Author details
[1]Department of Obstetrics and Gynecology, National Defense Medical College, Tokorozawa, Saitama 359-8513, Japan. [2]Department of Obstetrics and Gynecology, School of Medicine, Keio University, Shinano-machi 35, Shinjuku-ku, Tokyo 160-8582, Japan. [3]Department of Obstetrics and Gynecology, Iwate Medical University, Morioka, Iwate 020-8505, Japan.

Authors' contributions
Dr Takano and Dr Tsuda wrote the manuscript. Dr Takano, Dr Tsuda, and Dr Sugiyama approved it. All authors read and approved the final manuscript.

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Clinicopathological prognostic factors and the role of cytoreduction in surgical stage IVb endometrial cancer: A retrospective multi-institutional analysis of 248 patients in Japan

Takako Eto [a,*], Toshiaki Saito [a], Takahiro Kasamatsu [b], Toru Nakanishi [c], Harushige Yokota [d], Toyomi Satoh [e], Takayoshi Nogawa [f], Hiroyuki Yoshikawa [e], Toshiharu Kamura [g], Ikuo Konishi [h]

[a] Gynecology Service, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
[b] Department of Gynecology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[c] Department of Gynecology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa Nagoya, Aichi, 464-8681, Japan
[d] Department of Gynecology, Saitama Cancer Center Hospital, 818 Komuro Ina, Kita-Adachi, Saitama, 362-0806, Japan
[e] Department of Obstetrics and Gynecology, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan
[f] Gynecology Service, National Hospital Organization Shikoku Cancer Center, 160 Koh, Minami Umemoto-machi, Matsuyama, Ehime 791-0280, Japan
[g] Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahi machi, Kurume, 830-0011, Japan
[h] Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

HIGHLIGHTS

- A total of 248 patients with surgical stage IVb endometrial cancer were reviewed.
- Low grade endometrioid type was a good prognostic factor in this group.
- Cytoreduction and chemotherapy may improve survival even in metastatic disease.

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ABSTRACT

Objective. To evaluate clinicopathological prognostic factors and the impact of cytoreduction in patients with surgical stage IVb endometrial cancer (EMCA).

Methods. The records of 248 patients with stage IVb EMCA who underwent primary surgery including hysterectomy at multiple institutions from 1996 to 2005 were retrospectively analyzed. Data regarding disease distribution, surgical procedures, adjuvant therapy, and survival times were collected. Univariate and multivariate analyses were performed to identify factors associated with overall survival (OS).

Results. The median OS was 24 months. The most common histological types were endometrioid (grade 1: 15%, grade 2: 20%, grade 3: 24%) and serous (17%). The most common sites of intra-abdominal metastases were pelvis (65%), ovaries (58%), omentum (58%), retroperitoneal lymph nodes (52%), and upper abdominal peritoneum (44%). In 93 patients with extra-abdominal metastases, the most common site was the lung (n = 49). Complete resection of extra-abdominal metastases was achieved in only 13 patients. Complete resection of intra-abdominal metastases was achieved in 101 patients, 52 had ≤1 cm residual disease, and 95 had >1 cm residual disease; the median OS times in these groups were 48, 23, and 14 months, respectively ($p<0.0001$). Multivariate analysis showed that performance status, histology/grade, adjuvant treatment, and intra-abdominal residual disease were independent prognostic factors. Intra-abdominal residual disease was an independent prognostic factor in patients with and without extra-abdominal metastases.

Conclusions. Cytoreductive surgery and adjuvant therapy may improve survival in stage IVb EMCA, particularly in patients with favorable prognostic factors, even in the presence of extra-abdominal metastases.

Introduction

Endometrial cancer (EMCA) is commonly diagnosed at an early stage and has a favorable prognosis [1,2]. The treatment of early-stage EMCA is well established, but the most effective treatment strategies for stage IVb EMCA remain unclear. Stage IVb disease is rare, and the prognosis remains very poor. According to the International Federation of Gynecology and Obstetrics (FIGO) Annual Report, approximately 3% of EMCA patients are classified as stage IV [3]. The 5-year survival rate of surgical stage IVb patients is reportedly 20.1%, and the 4-year survival rate of clinical stage IVb patients is 7.7%. There are no data to

* Corresponding author. Fax: +81 92 551 4585.
E-mail address: teto@nk-cc.go.jp (T. Eto).

