Original paper

Prognostic and clinicopathological implications of expression of Beclin-1 and hypoxia-inducible factor 1α in serous ovarian carcinoma: an immunohistochemical study

Mohamed Alabiad[1], Ola Harb[1], Doaa Mandour[2], Rehab Hemeda[2], Rham Z. Ahmed[3], Ahmed El-Taher[4], Gamal Osman[4], Amany Shalaby[5], Amr A.A. Alnemr[6], Mostafa T. Abdelfattah[6]

[1]Pathology Department, Faculty of Medicine, Zagazig University, Egypt
[2]Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University, Egypt
[3]Medical Oncology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[4]General Surgery Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[5]Histology and Cell Biology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
[6]Obstetrics and Gynecology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Serous ovarian carcinoma (SOC) is an ovarian cancer with a high fatality rate. Therefore, a lot of researchers have tried to identify novel prognostic biomarkers which might improve the patient prognosis.

The aims of the study were to detect the tissue protein expression of Beclin-1 in addition to HIF-1α in SOC patients, to evaluate the relationship between their expression, the clinicopathological parameters, patients' prognosis, and the relation to chemotherapy resistance in SOC.

We evaluated the expression of Beclin-1 in addition to HIF-1α in 60 patients with SOC using immunohistochemistry, followed all patients for about 36 months, analyzed associations between both markers' expression, clinicopathological data, and patients' prognosis.

Beclin-1 expression was related to low grade ($p = 0.002$), early SOC stage, absence of peritoneal spread ($p = 0.006$), and absence of lymph nodes, and distant metastases ($p = 0.004$ and < 0.001 respectively), while HIF-1α expression was associated with higher grade and stage ($p = 0.007$), and presence of nodal and distant metastases ($p < 0.001$ and $= 0.012$ respectively).

High Beclin-1 expression and low HIF-1α expression were positively associated with good response to chemotherapy ($p = 0.047$ and $p = 0.022$ respectively), a lower recurrence rate after successful therapy ($p = 0.006$ and < 0.001 respectively), and increased three-year recurrence-free and overall survival rates ($p < 0.001$).

In SOC patients; Beclin-1 is a good prognostic marker, while HIF-1α is a poor prognostic marker.

Key words: serous ovarian carcinoma, Beclin-1, HIF-1α, survival, immunohistochemistry.

Introduction

Serous ovarian carcinoma (SOC) is a subtype of ovarian cancer with a high fatality rate. The absence of symptoms in the early stages of SOC leads to a delay in its diagnosis. As a result of that most patients are diagnosed in a late stage, with poorer prognosis. Cytoreductive surgery, in addition to currently used chemotherapeutics, is the 1st line of management of SOC patients, but many patients who receive chemotherapeutic agents develop chemo-resistance that subsequently worsens the prognosis [1]. Thus, a lot of researchers have tried to identify novel prognostic biomarkers which might improve survival and decrease the SOC recurrence rate. Recently, many studies have focused on the role of autophagy and its biomarkers in cancer research. Autophagy is a homeostatic recycling mechanism of intracellular structures, and it is a form of non-apoptotic programmed cell death. There is evidence that autophagy is required for normal cell survival and viability [2].

Many studies have tried to detect the role of abnormal regulation of autophagy in the initiation, promotion, or progression of malignancies [3], autophagy being considered a novel trend in research in the assessment of oncogenesis, cancer growth control, and clarification of how to overcome resistance to chemotherapy. However, the results are conflicting as to the exact mechanism of the regulation of autophagy as its role in cancer is complex [4, 5]. The autophagy-related protein Beclin1 was found to have a role in the induction thereof. Beclin-1 maps to the locus of a tumor susceptibility gene of 150 kb on chromosome 17q21 [6] which was primarily discovered as an onco-suppressor gene [7]. Beclin-1 loss leads to a decrease in autophagic activity with variable effects on different types of cancers [8].

Beclin1 expression fluctuates during malignant progression, so it was hypothesized that it might be related to it.

Ovarian cancer is a solid cancer that has a rapid rate of growth, and when angiogenesis fails to supply newly formed tumor cells with adequate and sufficient nutrients, tumor tissues will suffer from hypoxia that could induce tumor cell necrosis that could limit the increase in cancer size. However, marked hypoxia could lead to an increase in pro-survival stimulatory pathways and increased angiogenesis, which subsequently accelerated cancer cell invasion and metastasis [9].

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor formed of α and β subunits. It was the first detected mediator of the cellular hypoxia response in human cells [10]. Many researchers have contributed evidence that linked HIFs and carcinogenesis [11]. Moreover, previous studies have found important relations between autophagy and hypoxia, as it was found that under conditions of tumor tissue hypoxia, HIF-1α may lead to the induction of the autophagy cascade to remove damaged organelles, e.g. mitochondria, and decrease the degree of damage that is induced by reactive oxygen species to cells from apoptosis [12]. However, the possible combined role of autophagy and hypoxia in cancer has not been explained yet, particularly in cancer progression [13].

In the current study, we aimed to detect the tissue protein expression of Beclin-1 and HIF-1α in SOC patients using immunohistochemistry to evaluate the relationship between their expression, the clinicopathological parameters, patients' prognosis, and the relation to chemotherapy

Material and methods

The study was approved by the local ethical committee of the Faculty of Medicine, Zagazig University. 60 patients who were diagnosed with SOC were included. The patients were admitted to the General Surgery Department, and Gynecology and Obstetrics Department, Faculty of Medicine, Zagazig University Hospitals, for surgical resection of the tumor. The samples were sent to the Pathology Department, Faculty of Medicine, Zagazig University, for pathology examination and assessment of tumor grade and stage. After obtaining informed consent, patients with an established diagnosis of SOC were included in the study, while patients with other histopathological subtypes were excluded. TNM and FIGO systems were used for the staging of SOC [14] and the WHO system for grading [15]. Representative sections from 60 paraffin blocks were stained with primary antibodies against Beclin-1 and HIF-1α using immunohistochemistry, and assessed for tissue expression of each marker. Associations between markers' expression with clinical, pathological, prognostic, and follow-up parameters were analyzed.

The patients were divided into 2 groups as follows: i) patients who had achieved complete remission after an initial platinum-based regime of chemotherapy, and ii) patients who had recurrence at 6 months or more after finishing chemotherapy. These were considered chemo-sensitive. We considered patients who had a recurrence within 6 months as chemo-resistant. We followed our patients at the Clinical Oncology and Nuclear Medicine Department and Medical Oncology Department, Faculty of Medicine, Zagazig University for 3 years from March 2017 to April 2020. The follow-up was performed.

Immunohistochemical staining

We used the streptavidin-biotin method for immunohistochemistry [16]. Sections were stained with primary rabbit monoclonal anti-Beclin-1 antibody

Table I. Clinicopathological features and outcome

Characteristics	All patients (n = 60)
Age (years)	
Mean ±SD	55.53 ±10.53
Median (range)	57 (25-75)
< 40 years	4 (6.7%)
41-59 years	34 (56.7%)
≥ 60 years	22 (36.7%)
Positive cytology	
Absent	39 (65%)
Present	21 (35%)
CA125	
≤ 35 U/ml	21 (35%)
> 35 U/ml	39 (65%)
Bilaterality	
Unilateral	44 (73.3%)
Bilateral	16 (26.7%)
Implants	
Absent	38 (63.3%)
Present	22 (36.7%)
Ascites	
Absent	38 (63.3%)
Present	22 (36.7%)
Grade	
Low	23 (38.3%)
High	37 (61.7%)
LN	
Node negative	21 (35%)
Node positive	39 (65%)
M	
M0 (non-metastatic)	46 (76.7%)
M1 (metastatic)	14 (23.3%)
FIGO stage	
Stage IA	2 (3.3%)
Stage IB	1 (1.7%)
Stage IC	2 (3.3%)
Stage IIA	3 (5%)
Stage IIB	7 (11.7%)
Stage IIC	6 (10%)
Stage IIIA	9 (15%)
Stage IIIB	12 (20%)
Stage IIIC	4 (6.7%)
Stage IV	14 (23.3%)

Table I. Cont.

