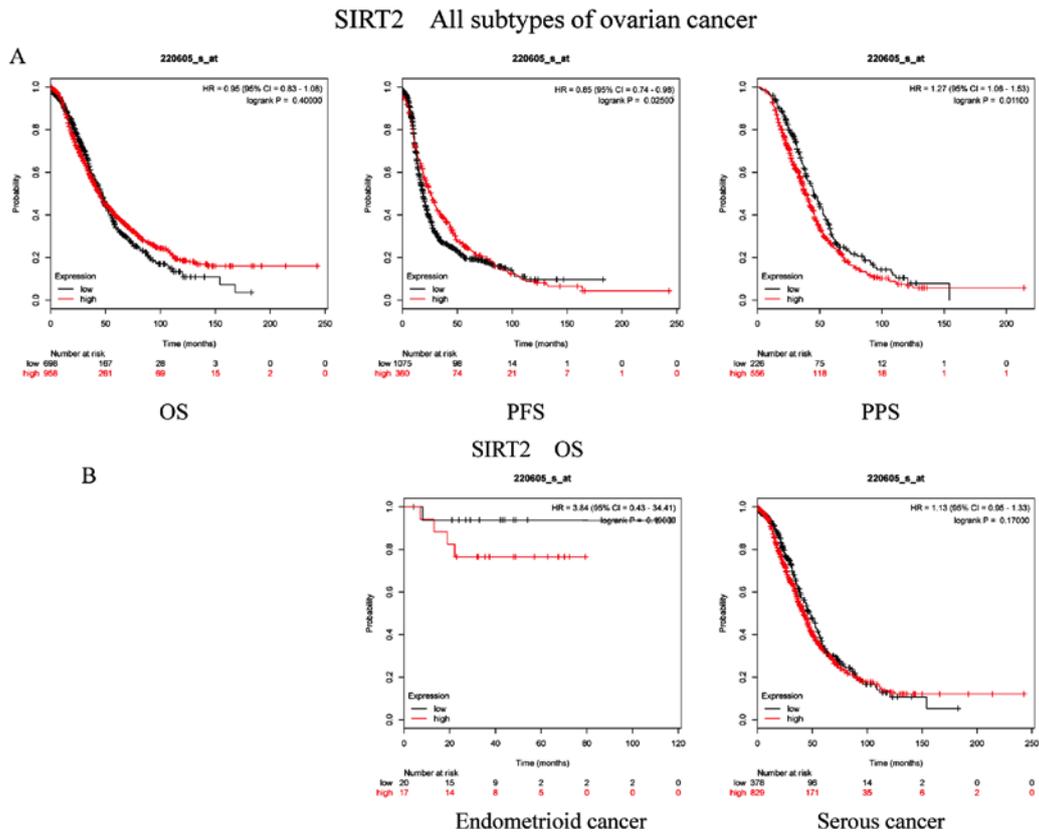


Figure 2. Prognostic significance of SIRT2 mRNA expression in OC patients. Prognostic significance of SIRT2 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