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aid therapeutic decision-making in these patients, and there is no consensus regarding the most effective treatment strategies.

The population of patients with stage IVb EMCA is heterogeneous, as this stage includes patients with upper intra-abdominal dissemination and extra-abdominal metastases. Patients with stage IVb EMCA can therefore be divided into subgroups according to intra- and extra-abdominal disease. However, few published reports have described the specific disease distribution of surgical stage IVb EMCA patients [4–7].

Although the treatment of advanced EMCA is developing in a similar direction to the treatment of ovarian cancer, the role of surgery in the treatment of stage IVb EMCA is unresolved. Recently, several investigators have retrospectively evaluated the role of surgical cytoreduction in patients with stage IVb disease [4,7–12]. A meta-analysis [13] demonstrated that complete cytoreduction is associated with superior overall survival. However, previous studies have been based on populations selected for surgery, with relatively few extra-abdominal metastases. The lung is reportedly the main site of extrapelvic tumor spread, followed by multiple other sites [14]. The effectiveness of intra-abdominal cytoreductive surgery in patients with extra-abdominal metastases considered to be unresectable is unknown.

We hypothesized that clinicopathological characteristics and disease distribution are important when establishing treatment strategies for this disease. We conducted a multicenter study of stage IVb EMCA patients treated in Japan Clinical Oncology Group-related institutions. The primary objective of this study was to clarify the clinicopathological characteristics and disease distribution of surgical stage IVb EMCA patients. The secondary objective was to identify prognostic factors which affect survival and evaluate the impact of cytoreductive surgery on prognosis, including surgery in patients with extra-abdominal metastases.

Methods

Patients

We performed a retrospective review of all patients diagnosed with clinical or surgical FIGO 1988 stage IVb EMCA from 1996 to 2005 who were treated in 30 Gynecologic Cancer Study Group of Japan Clinical Oncology Group-related institutions. Patients with sarcoma were excluded. Patients with stage IVb EMCA who underwent primary surgery including hysterectomy and bilateral salpingo-oophorectomy were eligible.

A case report form was developed using data software (FileMaker-pro Version 6/8) to obtain equivalent data from multiple institutions. The investigation protocol, including the case report form, was approved by the Institutional Review Board of each institution.

Complete clinical data were collected by reviewing inpatient charts, operative records, original pathology reports, and outpatient records from each institution. The demographic data collected included: age, Eastern Cooperative Oncology Group (ECOG) performance status (PS), reproductive history, medical comorbidities, and body mass index (BMI). Pathological information was collected from the pathology reports of the preoperative endometrial biopsy and hysterectomy specimens. The sites and sizes of metastases, surgical procedures, and sites and maximum diameter of residual disease after surgery were collected from radiology reports, intraoperative findings, and pathology reports. Treatment data collected included details of postoperative adjuvant treatment. Follow-up was continued regularly at each institution. Follow-up information included the date and disease status at the last follow-up, or the date and cause of death.

Stage IVb metastases were divided into intra- and extra-abdominal disease. Metastasis to the liver surface was classified as intra-abdominal disease, and metastasis to the liver parenchyma was classified as extra-abdominal disease, following the classification for ovarian cancer. Postoperative residual disease was also divided into intra- and extra-abdominal disease. Remaining retroperitoneal lymphadenopathy and intrapelvic disease were classified as intra-abdominal residual disease. Patient outcomes were analyzed by overall survival (OS) time. OS was calculated from the date of surgery to the date of death or last contact.

Statistical analysis

The Kaplan–Meier method was used to estimate OS curves, and survival was compared among groups using the log-rank test. A p value of <0.05 was considered statistically significant. Multivariate Cox proportional hazards regression analyses were used to identify independent prognostic variables. Factors with a p value of <0.1 in univariate analyses were included in the multivariate analyses. All analyses were performed using SPSS statistical software (11.0.1 J; SPSS Inc., Chicago, IL).

Results

Patients and characteristics

We identified a total of 426 patients with stage IVb EMCA, of which 279 underwent primary surgery with curative intent as the initial treatment. After excluding 31 patients who did not undergo hysterectomy and cytoreductive surgery, 248 patients met the study inclusion criteria.