Characteristics	All patients (n = 60)
Operation	
Radical surgery	15 (25%)
Suboptimal	18 (30%)
Optimal	27 (45%)
ECOG PS	
ECOG 1	42 (70%)
ECOG 2	18 (30%)
Number of cycles	(n = 57)
4 cycles	10 (17.5%)
6 cycles	8 (14%)
8 cycles	39 (68.4%)
Response	(n = 45)
NR	10 (22.2%)
OAR	35 (77.8%)
Response after 4-6 cycles	(n = 45)
PD	3 (6.7%)
SD	10 (22.2%)
PR	29 (64.4%)
CR	3 (6.7%)
Response after 8 cycles	(n = 45)
PD	3 (6.7%)
SD	7 (15.6%)
PR	7 (15.6%)
CR	28 (62.2%)
Follow-up duration (months)	
Mean ±SD	17.01 ±9.15
Median (Range)	11 (10-36)
Recurrence	(n = 43)
Absent	12 (27.9%)
Present	31 (72.1%)
Chemosensitivity	(n = 31)
Chemosensitive	11 (35.5%)
Chemorefractory	20 (64.5%)
Death	
Alive	28 (46.7%)
Died	32 (53.3%)

(1 : 100, Abcam, EPR1733Y) and mouse monoclonal anti-HIF-1α antibody (1 : 200, Abcam, 1A3). Normal breast tissue and adenocarcinoma of the lung were considered positive controls for Beclin-1 and HIF-1α, respectively. For the negative control, we removed the primary antibodies, replacing them with phosphate-buffered saline (PBS).

Table IIA. Correlations between clinicopathological criteria and Beclin-1 expression in SOC

Characteristics	All (n = 60)	Beclin-1		p-value
		Low (n = 35)	High (n = 25)	
	No. (%)	No. (%)	No. (%)	
Age (years)				
Mean ±SD	55.53 ±10.53	61.20 ±7.23	47.60 ±9.28	< 0.001•
Median (range)	57 (25-75)	60 (48-75)	45 (25-60)	
< 40 years	4 (6.7%)	0 (0%)	4 (100%)	0.003‡
41-59 years	34 (56.7%)	16 (47.1%)	18 (52.9%)	
≥ 60 years	22 (36.7%)	19 (86.4%)	3 (13.6%)	
Positive cytology				
Absent	39 (65%)	14 (35.9%)	25 (100%)	0.009‡
Present	21 (35%)	21 (100%)	0 (0%)	
CA125				
≤ 35 U/ml	21 (35%)	3 (14.3%)	18 (85.7%)	0.02‡
> 35 U/ml	39 (65%)	32 (82.1%)	7 (17.9%)	
Bilaterality				
Unilateral	44 (73.3%)	22 (50%)	22 (50%)	0.030‡
Bilateral	16 (26.7%)	13 (81.3%)	3 (18.8%)	
Implants				
Absent	38 (63.3%)	14 (36.8%)	24 (63.2%)	0.006‡
Present	22 (36.7%)	21 (95.5%)	1 (4.5%)	
Ascites				
Absent	38 (63.3%)	17 (44.7%)	21 (55.3%)	0.005‡
Present	22 (36.7%)	18 (81.8%)	4 (18.2%)	
Grade				
Low	23 (38.3%)	3 (13%)	20 (87%)	0.002‡
High	37 (61.7%)	32 (86.5%)	5 (13.5%)	
LN				
Node negative	21 (35%)	3 (14.3%)	18 (85.7%)	0.004‡
Node positive	39 (65%)	32 (82.1%)	7 (17.9%)	
M				
M0 (non-metastatic)	46 (76.7%)	21 (45.7%)	25 (54.3%)	< 0.001‡
M1 (metastatic)	14 (23.3%)	14 (100%)	0 (0%)	
FIGO Stage				
Stage IA	2 (3.3%)	0 (0%)	2 (100%)	< 0.001§
Stage IB	1 (1.7%)	0 (0%)	1 (100%)	
Stage IC	2 (3.3%)	0 (0%)	2 (100%)	
Stage IIA	3 (5%)	0 (0%)	3 (100%)	
Stage IIB	7 (11.7%)	0 (0%)	7 (100%)	
Stage IIC	6 (10%)	3 (50%)	3 (50%)	
Stage IIIA	9 (15%)	6 (66.7%)	3 (33.3%)	
Stage IIIB	12 (20%)	9 (75%)	3 (25%)	
Stage IIIC	4 (6.7%)	3 (75%)	1 (25%)	
Stage IV	14 (23.3%)	14 (100%)	0 (0%)	

Table IIA. Cont.

Characteristics	All (N=60)	Beclin-1		p-value
		Low (N=35)	High (N=25)	
	No. (%)	No. (%)	No. (%)	
Beclin-1				
Low	35 (58.3%)			
High	25 (41.7%)			
HIF-1α				
Low	26 (43.3%)	4 (15.4%)	22 (84.6%)	< 0.001‡
High	34 (56.7%)	31 (91.2%)	3 (8.8%)	

**independent samples Student's t-test; • Mann-Whitney U test; ‡ χ^2 test; § χ^2 test for trend; p < 0.05 is significant.*