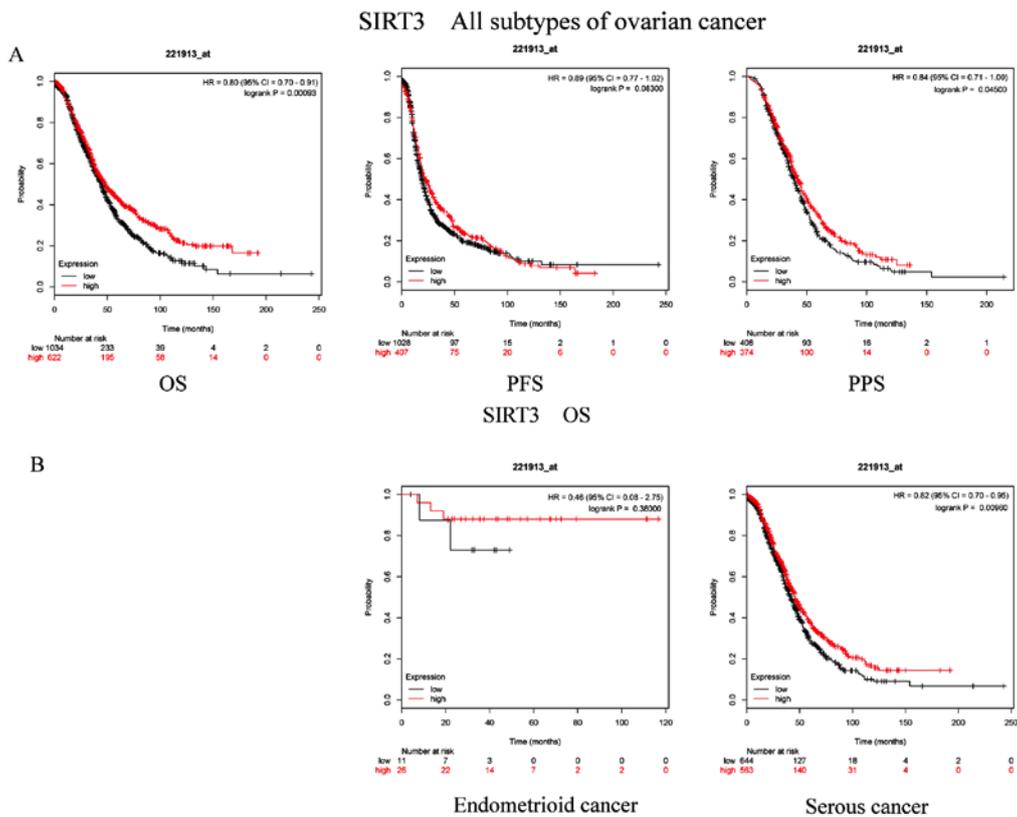


Figure 3. Prognostic significance of SIRT3 mRNA expression in OC patients. Prognostic significance of SIRT3 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

SIRT4 All subtypes of ovarian cancer

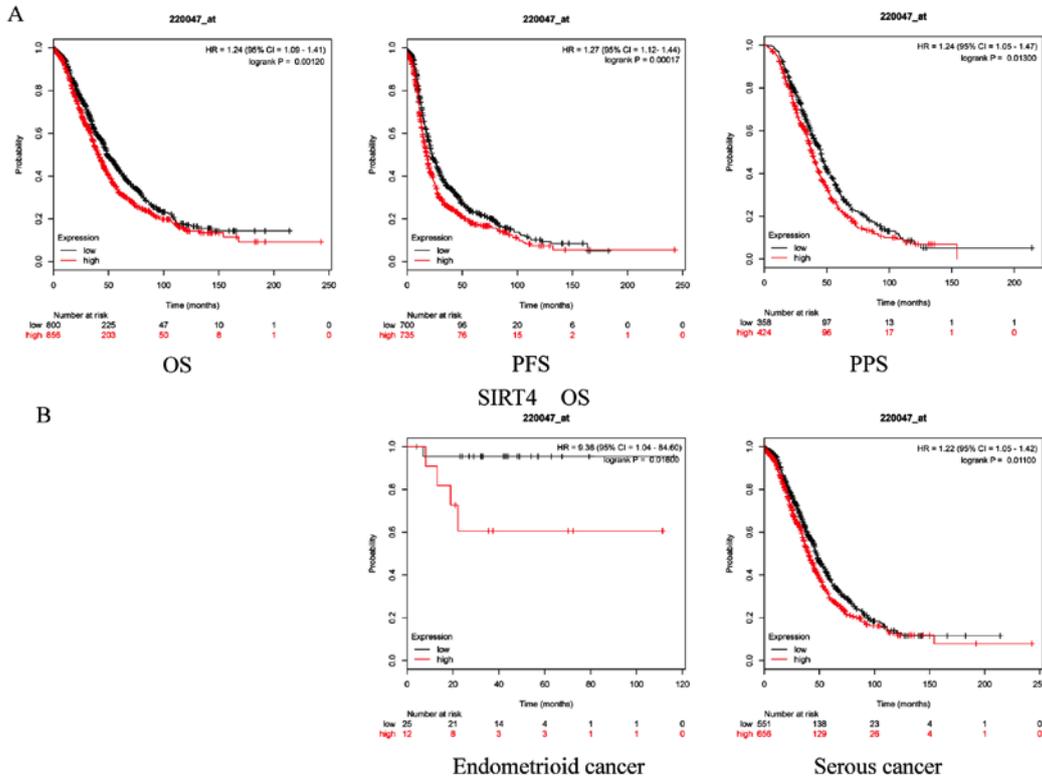


Figure 4. Prognostic significance of SIRT4 mRNA expression in OC patients. Prognostic significance of SIRT4 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