Detailed clinicopathological characteristics of the patients are listed in Table 1. The median age was 59 years (range: 30–89 years), and 91% had a pretreatment ECOG PS of 0/1. Mean BMI was 23.2 kg/m^2 (range: 15.1–35.8 kg/m^2). Medical comorbidities included hypertension in 19% of patients and diabetes in 9%. The most common histological subtype was endometrioid. There was a high frequency of poor histological factors, with endometrioid grade 3 (EMG3) or non-endometrioid

Table 1
Clinicopathological characteristics (n = 248).

Characteristic	n	(%)
Median age, years (range)	59(30–89)	
ECOG performance status		
0–1	226	(91)
2	17	(7)
3–4	4	(2)
Unknown	1	(<1)
Diabetes mellitus	22	(9)
Hypertension	47	(19)
Histological type		
Endometrioid	149	(61)
Serous	43	(17)
Clear cell	15	(6)
Carcinosarcoma	23	(9)
Other	18	(7)
Grade		
Endometrioid G1	36	(15)
Endometrioid G2	50	(20)
Endometrioid G3	60	(24)
Non-endometrioid	99	(40)
Unknown	3	(1)
Deep myometrial invasion	170	(69)
LVSI	173	(70)
Adjuvant therapy		
CT alone	185	(75)
RT alone	11	(4)
CT + RT	24	(10)
None	28	(11)
Chemotherapy regimen		
Taxane + platinum	115	(46)
AP ± α	79	(32)
Other	15	(6)

ECOG, Eastern Cooperative Oncology Group.
LVSI, lymphovascular space involvement.
CT, chemotherapy; RT, radiotherapy.
A P ± α, doxorubicin + platinum ± other.

histology, deep myometrial invasion, and positive lymphovascular space invasion (LVSI), each found in more than 60% of patients. Only 36 patients (15%) were classified as EMG1. The preoperative histological diagnosis was identical to the postoperative diagnosis in 150 patients (60%). Only 8 of 23 patients (35%) with carcinosarcoma were correctly diagnosed by preoperative endometrial biopsy.

Disease distribution

Disease distribution by anatomical region is shown in Table 2. Extra-abdominal metastases were documented in 93 patients (38%), of whom 71 (75%) had metastasis in only one anatomical region. The most common sites of extra-abdominal metastases were the lungs, supraclavicular lymph nodes, liver, and mediastinal lymph nodes. The majority of lung metastases were bilateral (36/49, 74%) and multiple (40/49, 82%). The diameter of lung metastasis was ≤1 cm in 19 patients, 1–2 cm in 20 patients, >2 cm in 9 patients, and unknown in 1 patient.

Intra-abdominal metastases beyond the pelvis were documented in 191 patients. The diameter of upper intra-abdominal metastases was >2 cm in 105 patients (55%), ≤2 cm in 72 patients (38%), and microscopic in 14 patients (7%). Intra-abdominal stage IVb disease was diagnosed on preoperative imaging in only 47 patients. Other intra-abdominal metastases not categorized as stage IVb disease were also frequently recognized (pelvic peritoneum, positive peritoneal washing cytology, ovaries, and retroperitoneal lymph nodes).

Table 2
Disease distribution.

Site of metastases	n	(%)
Stage IVb disease site		
Intra-abdominal alone	155	(62)
Extra-abdominal alone	57	(23)
Both	36	(15)
Extra-abdominal metastasis	93	(38)
1 region	71	(29)
2 regions	17	(7)
≥3 regions	5	(2)
Lung	49	(20)
Liver	12	(5)
Bone	7	(3)
Brain	3	(1)
Skin, umbilicus, breast	4	(2)
Conjunctiva	1	(<1)
Malignant pleural effusion	5	(2)
Supraclavicular lymph node	15	(6)
Mediastinal/axillary node	12	(5)
Inguinal node	10	(4)
Intra-abdominal metastasis	191	(77)
Sites staged as IVb		
Omentum	143	(58)
Macroscopic	125	(50)
Microscopic	18	(7)
Diaphragm	54	(22)
Peritoneum (upper abdomen)	109	(44)
Colon	7	(3)
Small intestine	3	(1)
Mesentery	6	(2)
Appendix	7	(3)
Sites staged as non-IVb		
Peritoneum (pelvis)	160	(65)
Retroperitoneal node	129	(52)
Para-aortic node	91	(37)
Pelvic node	115	(46)
Peritoneal washing cytology	156	(63)
Bowel mucosa	9	(4)
Bladder mucosa	2	(<1)
Ovary	144	(58)
Parametrium	28	(11)
Vagina	11	(4)