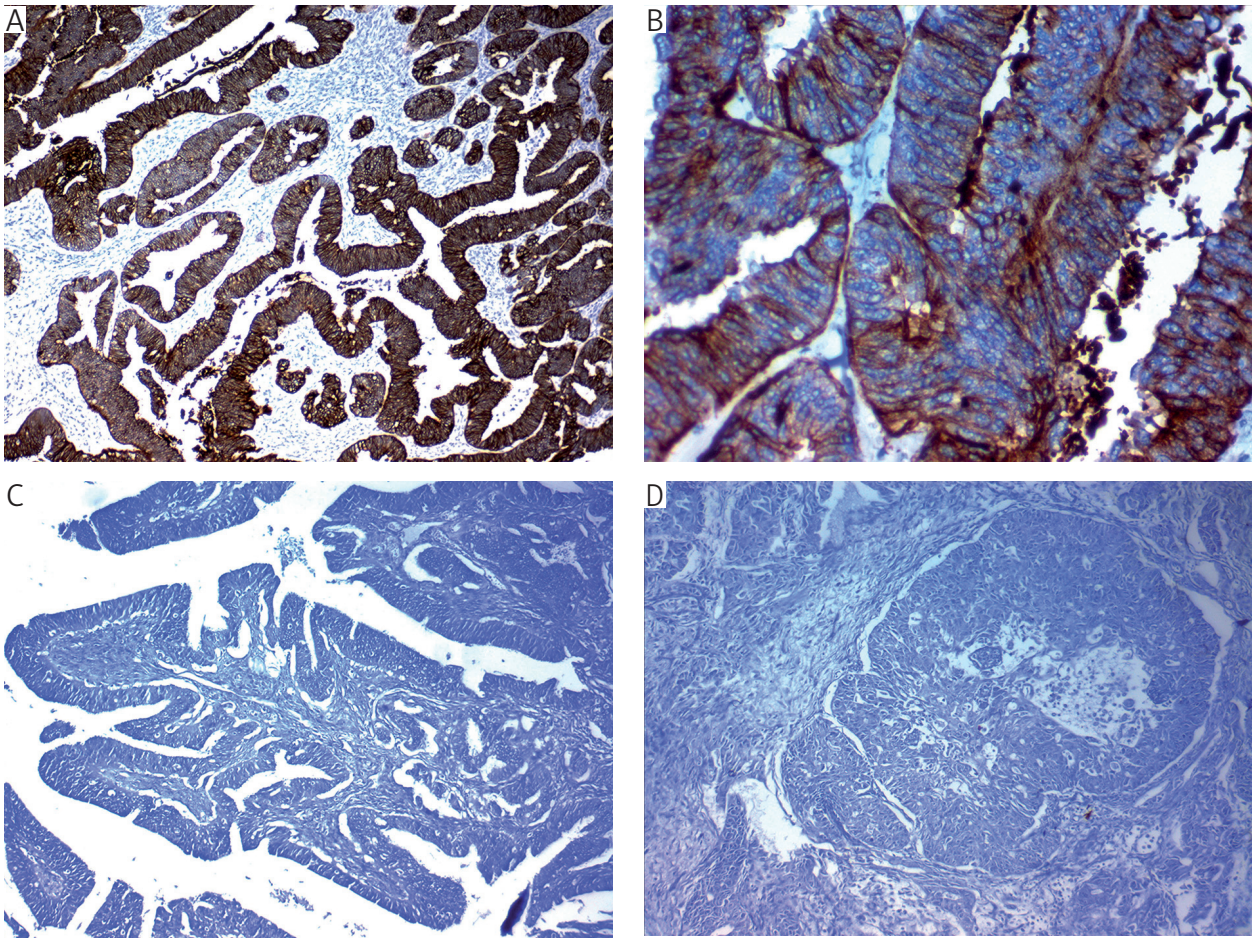

Fig. 1. Immunohistochemical expression of Beclin-1 in serous ovarian carcinoma (SOC): A) Expression in the cytoplasm of low grade and early stage SOC, 100×. B) Expression in the cytoplasm of low grade and early stage SOC, 400×. C) No expression in the cytoplasm of high grade and advanced stage SOC, 100×. D) No expression in the cytoplasm of high grade and advanced stage SOC, 400×

Evaluation of Beclin-1 and hypoxia-inducible factor 1α staining

The expression of Beclin1positive cells was assessed by evaluation of the cytoplasmic expression. The amount of positively stained cells among all cells in the sections was detected and given scores as follows: < 10%, 0; 10-20%, 1; 21-50%, 2; and > 50%, 3. The intensity of stain was also given scores as follows: no evidence of brown color, 0; pale yellow color, 1; light brown color, 2; and dark brown color, 3. The total final score was the sum of scores for the number of positively stained cells and the staining intensity. Cases with a score of ≤ 3 were consid-

Table IIB. Correlations between clinicopathological criteria and HIF-1α expression in SOC

Characteristics	All (n = 60)	HIF-1α		
		Low (n = 26)	High (n = 34)	p-value
	No. (%)	No. (%)	No. (%)	
Age (years)				
Mean ± SD	55.53 ±10.53	48.88 ±10.06	60.61 ±7.75	< 0.001•
Median (range)	57 (25-75)	47 (25-65)	60 (46-75)	
< 40 years	4 (6.7%)	4 (100%)	0 (0%)	0.002‡
41-59 years	34 (56.7%)	18 (52.9%)	16 (47.1%)	
≥ 60 years	22 (36.7%)	4 (18.2%)	18 (81.8%)	
Positive cytology				
Absent	39 (65%)	24 (61.5%)	15 (38.5%)	0.008‡
Present	21 (35%)	2 (9.5%)	19 (90.5%)	
CA125				
≤ 35 U/ml	21 (35%)	17 (81%)	4 (19%)	0.031‡
> 35 U/ml	39 (65%)	9 (23.1%)	30 (76.9%)	
Bilaterality				
Unilateral	44 (73.3%)	20 (45.5%)	24 (54.5%)	0.582‡
Bilateral	16 (26.7%)	6 (37.5%)	10 (62.5%)	
Implants				
Absent	38 (63.3%)	23 (60.5%)	15 (39.5%)	0.044‡
Present	22 (36.7%)	3 (13.6%)	19 (86.4%)	
Ascites				
Absent	38 (63.3%)	20 (52.6%)	18 (47.4%)	0.036‡
Present	22 (36.7%)	6 (27.3%)	16 (72.7%)	
Grade				
Low	23 (38.3%)	19 (82.6%)	4 (17.4%)	0.006‡
High	37 (61.7%)	7 (18.9%)	30 (81.1%)	
LN				
Node negative	21 (35%)	17 (81%)	4 (19%)	< 0.001‡
Node positive	39 (65%)	9 (23.1%)	30 (76.9%)	
M				
M0 (non-metastatic)	46 (76.7%)	24 (52.2%)	22 (47.8%)	0.012‡
M1 (metastatic)	14 (23.3%)	2 (14.3%)	12 (85.7%)	
FIGO Stage				
Stage IA	2 (3.3%)	2 (100%)	0 (0%)	0.007§
Stage IB	1 (1.7%)	1 (100%)	0 (0%)	
Stage IC	2 (3.3%)	2 (100%)	0 (0%)	
Stage IIA	3 (5%)	3 (100%)	0 (0%)	
Stage IIB	7 (11.7%)	5 (71.4%)	2 (28.6%)	
Stage IIC	6 (10%)	4 (66.7%)	2 (33.3%)	
Stage IIIA	9 (15%)	3 (33.3%)	6 (66.7%)	
Stage IIIB	12 (20%)	2 (16.7%)	10 (83.3%)	
Stage IIIC	4 (6.7%)	2 (50%)	2 (50%)	
Stage IV	14 (23.3%)	2 (14.3%)	12 (85.7%)	

Table IIB. Cont.

Characteristics	All (n = 60)	HIF-1α		
		Low (n = 26)	High (n = 34)	p-value
	No. (%)	No. (%)	No. (%)	
Beclin-1				
Low	35 (58.3%)	4 (11.4%)	31 (88.6%)	< 0.001‡
High	25 (41.7%)	22 (88%)	3 (12%)	
HIF-1α				
Low	26 (43.3%)			
High	34 (56.7%)			

**independent samples Student's t-test; • Mann-Whitney U test; ‡ χ^2 test; § χ^2 test for trend; p < 0.05 is significant.*