SIRT5 All subtypes of ovarian cancer

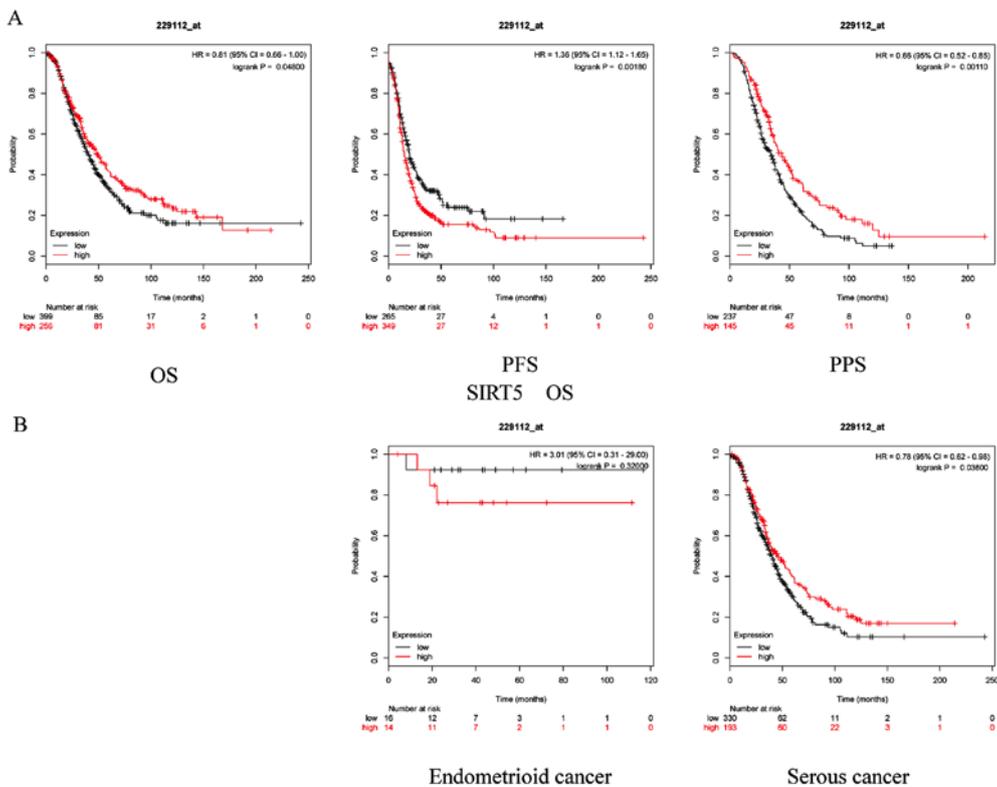


Figure 5. Prognostic significance of SIRT5 mRNA expression in OC patients. Prognostic significance of SIRT5 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