Surgical procedures and results

All 248 patients underwent surgical staging (Table 3). In addition to hysterectomy and bilateral salpingo-oophorectomy, cytoreductive procedures with the intent of maximum cytoreduction were performed in most patients. Resection of the colon, ileum, spleen, or diaphragmatic peritoneum was performed in 19 patients. After surgery, 101 patients (41%) had complete gross intra-abdominal resection and 52 (21%) had ≤1 cm residual disease.

To remove extra-abdominal metastases, some patients underwent resection of inguinal/supraclavicular lymph nodes, the umbilicus, or the abdominal wall. No patients underwent resection of lung or liver metastases. Complete resection of extra-abdominal metastases was achieved in only 13 patients.

Postoperatively, one patient with >2 cm residual disease died of disease progression on postoperative day 26. No major life-threatening complications occurred within 30 days after surgery.

Postoperative adjuvant therapy

Postoperative adjuvant therapy was administered to 220 patients (89%). The majority of these (n=185) were treated with chemotherapy alone. A variety of chemotherapy regimens were used including paclitaxel, docetaxel, carboplatin, cisplatin, doxorubicin, cyclophosphamide, ifosfamide, etoposide, CPT11, and 5-fluorouracil. The most commonly administered regimen was taxanes + platinum ± doxorubicin (n=115), followed by doxorubicin + platinum (AP) ± cyclophosphamide ± ifosfamide (n=79). Radiotherapy was administered to 35 patients, including external beam radiotherapy to the whole pelvis (n=23), para-aortic lesions (n=16), neck (n=6), bone (n=3), brain (n=2), and vaginal brachytherapy (n=2).

Clinical and pathological risk factors for survival

The median follow-up time among the censored patients was 41 months, and the median OS was 24 months (95% confidence interval [CI], 20–29 months). The causes of death were EMCA in 157 patients, other diseases in 2, and unknown in 3. At the last follow-up, 48 patients were alive with no evidence of disease, 33 were alive with disease, and 5 were alive with unknown disease status. There were no treatment-related deaths.

Table 3
Surgical procedures performed.

Procedure	n	(%)
Intra-abdominal		
Hysterectomy + BSO	248	(100)
Type of hysterectomy		
Simple	184	(74)
Subtotal	9	(4)
Modified-radical	49	(20)
Radical	6	(2)
Omentectomy/biopsy	157	(63)
Pelvic lymphadenectomy	157	(63)
Para-aortic lymphadenectomy	82	(33)
Resection of peritoneum	90	(36)
Appendectomy	30	(12)
Resection of colon/ileum	17	(7)
Colostomy/ileostomy	3	(1)
Splenectomy	1	(<1)
Diaphragm peritonectomy	1	(<1)
Resection of internal iliac artery	1	(<1)
Extra-abdominal		
Mastectomy	1	(<1)
Resection of umbilicus/skin	3	(1)
Resection of supraclavicular nodes	3	(1)
Resection of inguinal nodes	7	(3)

BSO, bilateral salpingo-oophorectomy.

Table 4
Univariate analyses for overall survival.