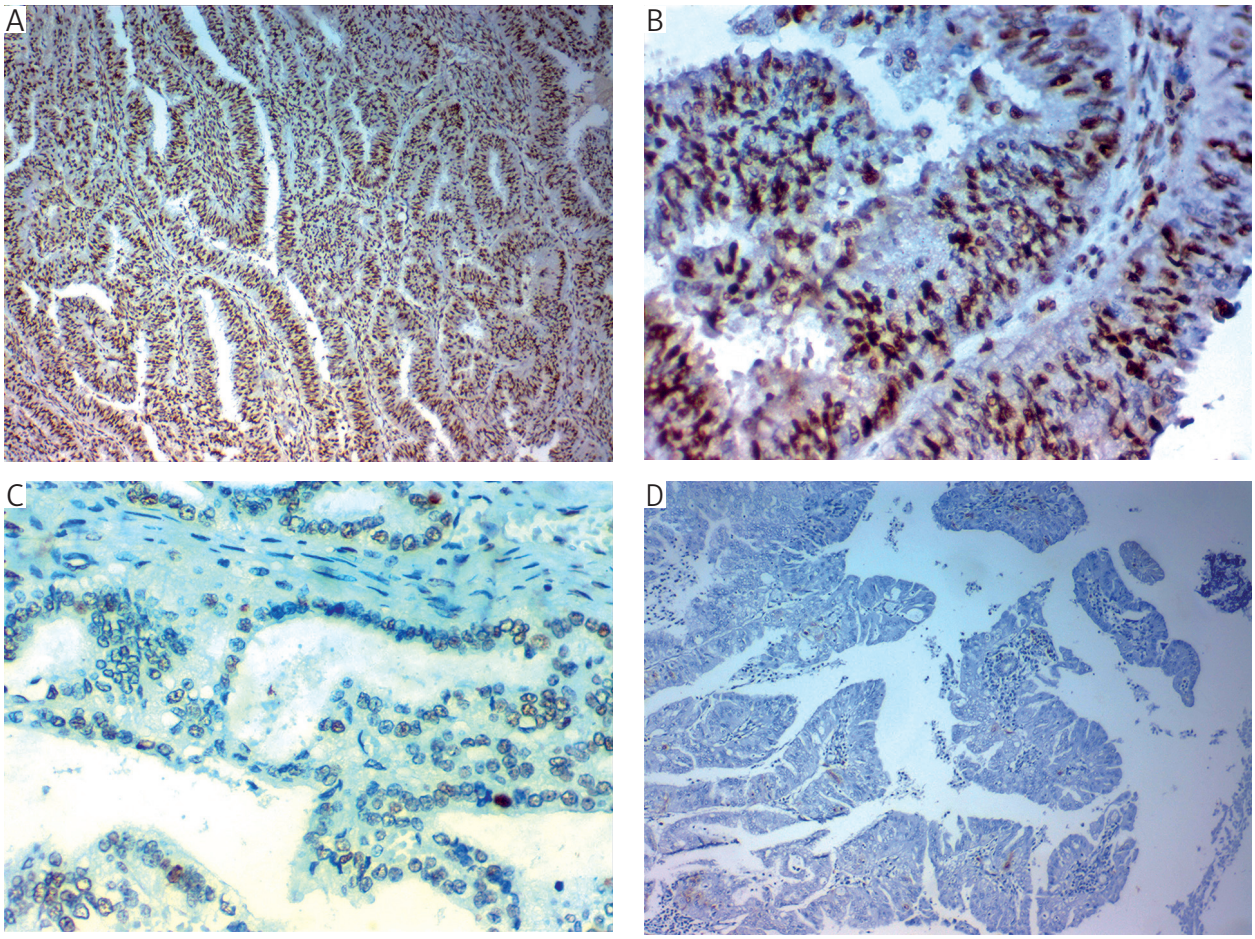

Fig. 2. Immunohistochemical staining of HIF-1α serous ovarian carcinoma (SOC): A) High nuclear expression in high grade SOC, 100 ×. B) High nuclear expression in high grade SOC, 400 ×. C, D) Low nuclear expression in low grade SOC, 400 ×

ered Beclin-1 negative, and those with a score of > 3 were considered Beclin-1 positive [1].

Regarding HIF-1α expression, positive tumor cells were assessed by evaluation of brown nuclear stain; low expression was defined if < 10% of tumor cells were positive, and high expression was defined if ≥ 10% of cells were positive [17].

Statistical analysis

Continuous variables were analyzed by calculation of the mean ± SD and median (range). We used the Mann-Whitney U test, Student's t-test, Shapiro-Wilk, χ^2, and Fisher's exact tests according to the type of data. The overall survival (OS) rate is the interval from cancer diagnosis to death. The re-

Table IIIA. Correlations between Beclin-1 expression in SOC and outcome of patients

Characteristics	All	Beclin-1		p-value
		Low	High	
	No. (%)	No. (%)	No. (%)	
Operation	(n = 60)	(n = 35)	(n = 25)	
Radical surgery	15 (25%)	0 (0%)	15 (60%)	< 0.001‡
Suboptimal	18 (30%)	17 (48.6%)	1 (4%)	
Optimal	27 (45%)	18 (51.4%)	9 (36%)	
ECOG PS	(n = 60)	(n = 35)	(n = 25)	
ECOG 1	42 (70%)	22 (62.9%)	20 (80%)	0.153‡
ECOG 2	18 (30%)	13 (37.1%)	5 (20%)	
Number of cycles	(n = 57)	(n = 35)	(n = 22)	
4 cycles	10 (17.5%)	0 (0%)	10 (45.5%)	< 0.001‡
6 cycles	8 (14%)	5 (14.3%)	3 (13.6%)	
8 cycles	39 (68.4%)	30 (85.7%)	9 (40.9%)	
Response	(n = 45)	(n = 35)	(n = 10)	
NR	10 (22.2%)	10 (28.6%)	0 (0%)	0.047‡
OAR	35 (77.8%)	25 (71.4%)	10 (100%)	
Response after 4-6	(n = 45)	(n = 35)	(n = 10)	
PD	3 (6.7%)	3 (8.6%)	0 (0%)	0.039‡
SD	10 (22.2%)	10 (28.6%)	0 (0%)	
PR	29 (64.4%)	19 (54.3%)	10 (100%)	
CR	3 (6.7%)	3 (8.6%)	0 (0%)	
Response after 8	(n = 45)	(n = 35)	(n = 10)	
PD	3 (6.7%)	3 (8.6%)	0 (0%)	0.050‡
SD	7 (15.6%)	7 (20%)	0 (0%)	
PR	7 (15.6%)	7 (20%)	0 (0%)	
CR	28 (62.2%)	18 (51.4%)	10 (100%)	
Recurrence	(n = 43)	(n = 18)	(n = 25)	
Absent	12 (27.9%)	1 (5.6%)	11 (44%)	0.006‡
Present	31 (72.1%)	17 (94.4%)	14 (56%)	
Chemosensitivity	(n = 31)	(n = 17)	(n = 14)	
Chemosensitive	11 (35.5%)	4 (23.5%)	7 (50%)	0.043‡
Chemorefractory	20 (64.5%)	13 (76.5%)	7 (50%)	
RFS	(n = 43)	(n = 17)	(n = 14)	
Mean (months)	20.2	14	24.4	< 0.001†
(95% CI)	(16.9-23.5)	(10.9-17.2)	(19.9-28.9)	
1 year RFS	48.8%	27.8%	64%	
2 year RFS	31.4%	6.9%	48%	
3 year RFS	23.2%	–	40%	
Death	(n = 60)	(n = 35)	(n = 25)	
Alive	28 (46.7%)	6 (17.1%)	22 (88%)	< 0.001‡
Died	32 (53.3%)	29 (82.9%)	3 (12%)	

Table IIIA. Cont.

Characteristics	All	Beclin-1		p-value
		Low	High	
	No. (%)	No. (%)	No. (%)	
OS	(n = 60)	(n = 35)	(n = 25)	
Mean (months)	22.3 months	14.1 months	33 months	< 0.001†
(95% CI)	(19-25.5)	(11.5-16.8)	(29.8-36.2)	
1 year OS	44.9%	14.7%	88%	
2 year OS	44.9%	14.7%	88%	
3 year OS	44.9%	14.7%	88%	

NR – no response; PD – progressive disease; SD – stable disease; PR – partial response; CR – complete response; 95% CI – 95% confidence interval; ‡ χ^2 test; † log-rank test; p < 0.05 is significant

currencefree survival (RFS) rate is the time from starting treatment to recurrence. The calculation and correlation of OS and RFS rates were performed according to markers estimated using the Kaplan-Meier plots then were compared using the log-rank test. The statistical analysis was done using SPSS 22.0 for Windows.