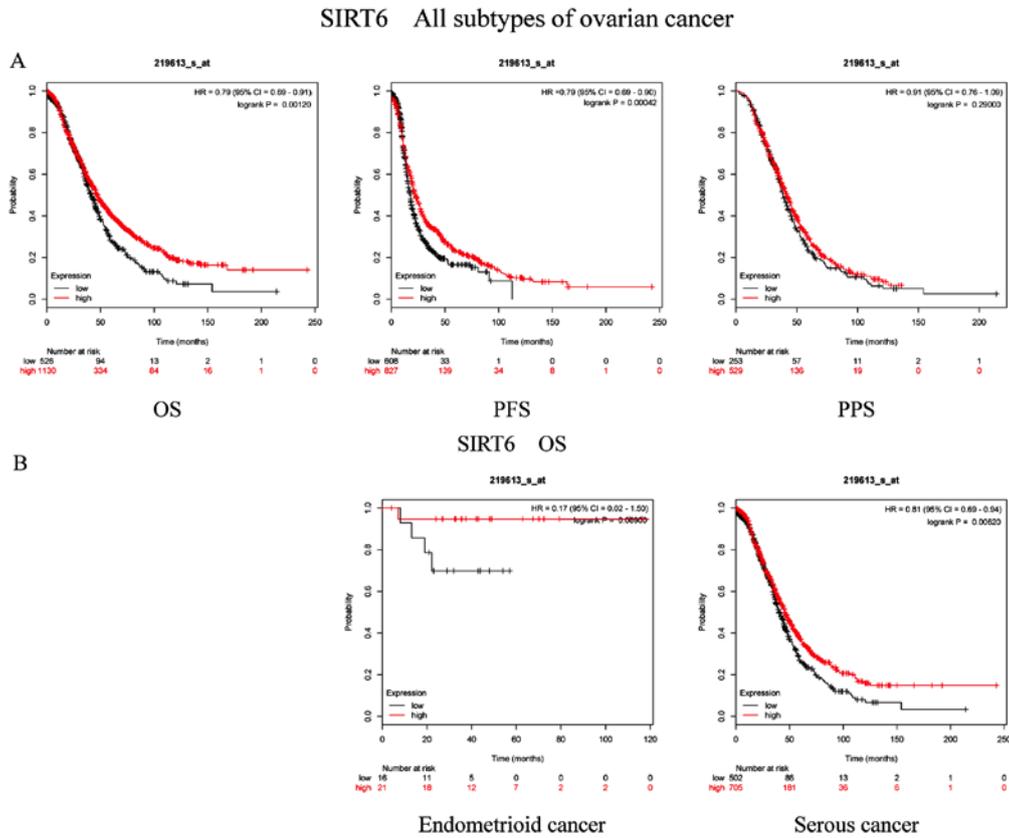


Figure 6. Prognostic significance of SIRT6 mRNA expression in OC patients. Prognostic significance of SIRT6 mRNA expression (A) in all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

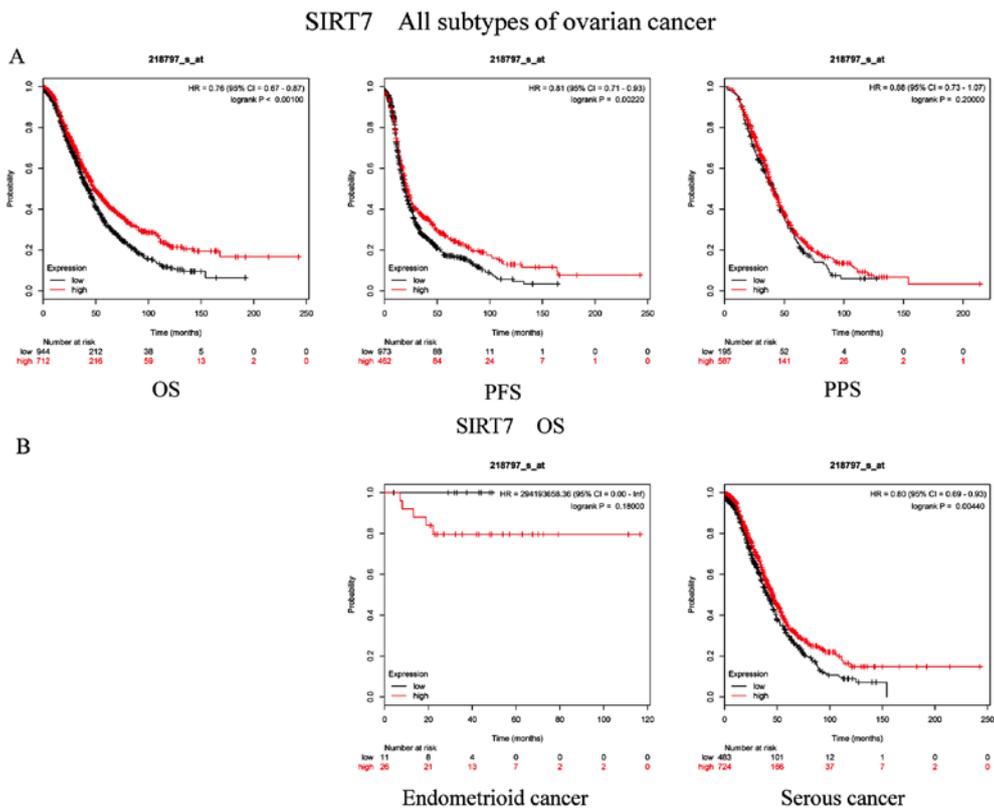


Figure 7. Prognostic significance of SIRT7 mRNA expression in OC patients. Prognostic significance of SIRT7 mRNA expression in (A) all patients with OC and (B) in patients with different subtypes of OC. All patients with OC, n=1,656; patients with endometrioid cancer, n=37; patients with serous cancer, n=1,207. OC, ovarian cancer; SIRT, sirtuin; OS, overall survival; PFS, progression-free survival; PPS, post-progression survival; HR, hazard ratio.