Variable	n	(%)[a]	Median OS (months) (95% CI)	Log-rank p[b]
Age				
≤59 years	134	(54)	29 (22–36)	
≥60 years	114	(46)	20 (13–28)	0.0675
ECOG performance status				
0–1	226	(91)	25 (20–31)	
2–4	21	(8)	11 (5–16)	0.0150
Stage IVb disease site				
Intra-abdominal alone	155	(62)	24 (19–29)	
Extra-abdominal alone	57	(23)	30 (0–61)	
Both	36	(15)	20 (9–31)	0.1283
Histological type				
Endometrioid	149	(60)	31 (21–40)	
Non-endometrioid	99	(40)	14 (7–22)	<0.0001
Histology and grade				
EMG1	36	(15)	79 (not estimated)	
EMG2	50	(20)	48 (28–68)	
EMG3 + non-EM	159	(64)	14 (8–21)	<0.0001
Myometrial invasion				
≤1/2	59	(24)	40 (28–53)	
>1/2	170	(69)	22 (16–7)	0.0108
LVSI				
Present	173	(70)	24 (19–8)	
Absent	27	(11)	58 (not estimated)	0.0037
Stage IVb disease site				
Extra-abdominal metastasis				
Positive	93	(38)	24 (15–4)	
Negative	155	(62)	24 (19–9)	0.3722
Intra-abdominal metastasis				
Positive	191	(77)	23 (19–27)	
Negative	57	(23)	30 (0–61)	0.0609
Site of metastasis				
Para-aortic lymph node				
Positive	91	(37)	21 (14–27)	
Negative	119	(48)	31 (17–45)	0.0086
Pelvic lymph node				
Positive	115	(46)	21 (14–28)	
Negative	104	(42)	32 (18–47)	0.0134
Omentum				
Positive	143	(58)	24 (20–29)	
Negative	92	(37)	24 (10–38)	0.3877
Diaphragm				
Positive	54	(22)	22 (16–29)	
Negative	172	(69)	25 (18–32)	0.1077
Peritoneum (upper abdomen)				
Positive	109	(44)	18 (12–25)	
Negative	131	(53)	29 (24–35)	0.0070
Bone				
Positive	7	(3)	6 (3–9)	
Negative	241	(97)	25 (20–31)	<0.0001
Parametrium				
Positive	28	(11)	18 (11–26)	
Negative	202	(81)	25 (20–30)	0.0338
Postoperative residual disease				
None	62	(25)	48 (27–69)	
≤1 cm	63	(25)	25 (19–31)	
>1 cm	123	(50)	17 (11–22)	0.0004
Intra-abdominal residual disease				
None	101	(41)	48 (30–66)	
≤1 cm	52	(21)	23 (18–27)	
>1 cm	95	(38)	14 (10–19)	<0.0001
Extra-abdominal residual disease				
None	168	(67)	26 (21–31)	
≤1 cm	24	(10)	38 (0–100)	
>1 cm	56	(23)	21 (8–34)	0.3553
Adjuvant therapy				
Yes	220	(89)	26 (21–31)	
No	28	(11)	6 (4–9)	<0.0001
Type of adjuvant therapy				
CT alone	185	(75)	27 (22–32)	
RT alone	11	(4)	12 (0–50)	
CT + RT	24	(10)	26 (4–48)	0.9816
Chemotherapy regimen				
Taxane + platinum	115	(46)	30 (23–37)	
AP ± α	79	(32)	27 (20–34)	
Other	15	(6)	8 (0–16)	0.0470

Univariate analyses

Univariate analyses were performed to identify relationships between OS and demographic, clinicopathological, surgical, and therapeutic variables (Table 4). Of the demographic and clinicopathological variables, PS, histology/grade, myometrial invasion, and LVSI were significantly associated with OS. Fig. 1 shows OS curves according to PS, histology/grade, and adjuvant therapy. Median OS was 79 months in patients with EMG1, 48 months in EMG2, and 14 months in EMG3 + non-EM ($p<0.0001$).

Metastases to para-aortic lymph nodes, pelvic lymph nodes, upper abdominal peritoneum/mesentery, bone, and parametrial invasion were inversely related to OS. The median OS according to stage IVb disease site was 30 months in patients with extra-abdominal metastases alone (n = 57) and 24 months with intra-abdominal metastases alone (n = 155). In the 155 patients with intra-abdominal metastases alone, the median OS was 42 months (95% CI, 0–86) in patients with microscopic disease, 24 months (95% CI, 16–33) with ≤2 cm disease, and 20 months (95% CI, 14–27) with >2 cm disease. This was not significantly different among groups ($p = 0.1527$).

Residual disease showed a significant association with OS ($p = 0.0004$). In patients with intra-abdominal residual disease, smaller size of residual disease was associated with longer OS. In contrast, extra-abdominal residual disease was not related to OS. Median OS was 48 months in patients with no gross intra-abdominal residual disease, 23 months with ≤1 cm residual disease, and 14 months with >1 cm residual disease ($p<0.0001$) (Fig. 2A).

Further stratification according to the presence of extra-abdominal metastases showed that patients with no gross intra-abdominal residual disease survived significantly longer than patients with intra-abdominal residual disease, with or without extra-abdominal metastases (Fig. 2B).