Results

Detailed pathological and demographic data are presented in Table I.

Immunohistochemistry

Beclin-1 tissue expression

Beclin-1 tissue expression in SOC was associated with younger patients' age (p = 0.003), lower grade (p = 0.002), early SOC stage, absence of lymph nodes and distant metastases (p = 0.004 and < 0.001 respectively), absence of peritoneal deposits (p = 0.006), negative peritoneal invasion (p = 0.009), absence of bilaterality (p = 0.03) and absence of ascites (p = 0.005) (Table IIA, Fig. 1).

Hypoxia-inducible factor 1α expression

HIF-1α expression in SOC was associated with older patients' age (p = 0.002), higher SOC grade, presence of peritoneal deposits (p = 0.006), advanced SOC stage (p = 0.007), malignant ascites (p = 0.036), occurrence of lymph nodes and distant metastases (p < 0.001 and = 0.012 respectively) (Table IIB, Fig. 2).

No statistically significant differences were detected between the expression of HIF-1α and the presence of disease bilaterality in SOC patients.

High Beclin-1 expression and low HIF-1α expression were positively associated with a good response to chemotherapy (p = 0.047 and = 0.022 respectively), tumor chemo-sensitivity, and a lower recurrence rate after therapy success (p = 0.006 and < 0.001 respectively), adequacy of surgical excision, and raised three-year recurrence-free and overall survival rates (p < 0.001) (Table IIIA, Fig. 3).

Tissue protein expression levels of both Beclin-1 and HIF-1α were negatively correlated with each other; r = –0.762 (p < 0.001) (Table IVA, B).

Discussion

Beclin-1 expression and its role in carcinogenesis were only recently recognized. Beclin-1 is found to be over-expressed in certain cancers, but its expression is down-regulated in others, which suggested its different roles in different cancers [1].

Autophagy has a variable role in carcinogenesis that depends on cellular properties and type, showing an inhibitory role in the early stages of oncogenesis by inhibiting inflammation and maintaining genome stability. However, it might act as a pro-survival process to protect malignant cells from death due to cellular stress. Moreover, if cellular stressful conditions continue to result in maintaining autophagy, it will be followed by death of cancer cells [18, 19]. Recently detected evidence that has linked autophagy to cancer suppression is that one of the oncogenesis regulators, Beclin-1, had a cancer suppressor role and it was found to be disabled or deleted in many malignant tumors [18].

Beclin-1 and clinical-pathological correlation in serous ovarian carcinoma

In the present study, we correlated SOC Beclin-1 tissues expression with pathological parameters, clinical findings, and follow-up and found that Beclin-1 expression in SOC was correlated with favorable findings as a high degree of differentiation and early stage.

Similarly, Osman *et al*., Qiu *et al*., Cai *et al*., and Wu *et al*. [17, 19. 20, 21] found that the expression of Beclin-1 was positively associated with a higher

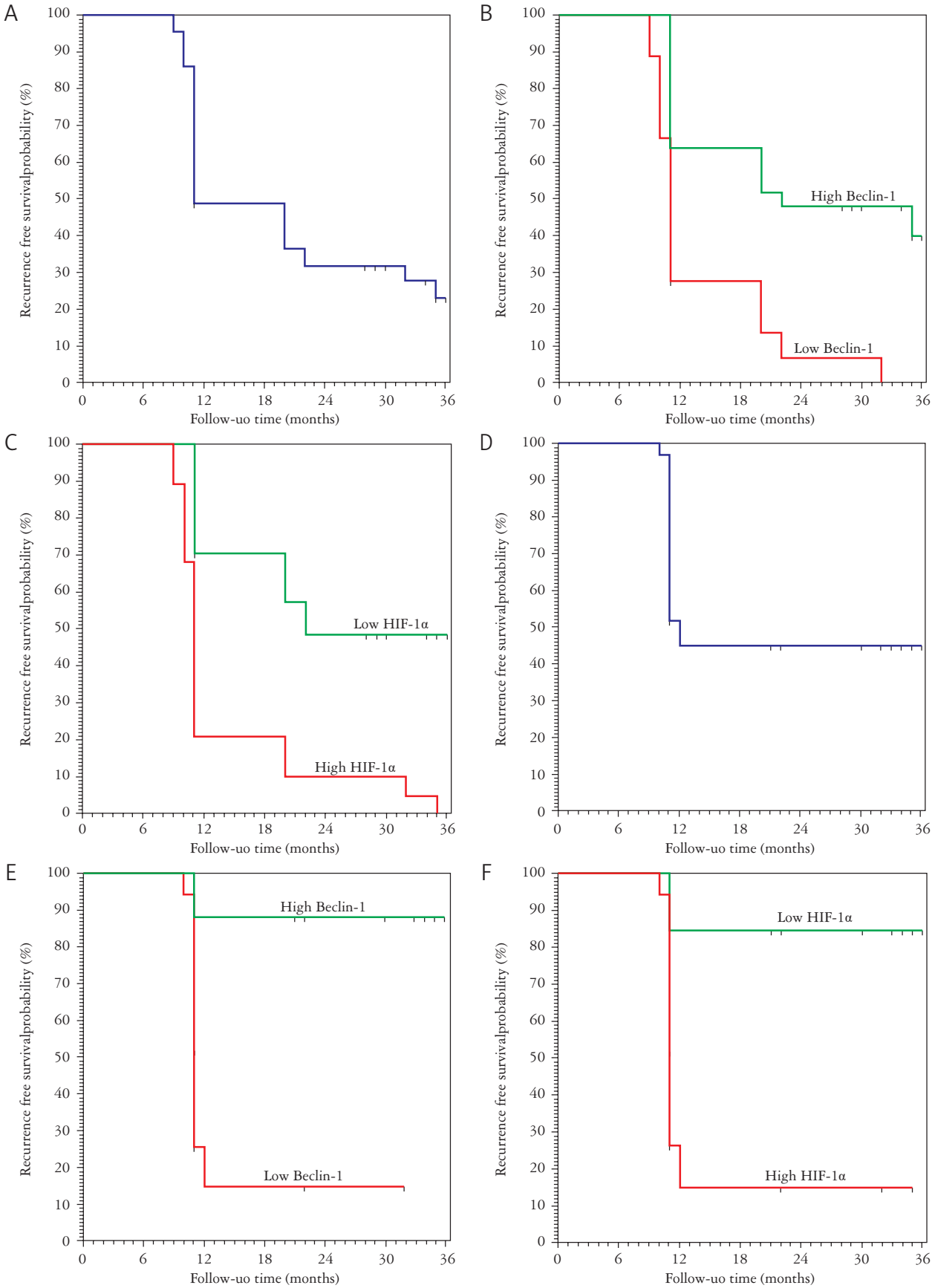

Fig. 3. Kaplan-Meier plot: Recurrence-free survival (A-C); A) All patients, B) Material stratified according to Beclin-1 expression in tumor tissues, C) Material stratified by according to HIF-1 expression in tumor tissues. Overall survival (D-F); D) All patients, E) Stratified according to Beclin-1 expression in tumor tissues, F) Stratified by according to HIF-1 expression in tumor tissues

Table IIIB. Correlations between HIF-1α expression in SOC and outcome of patients