Table I. Associations between elevated SIRT mRNA expression and overall survival of all patients with ovarian cancer, according to different pathological grades.

SIRT	Pathological grade	Cases, n	HR (95% CI)	P-value
SIRT1	I	56	0.68 (0.25-1.68)	0.4498
	II	324	1.29 (0.95-1.77)	0.1025
	III	1,015	1.18 (0.99-1.41)	0.0719
SIRT2	I	56	3.04 (0.98-9.38)	0.0425
	II	324	0.82 (0.58-1.15)	0.2536
	III	1,015	1.10 (0.92-1.33)	0.3024
SIRT3	I	56	1.75 (0.67-4.55)	0.2446
	II	324	0.58 (0.43-0.79)	0.0005
	III	1,015	0.84 (0.70-1.00)	0.0522
SIRT4	I	56	0.71 (0.28-1.81)	0.4768
	II	324	1.24 (0.91-1.69)	0.1805
	III	1,015	1.32 (1.10-1.58)	0.0026
SIRT5	I	41	2.06 (0.71-6.96)	0.1744
	II	162	0.71 (0.44-1.13)	0.1488
	III	392	0.81 (0.61-1.08)	0.1560
SIRT6	I	56	0.43 (0.16-1.13)	0.0792
	II	324	0.61 (0.45-0.83)	0.0013
	III	1,015	0.82 (0.69-0.97)	0.0183
SIRT7	I	56	0.63 (0.24-1.64)	0.3374
	II	324	0.78 (0.57-1.05)	0.1052
	III	1,015	0.73 (0.61-0.88)	0.0008

Pathological grades were classified according to the pathological classification (52). SIRT, sirtuin; HR, hazard ratio.

with improved OS (P=0.0012; HR, 0.79; 95% CI, 0.69-0.91) and PFS (P=0.00042; HR, 0.79; 95% CI, 0.69-0.90) in patients with OC. Despite these results, SIRT6 exhibited no effect on PPS (P=0.29; HR, 0.91; 95% CI, 0.76-1.09) in patients with OC (Fig. 6A). With respect to the histological subtype of OC, elevated SIRT6 mRNA expression was associated with good OS in patients with serous cancer (P=0.0062; HR, 0.81; 95% CI, 0.69-0.94), but not in patients with endometrioid cancer (P=0.069; HR, 0.17; 95% CI, 0.02-1.50; Fig. 6B).

Finally, the prognostic role of SIRT7 (Affymetrix ID, 218797_s_at) was studied. High SIRT7 mRNA expression was significantly associated with good OS (P=5.3x10⁻⁵; HR, 0.76; 95% CI, 0.67-0.87) and PFS (P=0.0022; HR, 0.81; 95% CI, 0.71-0.93) in all patients with OC. However, there was no association between SIRT7 expression and PPS (P=0.2; HR, 0.88; 95% CI, 0.73-1.07) in patients diagnosed with OC (Fig. 7A). Regarding the subtypes, increased SIRT7 mRNA levels were significantly associated with improved OS in patients with serous cancer (P=0.0044; HR, 0.8; 95% CI, 0.69-0.93), but not in patients with endometrioid cancer [P=0.18; HR, 294,193,658.36 (0-inf); Fig. 7B].

Associations between high mRNA expression levels of SIRT members and other clinicopathological characteristics. Additionally, the associations of each SIRT family

Table II. Associations between elevated SIRT mRNA expression and overall survival of all patients with ovarian cancer, according to different clinical stages.