Furthermore, stratification by histology/grade showed a survival advantage in patients who underwent cytoreduction of intra-abdominal disease. Among patients with EMG1/EMG2 type, those with no residual intra-abdominal disease had a longer median OS than those with gross residual intra-abdominal disease (79 vs. 36 months, $p = 0.0226$). The results were similar among patients with EMG3/non-EM type (24 vs. 13 months, $p = 0.0022$).

OS was significantly longer in patients who received postoperative adjuvant chemotherapy and/or radiotherapy than patients who did not receive adjuvant therapy (Fig. 1C). In patients who received postoperative chemotherapy, there was no difference in OS between those who received taxanes plus platinum and those who received AP ($p = 0.5658$).

Multivariate analysis

Cox multivariate analysis was used to simultaneously examine the independent effects on OS of age, PS, histology/grade, myometrial invasion, parametrial invasion, para-aortic lymph node metastasis, pelvic lymph node metastasis, upper abdominal peritoneal/mesenteric metastasis, adjuvant therapy, and intra-abdominal residual disease. Bone metastasis showed a strong correlation with poor prognosis by univariate analysis, but was excluded from the multivariate analysis because there were only 7 patients in this group. The results showed that PS, histology/grade, adjuvant therapy, and intra-abdominal residual disease were independent prognostic factors for OS. The significance of these

Notes to Table 4:
ECOG, Eastern Cooperative Oncology Group; LVSI, lymphovascular space invasion; AP ± α, doxorubicin + platinum ± ifosfamide/cyclophosphamide/5FU/VP16; CT, chemotherapy; RT, radiotherapy.
[a] Numbers may not add up to the total because some data are unknown.
[b] Patients with unknown status were excluded from the calculation of log-rank p-values.

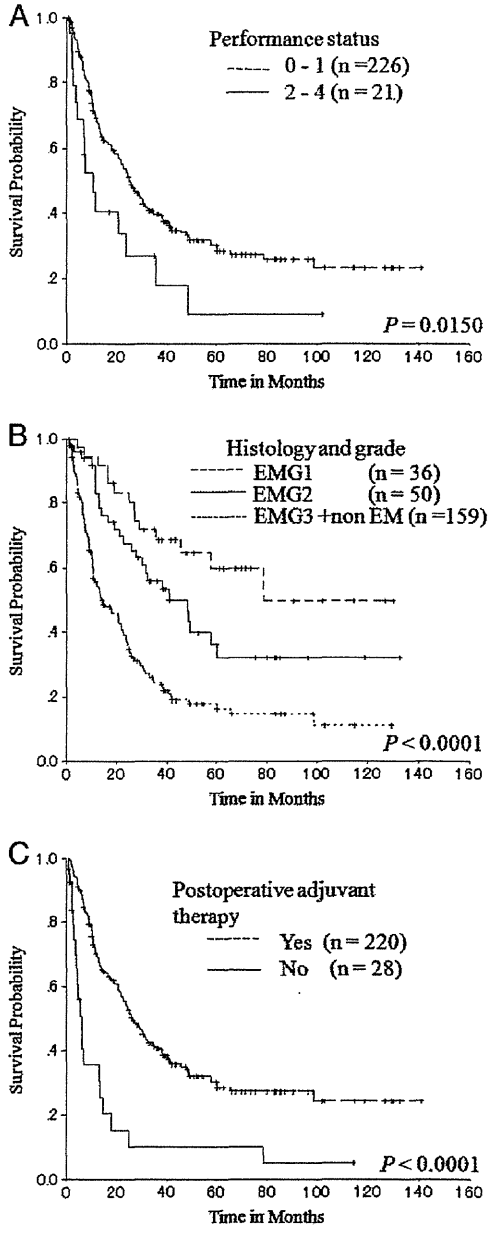

Fig. 1. Kaplan–Meier curves for overall survival (OS). A: Median OS time according to performance status (PS): PS 0–1 (dashed line), 25 months; PS2–4 (solid line), 11 months. B: Median OS time according to histology/grade: endometrioid (EM) grade 1 (dashed line), 79 months; EM grade 2 (solid line), 48 months; EM grade 3 + non-EM (dotted line), 14 months. C: Median OS time according to adjuvant therapy: yes (dashed line), 26 months; no (solid line), 6 months.

variables was as follows: PS (0–1 vs. 2–4) (hazard ratio [HR] = 1.988; 95% CI, 1.108–3.569; $p = 0.021$), histology/grade (EMG1 vs. EMG2 vs. EMG3 + non EM) (HR = 2.245; 95% CI, 1.652–3.050; $p < 0.001$), adjuvant therapy (yes vs. no) (HR = 3.396; 95% CI, 1.898–6.076; $p < 0.001$), and intra-abdominal residual disease (none vs. ≤1 cm vs. >1 cm) (HR = 1.499; 95% CI, 1.203–1.867; $p < 0.001$).