Characteristics	All	HIF-1a		p-value
		Low	High	
	No. (%)	No. (%)	No. (%)	
Operation	(n = 60)	(n = 26)	(n = 34)	
Radical surgery	15 (25%)	13 (50%)	2 (5.9%)	< 0.001‡
Suboptimal	18 (30%)	4 (15.4%)	14 (41.2%)	
Optimal	27 (45%)	9 (34.6%)	18 (52.9%)	
ECOG PS	(n = 60)	(n = 26)	(n = 34)	
ECOG 1	42 (70%)	20 (76.9%)	22 (64.7%)	0.306‡
ECOG 2	18 (30%)	6 (23.1%)	12 (35.3%)	
Number of cycles	(n = 57)	(n = 23)	(n = 34)	
4 cycles	10 (17.5%)	8 (34.8%)	2 (5.9%)	0.010‡
6 cycles	8 (14%)	4 (17.4%)	4 (11.8%)	
8 cycles	39 (68.4%)	11 (47.8%)	28 (82.4%)	
Response	(n = 45)	(n = 13)	(n = 32)	
NR	10 (22.2%)	0 (0%)	10 (31.3%)	0.022‡
OAR	35 (77.8%)	13 (100%)	22 (68.8%)	
Response after 4-6	(n = 45)	(n = 13)	(n = 32)	
PD	3 (6.7%)	0 (0%)	3 (9.4%)	0.027‡
SD	10 (22.2%)	2 (15.4%)	8 (25%)	
PR	29 (64.4%)	10 (76.9%)	19 (59.4%)	
CR	3 (6.7%)	1 (7.7%)	2 (6.3%)	
Response after 8	(n = 45)	(n = 13)	(n = 32)	
PD	3 (6.7%)	0 (0%)	3 (9.4%)	0.009‡
SD	7 (15.6%)	0 (0%)	7 (21.9%)	
PR	7 (15.6%)	2 (15.4%)	5 (15.6%)	
CR	28 (62.2%)	11 (84.6%)	17 (53.1%)	
Recurrence	(n = 43)	(n = 24)	(n = 19)	
Absent	12 (27.9%)	12 (50%)	0 (0%)	< 0.001‡
Present	31 (72.1%)	12 (50%)	19 (100%)	
Chemosensitivity	(n = 31)	(n = 12)	(n = 19)	
Chemosensitive	11 (35.5%)	7 (58.3%)	4 (21.1%)	0.036‡
Chemorefractory	20 (64.5%)	5 (41.7%)	15 (78.9%)	
RFS	(n = 43)	(n = 24)	(n = 19)	
Mean (months)	20.2 months	25.3 months	13.9 months	< 0.001†
(95%CI)	(16.9-23.5)	(20.9-29.8)	(10.5-17.3)	
1 year RFS	48.8%	70.8%	21.1%	
2 year RFS	31.4%	48.7%	10.5%	
3 year RFS	23.2%	48.7%	–	
Death	(n = 60)	(n = 26)	(n = 34)	
Alive	28 (46.7%)	22 (84.6%)	6 (17.6%)	< 0.001‡
Died	32 (53.3%)	4 (15.4%)	28 (82.4%)	

Table IIIB. Cont.

Characteristics	All	HIF-1a		p-value
		Low	High	
	No. (%)	No. (%)	No. (%)	
OS	(n = 60)	(n = 26)	(n = 34)	
Mean (months)	22.3 months	32.2 months	14.7 months	< 0.001†
(95% CI)	(19-25.5)	(28.7-35.6)	(11.6-17.8)	
1 year OS	44.9%	84.6%	15.1%	
2 year OS	44.9%	84.6%	15.1%	
3 year OS	44.9%	84.6%	15.1%	

NR – no response; PD – progressive disease; SD – stable disease; PR – partial response; CR – complete response; 95% CI – 95% confidence interval; ‡ χ^2 test; † log-rank test; $p < 0.05$ is significant

degree of cancer differentiation, suggesting that Beclin-1 could be considered a protective factor against SOC progression They also found that Beclin-1 expression was more frequently associated with low grade than high grade tumors and was negatively associated with unfavorable pathological parameters, and clinical findings.

The current study revealed that high expression of Beclin-1 was correlated with such factors as better clinical and prognostic parameters, low stage of SOCS, a response to therapy, increased SOC chemo-sensitivity, low incidence of recurrence after therapy, a longer survival period, favorable three-year recurrence-free survival, and overall survival rates. Similarly, the study by Ying *et al.* [1] revealed that downregulation of tissue expression of Beclin1 was detected in drug-resistant SOC patients in comparison with its expression in drug-sensitive SOC patients, which suggests that low Beclin1 expression may be associated with resistance to chemotherapy and poor prognoses in patients with ovarian cancer, while Beclin1 overexpression may increase the chemo-sensitivity of SOC patients, which could lead to improvement of treatment efficiency and prognosis. Similarly, in gastric and brain cancers, the results of Fei *et al*. [22] and Miracco *et al*. [23] showed that Beclin1 expression decreased with the progression of cancer as the malignant cells tend to lose their autophagy capacity during cancer progression, gradually acquiring the ability to escape cell death and likely invasion and spread.

However, other authors reported different results. Zhao *et al*. [24] found that Beclin-1 is not a good predictive or prognostic factor for ovarian carcinoma. However, their patients were followed for a shorter period than ours.

Similarly to us, Cai *et al*. [20] found that high Beclin-1 levels are associated with a decreased risk of death in patients with ovarian cancer. They explained their results by: 1) Beclin-1 increasing autophagy in malignant ovarian cells with absence of apoptotic ability; 2) Beclin-1 stabilizing the structure of mitochondria and decreasing additional gene mutations; 3) inhibition of cell cycle and proliferation of malignant cells, and stimulation of apoptosis and autophagy, thus 4) delaying cell cycle progression. Many previous studies proved the protective role of Beclin-1 in cancers of other organs [25]. In this context our results could be explained by the up-regulation of Beclin-1 expression, increased p53, and Bcl-2 phosphorylation that finally enhanced autophagy-related cell death. Also, Beclin-1 promoted apoptosis by increasing caspase-9 activity [27]. Moreover, reduced tissue expression of Beclin-1 in liver cancer is associated with subsequent elevated expression of anti-apoptotic Bcl-XL that allows increased hepatocellular carcinoma cell survival. Bcl-XL and Beclin-1 interaction might be able to inhibit autophagy and subsequently might promote the progression of cancer [28].

By contrast to our results, other studies have demonstrated that Beclin-1 overexpression was associated with prognosis of nasopharyngeal carcinoma patients [29] and squamous cell carcinoma of the mouth [30].