SIRT	Clinical stages	Cases, n	HR (95% CI)	P-value
SIRT1	I+II	135	1.96 (0.90-4.28)	0.0862
	III+IV	1,220	1.18 (1.02-1.37)	0.0298
SIRT2	I+II	135	0.50 (0.23-1.09)	0.0763
	III+IV	1,220	1.11 (0.94-1.31)	0.2335
SIRT3	I+II	135	0.51 (0.19-1.35)	0.1682
	III+IV	1,220	0.75 (0.64-0.89)	0.0007
SIRT4	I+II	135	0.52 (0.20-1.40)	0.1883
	III+IV	1,220	1.26 (1.07-1.49)	0.0052
SIRT5	I+II	83	1.93 (0.70-5.36)	0.1976
	III+IV	487	0.68 (0.54-0.87)	0.0015
SIRT6	I+II	135	0.23 (0.07-0.77)	0.009
	III+IV	1,220	0.77 (0.67-0.90)	0.0008
SIRT7	I+II	135	0.37 (0.17-0.81)	0.0097
	III+IV	1,220	0.83 (0.71-0.96)	0.0130

The number of patients expressing SIRT5 is different compared with all the other SIRT genes. The expression level of SIRT5 is affected by factors such as study design, patient selection and statistical analyses. Clinical stage was classified according to the FIGO (International Federation of Gynecology and Obstetrics) classification (53). SIRT, sirtuin; HR, hazard ratio.

Table III. Associations between elevated SIRT mRNA expression and overall survival of all patients with ovarian cancer, according to the TP53 mutation condition.

SIRT	TP53 mutation	Cases, n	HR (95% CI)	P-value
SIRT1	Mutant	506	1.21 (0.95-1.53)	0.1256
	Wild	94	1.53 (0.85-2.76)	0.1545
SIRT2	Mutant	506	1.55 (1.22-1.97)	0.0003
	Wild	94	0.64 (0.37-1.12)	0.1153
SIRT3	Mutant	506	0.51 (0.19-1.35)	0.0072
	Wild	94	0.54 (0.29-0.99)	0.0432
SIRT4	Mutant	506	1.19 (0.94-1.49)	0.1407
	Wild	94	1.69 (0.95-3.02)	0.0720
SIRT5	Mutant	124	0.50 (0.32-0.77)	0.0015
	Wild	19	0.51 (0.16-1.64)	0.2497
SIRT6	Mutant	506	1.17 (0.92-1.49)	0.2102
	Wild	94	0.67 (0.38-1.18)	0.1677
SIRT7	Mutant	506	1.19 (0.94-1.50)	0.1515
	Wild	94	1.30 (0.72-2.34)	0.3808

The number of patients expressing SIRT5 is different compared with all the other SIRT genes. The expression level of SIRT5 is affected by factors such as study design, patient selection and statistical analyses. SIRT, sirtuin; HR, hazard ratio.

member with pathological grade (Table I), clinical stage (Table II), TP53 mutation status (Table III) and chemotherapy

Table IV. Associations between elevated SIRT mRNA expression and overall survival of all patients with ovarian cancer, according to different chemotherapy methods.

SIRT	Chemotherapy method	Cases, n	HR (95% CI)	P-value
SIRT1	Contains Platinum	1,049	1.21 (1.05-1.40)	0.0092
	Contains Taxol	793	1.25 (1.03-1.51)	0.0207
	Contains Taxol+Platinum	776	1.28 (1.06-1.55)	0.0113
SIRT2	Contains Platinum	1,049	0.90 (0.76-1.05)	0.1792
	Contains Taxol	793	0.88 (0.73-1.07)	0.1980
	Contains Taxol+Platinum	776	0.88 (0.73-1.07)	0.2007
SIRT3	Contains Platinum	1,049	0.83 (0.70-0.97)	0.0199
	Contains Taxol	793	0.77 (0.64-0.93)	0.0069
	Contains Taxol+Platinum	776	0.81 (0.67-0.98)	0.0289
SIRT4	Contains Platinum	1,049	1.26 (1.09-1.44)	0.0014
	Contains Taxol	793	1.31 (1.07-1.60)	0.0090
	Contains Taxol+Platinum	776	1.30 (1.06-1.60)	0.0111
SIRT5	Contains Platinum	478	0.79 (0.62-1.00)	0.0540
	Contains Taxol	357	0.82 (0.61-1.10)	0.1903
	Contains Taxol+Platinum	356	0.82 (0.61-1.11)	0.2017
SIRT6	Contains Platinum	1,049	0.81 (0.70-0.93)	0.0031
	Contains Taxol	793	0.77 (0.63-0.94)	0.0094
	Contains Taxol+Platinum	776	0.75 (0.61-0.92)	0.0057
SIRT7	Contains Platinum	1,049	0.79 (0.68-0.90)	0.0008
	Contains Taxol	793	0.74 (0.61-0.89)	0.0014
	Contains Taxol+Platinum	776	0.74 (0.60-0.91)	0.0043

The number of patients expressing SIRT5 is different compared with all the other SIRT genes. The expression level of SIRT5 is affected by factors such as study design, patient selection and statistical analyses. Platinum, platinum-based therapies; SIRT, sirtuin; HR, hazard ratio.

choice (Table IV) were determined. Table II shows that SIRT1 and SIRT4 were significantly associated with poor OS in patients with stage III/IV OC, whereas SIRT3 and SIRT5 predicted improved OS. Moreover, SIRT6 and SIRT7 were associated with favorable OS in patients with stage I/II OC, as well as those with stage III/IV. Consistent to the KM outcomes, SIRT2 exhibited no association with OS in patients with stage I/II or III/IV. With regard to the pathological grade in Table I, SIRT2 and SIRT4 indicated poor OS in pathological grades I and III, respectively. However, SIRT3 and SIRT7 were associated with significantly improved OS in patients with OC of pathological grades II and III, respectively. Elevated levels of SIRT6 exhibited a significant association with improved OS in both pathological grades II and III. In terms of TP53 mutation (Table III), the results demonstrated the significant associations of SIRT2 and SIRT5 with OS in patients with OC that have a TP53 mutation. In addition, SIRT3 was associated with favorable OS in patients with mutant and wild-type TP53. With the exception of SIRT2 and SIRT5, the associations between high mRNA expression levels of other SIRT family members and chemotherapy agents were significant (Table IV).

Tumor xenograft and IHC results. Fig. S1 represents excised tumors with scale bars and tumor volume over time statistical graph (Fig. S1B and S1C). In addition, using IHC staining it was demonstrated that SIRT1, SIRT3, SIRT6 were expressed in various degrees in the tumor tissues (Fig. S1A).

Discussion

OC is one of the most lethal types of gynecological malignancies, which affects the health condition of female patients worldwide. Despite the development of medical technology, the incidence and OS rates remain unsatisfactory, due to the unique biological characteristics of OC (24). Thus, identifying a novel biomarker for the prognostic prediction of OC is necessary. Previously, the SIRT family has been reported to serve a critical part in the process of tumorigenesis (25-27). Each individual SIRT may act either as a tumor suppressor or an oncogene in different types of malignancies, potentially via tumor-associated signaling pathways or mitosis regulation (13,28,29). However, the specific association between SIRTs and OC remains controversial, and remains to be further clarified.

In the present study, the prognostic values of seven individual SIRTs were comprehensively assessed in OC via the KM plotter online database. According to the analysis, most of the SIRT members act as either tumor promoters or inhibitors in OC. As a consequence, the high mRNA expression levels of SIRT1 and SIRT4 were associated with an unfavorable prognosis in patients with OC. Similarly, previous studies have also verified the roles of the aforementioned two SIRTs in other carcinomas, such as lung cancer (30,31), breast cancer (32-34), gastric cancer (35,36), and prostate cancer (37). Specifically, Shin *et al.* (30) reported that high SIRT1 expression was identified in non-small cell

lung cancer, which was highly associated with drug resistance via the SIRT1-mitogen-activated protein kinase signaling pathway. Additionally, SIRT1 could aggravate invasion capacity and metastasis in prostate cancer via the induction of epithelial-to-mesenchymal transition (37). Consistently, the present study demonstrated that elevated mRNA levels of SIRT1 were associated with poor OS in patients with OC, notably those at a high clinical stage (III+IV) and those that received chemotherapy. SIRT4 has been demonstrated to be a tumor suppressor as it causes inhibition of cell proliferation and metastasis (38). To the best of our knowledge, no study has reported the prognostic value of SIRT4 in OC. The present study demonstrated that SIRT4 expression was inversely associated with prognosis in OC. In patients with OC of a high clinical stage (III+IV), poor differentiation (pathological grade III) and who received chemotherapy, high SIRT4 expression was associated with unfavorable OS. This observation may provide a novel insight into the effect of SIRT4 on the regulation of OC. Above all, these results support the use of SIRT1 and SIRT4 as potential biomarkers for prognostic prediction in OC.