Hypoxia-inducible factor 1α and clinical-pathological correlation in serous ovarian carcinoma

The current study showed that expression of the HIF-1α in SOC was significantly positively correlated with poor differentiation of the tumor cells and tumor invasion, metastases and spread, and advanced stage, which was similar to the results of Osman *et al*. [17] and Acs *et al*. [31]. The authors observed that HIF-1α over-expression was related to large tumor size, presence of multiple tumors and advanced stage and HIF-1A expression was significantly higher in SOC with FIGO stages III and IV than in cases of stages I and II. They explained that with larger tumor bulk

Table IVA. Correlations between Beclin-1 expression in SOC and clinicopathological parameters in our patients

	Beclin1 (Low, High)	
	r	p-value
Age (years)	–0.642	< 0.001
Cytology (negative, positive)	–0.620	< 0.001
CA125 (≤ 35 U/ml, > 35 U/ml)	–0.656	< 0.001
Laterality (unilateral, bilateral)	–0.280	0.030
Implants (absent, present)	–0.573	< 0.001
Ascites (absent, present)	–0.362	0.005
Grade (low, high)	–0.724	< 0.001
LN (node negative, node positive)	–0.656	< 0.001
M (M0, M1)	–0.466	< 0.001
FIGO stage (stage IA, stage IB, stage IC, stage….)	–0.698	< 0.001
Beclin1 (low, high)	———	———
HIF (low, high)	–0.762	< 0.001
Operation (radical surgery, suboptimal, ……)	–0.458	< 0.001
ECOG PS (ECOG 1, ECOG 2)	–0.184	0.153
Number of cycles (4 cycles, 6 cycles,……)	–0.567	< 0.001
Response (NR, OAR)	+0.286	0.055
Response after 4-6 (PD, SD, PR, CR)	+0.225	0.138
Response after 8 (PD, SD, PR, CR)	+0.369	0.013
Recurrence (absent, present)	–0.423	0.006
Chemosensitivity (chemosensitive, chemorefractory)	–0.275	0.125
Death (alive, died)	–0.700	< 0.001

r correlation coefficient; $p < 0.05$ is significant

in the high stage tumors, larger areas of resulting hypoxia are present.

We also found that HIF-1α expression was related to poor outcome and poor patients' survival That is similar to the results of Jin *et al*. [33], who found that the high expression of HIF-1α leads to chemo-resistance and poor survival of patients [32, 33]. Our results were also similar to those of Zheng *et al*. [34], who considered tissue protein expression of HIF-1α a poor HCC prognostic biomarker. Both the results of other researchers' studies and the results of the present study indicate therefore the poor prognosis of HIF-1α in SOC patients.

Many previous studies have detected higher expression of HIF-1 in SOC, and they attributed their results to the presence of papillary formation in that tumor, which is believed to lead to a more hypoxic microenvironment [35].

We found that low HIF-1α expression was positively associated with good prognostic parameters, good response to chemotherapy, increased three-year recurrence-free, and overall survival rates. Contrary to our results, Birner *et al*. [36] found no association between HIF-1α expression and chemotherapy response. Nakai *et al*. [37] found higher rates of response to platinum-based chemotherapeutics in patients with high HIF-1α expression. The differences between the results of these studies and our results could be due to differences in patient group composition, HIF-1α evaluation methods, and variability in treatment programs, as Nakai *et al*. performed their analysis using the Western blot method for determination of HIF-1α expression, so they did not consider the presence of non-functional cytoplasmic HIF-1α, with resulting possible overestimation of the association between expression of HIF-1α and therapy response [37].

Similarly to our results, Nakai *et al*. [37] found a shorter 5-year OS in patients with high HIF expression, whereas no effect on patients' survival was noted by others [35].

Ovarian cancer is the most fatal cancer of the female genital system [39], due to its invasiveness and migration that could accelerate its progression. Therefore, suppression of its invasion and spread is an essential step for ovarian carcinoma therapy. HIF-1α promoted tumor progression by increasing cancer cells' invasion and spread, so HIF-1α is

Table IVB. Correlations between HIF-1α expression in SOC and clinicopathological parameters in our patients

	HIF (low, high)	
	r	p-value
Age (years)	+0.557	< 0.001
Cytology (negative, positive)	+0.501	< 0.001
CA125 (≤ 35 U/ml, > 35 U/ml)	+0.557	< 0.001
Laterality (unilateral, bilateral)	+0.071	0.582
Implants (absent, present)	+0.456	< 0.001
Ascites (absent, present)	+0.247	0.056
Grade (low, high)	+0.625	< 0.001
LN (node negative, node positive)	+0.557	< 0.001
M (M0, M1)	+0.323	0.012
FIGO stage (stage IA, stage IB, stage IC, stage….)	+0.555	< 0.001
Beclin1 (low, high)	–0.762	< 0.001
HIF (low, high)	———	———
Operation (radical surgery, suboptimal, ……)	+0.381	0.003
ECOG PS (ECOG 1, ECOG 2)	+0.132	0.306
Number of cycles (4 cycles, 6 cycles,……)	+0.401	0.002
Response (NR, OAR)	–0.341	0.022
Response after 4-6 (PD, SD, PR, CR)	–0.197	0.195
Response after 8 (PD, SD, PR, CR)	–0.338	0.023
Recurrence (absent, present)	+0.554	< 0.001
Chemosensitivity (chemosensitive, chemorefractory)	+0.380	0.035
Death (alive, died)	+0.665	< 0.001

r correlation coefficient; $p < 0.05$ is significant.

considered a significant target for SOC therapy. Therefore, many recently discovered HIF-1α inhibitors were found to block tumor progression, e.g., Zhang *et al*. [40] observed digoxin to suppress tumor growth by inhibition of HIF-1α synthesis, and Zagzag *et al*. [41] reported that geldanamycin prevents migration of glioma cells by inhibiting the induction of HIF-1α.

Correlation between Beclin-1 expression and hypoxia-inducible factor 1α expression in serous ovarian carcinoma

We detected that Beclin-1 expression was inversely associated with HIF-1α expression. Also, the association between decreased Beclin-1 expression and the aggressive clinical pathological features was more significant in the hypoxic areas of the SOC, the results being therefore similar to Osman *et al*. [17] in hepatocellular carcinoma (HCC).

Lee *et al*. [42] reported that loss of Beclin-1 was related to aggressive behavior and increased angiogenesis in more hypoxic regions of the tumor cancer, while Dong *et al*. stated that the reduction of tissue Beclin-1 expression could be considered a predictive factor for poor (OS) in breast cancer patients with HIF-1α- over-expression [25]. This supports our hypothesis regarding the role of autophagy and hypoxia in SOC progression.

In contrast to our results, other studies demonstrated a positive correlation between autophagy Beclin-1 expression and high HIF-1α, where cancers with HIF-1α over-expression are more liable to develop Beclin-1 up-regulation than those with low HIF-1α expression in nasopharyngeal and breast carcinoma [25]. This may be explained by the role of hypoxia in the activation of autophagy, which is variable among different cancer types.

Therefore, the association between Beclin-1 and HIF-1α proved by our study confirms the essential role of hypoxia in autophagy induction. In addition, reduced Beclin-1 and higher levels of HIF-1α expression are related to aggressive pathological findings, clinical parameters, and poor prognosis, thus supporting their essential roles in SOC oncogenesis, progression, invasion, and spread. Both markers could be therefore used as beneficial novel targets for SOC molecular therapies in combination with the currently used treatment strategies.

In summary, expression levels of the autophagy-related protein Beclin-1 and the tissue hypoxia-related biomarker HIF-1α in SOC were shown to be associated with clinical-pathological parameters and patients' prognosis and might be able to have a vital role in participating in platinum-based chemo-resistance mechanisms that were found in SOC cancers. Further studies are required to investigate the possibility of targeting both of them in the treatment of ovarian cancer.

As specific mechanisms that regulate autophagy and its relation to hypoxia in SOC remain unclear, further studies are needed to aid in clarifying their roles in ovarian cancer progression, which may be able to provide novel solutions for the management of SOC with chemo-resistance and clarify a reliable theoretical basis for the detection of novel drugs.