The tumor suppressive effects of SIRT3, SIRT5, SIRT6 and SIRT7 in OC were also determined in the present study. To the best of our knowledge, mitochondrial localized SIRT3 (39), with its unique characteristic, serves as a crucial regulator of the Warburg effect (40) in breast cancer via the inhibition of reactive oxygen species and the destabilization of HIF-1 α . Previous studies have demonstrated that SIRT3 can suppress the metastasis capacity and induce apoptosis in OC (18,41). Consistently, the KM plotter results of the present study demonstrated that high SIRT3 expression may be associated with a favorable prognosis in patients. Additionally, patients with pathological grade II, a high clinical stage (III+IV), a TP53 mutation and those who received chemotherapy experienced improved OS, further indicating that increased SIRT3 expression predicts prolonged OS. SIRT5 has been reported to possess dual roles in the regulation of various types of carcinoma, suggesting that SIRT5 acts as a tumor suppressor in hepatic cancer by inhibiting acyl-CoA oxidase 1 (42). However, SIRT5 has also been demonstrated to function as an oncogene in the carcinogenesis of colorectal cancer (CRC), potentially by stimulating glutamine metabolism through the activation of dehydrogenase 1 during the tricarboxylic-acid cycle in CRC cells (43). The fact that SIRT5 exhibits dual effects on the regression of different tumors may depend on the dominant factor in the microenvironment and the signaling pathway. In the present study, SIRT5 overexpression was associated with a favorable prognosis in OC, and elevated SIRT5 levels in patients with clinical stage III+IV and TP53 mutations were associated with improved OS, implying that SIRT5 is a potential biomarker of OC. Previous studies referring to SIRT6 have investigated its role as a tumor suppressor, and its decreased expression is associated with poor OS in patients with pancreatic cancer (44), hepatic carcinoma (45) and colon cancer (46). The present study demonstrated that SIRT6 overexpression was associated with good prognosis in OC. An increased SIRT6 level also predicted favorable OS in patients with poor differentiation (pathological grades II+III), in all clinical stages and in patients who received chemotherapy. However, Bae *et al* (19) have demonstrated that SIRT6 expression is notably associated with a poor prognosis in OC. This

difference could be attributed to the patient selection, sample size, study design, statistical method and detection means (the present study focused on mRNA expression, whereas the former study focused on the protein degrees). According to the SIRT7 mRNA analysis, the present study revealed that increased levels of SIRT7 predicted a good prognosis in patients with OC, notably those with serous cancer and pathological grade II and in all clinical stages. Additionally, SIRT7 overexpression was associated with improved OS in patients who received any type of chemotherapy. The data in the present study differed from those described in previous studies, in that SIRT7 acted as an oncogene in tumor progression (14,47,48), which provides a novel basis for predicting the potential of SIRT7 in OC.

Furthermore, the analysis using the KM plotter of each of the SIRT members revealed contradictory associations with PFS, PPS and OS. Theoretically, OS may better represent patient prognosis (in contrast to PFS and PPS); however, because PFS and PPS compose OS, OS is in turn affected by these two factors (49). These conflicting associations may partially result from differences in the chemotherapy choice, sample size, sensitivity to diagnosis and treatment between two SIRT expression groups (50,51). Therefore, the PFS and PPS data may provide another direction for further studies investigating the role of the SIRT family in the prognosis of OC.

In conclusion, the present study suggested that the mRNA expression levels of SIRTs (SIRT1, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7) are associated with prognosis in patients with OC. Additionally, it was revealed that the SIRT family members demonstrated potential prediction capability for other clinicopathological features, including the histological type, pathological grade, clinical stage, TP53 mutation status and mainstream chemotherapy choice. However, these results are limited to the mRNA expression levels of SIRTs. Therefore, analysis of the protein expression levels is required in future studies. Overall, the present study provides a novel prospect for future studies on specific signaling pathways through which SIRTs may participate in the tumorigenesis, progression and metastasis of OC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LW and QH conceived and designed the present study. KC and PG analyzed the data. QH, RY and ND wrote the manuscript.

RY performed the animal experiments and histological examinations. ND revised the manuscript for intellectual content and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal study was approved by the Wenzhou Medical University Ethics Committee (approval no. wyd2019-0214).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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