The authors declare no conflict of interest

References

1. Ying H, Qu D, Liu C, et al. Chemo-resistance is associated with Beclin-1 and PTEN expression in epithelial ovarian cancers. Oncol Lett 2015; 9: 1759-1763.
2. KunduM, Thompson CB. Autophagy: basic principles and relevance to disease. Annu Rev Pathol 2008; 3: 427-455.
3. Koren I, Kimchi A. Cell biology. Promoting tumorigenesis by suppressing autophagy. Science 2012; 338: 889-890.
4. Baspinar S, Bircan S, Orhan H, et al. The relation of beclin 1 and bcl2 expressions in high grade prostatic intraepithelial neoplasia and prostate adenocarcinoma: A tissue microarray study. Pathol Res Pract 2014; 210: 412418.
5. Tong Y, You L, Liu H, et al. Potent antitumor activity of oncolytic adenovirus expressing Beclin1 via induction of autophagic cell death in leukemia. Oncotarget 2013; 4: 860874.
6. Sun Q, Fan W, Zhong Q. Regulation of Beclin 1 in autophagy. Autophagy 2009; 5: 713-716.
7. Edinger AL, Thompson CB. Defective autophagy leads to cancer. Cancer Cell 2003; 4: 422-424.
8. Kang KF, Wang XW, Chen XW, et al. Beclin 1 and nuclear factor-κBp65 are up-regulated in hepatocellular carcinoma. Oncol Lett 2013; 5: 1813-1818.
9. Wong CC, Kai AK, Ng IO. The impact of hypoxia in hepatocellular carcinoma metastasis. Front Med 2013; 8: 33-41.
10. Mabjeesh NJ, Amir S. Hypoxia-inducible factor (HIF) in human tumorigenesis. Histol Histopathol 2007; 22: 559-572.
11. Wilson KG, Tennant AD, McKeating AJ. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: current understanding and future directions. J Hepatol 2014; 61: 1397-1406.
12. Zhang H, Bosch-Marce M, Shimoda LA, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 2008; 283:10892–903.
13. Dong M, Wan X, Yuan ZY, et al. Low expression of Beclin 1 and elevated expression of HIF-1 α define distant metastasis risk and predict poor prognosis of ER- positive, HER2-negative breast cancer. Med Oncol 2013; 30: 355-365.
14. Prat J. FIGO Committee on Gynecologic Oncology. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet 2014; 124: 1-5.
15. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO Classification of Tumours of Female Reproductive Organs. 4th ed. International Agency for Research on Cancer, Lyon 2014.
16. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981; 29: 577-580.
17. Osman NA, Abd El-Rehim DM, Kamal IM. Defective Beclin-1 and elevated hypoxia-inducible factor (HIF)-1α expression are closely linked to tumorigenesis, differentiation, and progression of hepatocellular carcinoma. Tumour Biol 2015; 36: 4293-4299.
18. Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. Mol Cancer Ther 2011; 10: 1533-1541.
19. Cui J, Gong Z, Shen HM. The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets. Biochim Biophys Acta 2013; 1836: 15-26.
20. Cai M, Hu Z, Liu J, et al. Beclin 1 expression in ovarian tissues and its effects on ovarian cancer prognosis. Int J Mol Sci 2014; 15: 5292-5303.
21. Wu X, Chen J, Cao Q, et al. Beclin 1 activation enhances chemosensitivity and predicts a favorable outcome for primary duodenal adenocarcinoma. Tumor Biol 2013; 34: 713-722.
22. Fei B, Ji F, Chen X, et al. Expression and clinical significance of Beclin-1 in gastric cancer tissues of various clinical stages. Oncol Lett 2016; 11: 2271-2277.
23. Miracco C, Cosci E, Oliveri G, et al. Protein and mRNA expression of autophagy gene Beclin-1 in human brain tumours. Int J Oncol 2007; 30: 429-436.
24. Zhao Y, Chen S, Gou WF, et al. Aberrant Beclin 1 expression is closely linked to carcinogenesis, differentiation, progression, and prognosis of ovarian epithelial carcinoma. Tumour Biol 2014; 35: 1955-1964.
25. Dong LW, Hou YJ, Tan YX, et al. Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. Autophagy 2011; 7: 1222-1229.
26. Park KJ, Lee SH, Lee CH, et al. Upregulation of Beclin-1 expression and phosphorylation of Bcl-2 and p53 are involved in the JNK-mediated autophagic cell death. Biochem Biophys Res Commun 2009; 382: 726-729.
27. Furuya D, Tsuji N, Yagihashi A, Watanabe N. Beclin 1 augmented cis-diamminedichloroplatinum induced apoptosis via enhancing caspase-9 activity. Exp Cell Res 2005; 307: 26-40.
28. Shi YH, Ding ZB, Zhou J, Qiu SJ, Fan J. Prognostic significance of Beclin 1-dependent apoptotic activity in hepatocellular carcinoma. Autophagy 2009; 5: 380-382.
29. Wan XB, Fan XJ, Chen MY, et al. Elevated Beclin 1 expression is correlated with HIF-1alpha in predicting poor prognosis of nasopharyngeal carcinoma. Autophagy 2010; 6: 395-404.
30. Tang JY, Fang YY, Hsi E, et al. Immunopositivity of Beclin1 and ATG5 as indicators of survival and disease recurrence in oral squamous cell carcinoma. Anticancer Res 2013; 33: 5611-5616.
31. Acs G, Xu X, Chu C, Acs P, Verma A. Prognostic significance of erythropoietin expression in human endometrial carcinoma. Cancer 2004; 100: 2376-2786.
32. Qin J, Liu Y, Lu Y, et al. Hypoxia-inducible factor 1 alpha promotes cancer stem cells-like properties in human ovarian cancer cells by upregulating SIRT1 expression. Sci Rep 2017; 7: 10592.
33. Jin Y, Wang H, Liang X, et al. Pathological and prognostic significance of hypoxia-inducible factor 1alpha expression in epithelial ovarian cancer: a meta-analysis. Tumour Biol 2014; 35: 8149-8159.
34. Zheng SS, Chen XH, Yin X, Zhang BH. Prognostic significance of HIF-1alpha expression in hepatocellular carcinoma: a meta-analysis. PLoS One 2013; 8: e65753.
35. Shimogai R, Kigawa J, Itamochi H, et al. Expression of hypoxiainducible factor 1alpha gene affects the outcome in patients with ovarian cancer. Int J Gynecol Cancer 2008; 18: 499-505.

36. Birner P, Schindl M, Obermair A, et al. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res 2000; 60: 4693-4696.
37. Nakai H, WatanabeY, Ueda H, Hoshiai H. Hypoxia inducible factor 1- alpha expression as a factor predictive of efficacy of taxane/platinum chemotherapy in advanced primary epithelial ovarian cancer. Cancer Lett 2007; 251: 164-167.
38. Liu T, Zhao L, Hou H, Ding L, et al. Ginsenoside 20(S)-Rg3 suppresses ovarian cancer migration via hypoxia-inducible factor 1 alpha and nuclear factor-kappa B signals. Tumor Biol 2017; 39: 1010428317692225.
39. Ruscito I, Gasparri ML, Marchetti C, et al. Cediranib in ovarian cancer: state of the art and future perspectives. Tumour Biol 2016; 37: 2833-2839.
40. Zhang H, Qian DZ, Tan YS, et al. Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. Proc Natl Acad Sci U S A 2008; 105: 19579-19586.
41. Zagzag D, Nomura M, Friedlander DR, et al. Geldanamycin inhibits migration of glioma cells in vitro: a potential role for hypoxia-inducible factor (HIF-1alpha) in glioma cell invasion. J Cell Physiol 2003; 196: 394-402.
42. Lee SJ, Kim HP, Jin Y, et al. Beclin 1 deficiency is associated with increased hypoxia-induced angiogenesis. Autophagy 2011; 7: 829-839.

Address for correspondence

Mohamed Alabiad
Pathology Department
Faculty of Medicine
Zagazig University, Egypt
e-mail: alabiad@zu.edu.eg