Discussion

Our study is the largest retrospective series exploring the clinical outcome of surgical stage IVb EMCA, including patients with extra-abdominal metastases. Our study also investigated the clinicopathological variables of these patients.

Although surgical staging is the most basic treatment for EMCA [1,2], intra-abdominal metastases are poorly recognized without staging laparotomy. Although several reports have documented disease distribution in surgical stage IVb patients, the reports lacked detailed information, or did not classify patients according to intra- or extra-abdominal disease. Bristow et al. reported that the most common intra-abdominal metastatic sites were the pelvis, peritoneum, omentum, and retroperitoneal nodes [7]. The distribution of metastatic disease sites in our study was comparable with previously reported distributions. In the present study, 77% of surgical stage IVb patients had upper intra-abdominal disease, and 78% had intra-pelvic spread and/or retroperitoneal lymph node metastases. However, most upper intra-abdominal disease was not detected by preoperative imaging studies. Goff et al. reported that preoperatively unrecognized upper intra-abdominal disease occurred in 53% of surgical stage IV patients [8]. In our study, the size of intra-abdominal stage IVb disease was ≤2 cm in half of the patients, which seemed to be smaller than the disseminated disease found in cases of advanced ovarian cancer. This may be one of the reasons why preoperative diagnosis is difficult. Metastasis to the diaphragm was documented in 22% of patients in the present study. This may be valuable information for gynecologic oncologists. The importance of thorough observation of the entire abdominal cavity, including the diaphragm, is stressed.

Few reports of stage IVb EMCA patients have discussed the relationship between survival and histological factors, which is known to be significant in stage I–III patients [1,2]. This is the first study to report a detailed evaluation of histopathological factors in stage IVb EMCA. As the unfavorable histopathological factors such as serous subtype or LVSI have a higher propensity for extrauterine metastasis, patients with these factors are more likely to present with advanced-stage disease. Most patients in the present study had these factors. Univariate analyses showed that non-endometrioid type, high-grade endometrioid type, deep myometrial invasion, and positive LVSI were significantly associated with poor prognosis. Multivariate analysis showed that histology/grade was an independent prognostic factor. Patients with lower-grade endometrioid type are expected to have a longer survival time, even in stage IVb EMCA.

The favorable impact of surgical cytoreduction on survival has been well demonstrated in advanced ovarian cancer [15,16]. Greer and Hamberger first suggested the beneficial effect of cytoreductive surgery and postoperative radiotherapy in advanced EMCA [17]. Subsequently, several reports on advanced EMCA have demonstrated improved OS in patients who undergo optimal cytoreductive surgery, including all histological subtypes [4,7–9,18], endometrioid subtype [10], and serous subtype [11,12,19]. Barlin et al. performed a meta-analysis of 14 retrospective cohort studies including 672 patients with advanced or recurrent EMCA who underwent cytoreductive surgery [13]. Although primary stage IV patients accounted for only 60% of the patients in their analysis, complete cytoreduction to no gross residual disease was associated with superior OS.

Generally, distant metastases are considered to be a poor prognostic factor. The association between extra-abdominal metastases and prognosis has not previously been discussed, because studies have included few patients in this group. In our study, the frequency of extra-abdominal metastases was 38%, which is the highest reported frequency compared with previous reports of surgical stage IVb EMCA. Ayhan et al. reported that the prognosis of patients with extra-abdominal metastases was poor [4]. Bristow et al. reported that optimal debulking was not achieved in patients with extra-abdominal metastases [7]. Most extra-abdominal metastases are unresectable, and in our study complete resection of extra-abdominal metastases was achieved in 13 patients (14%). It is unclear whether laparotomy benefits patients with unresectable extra-abdominal metastases. Recently, Ueda et al. reported a small study which demonstrated that optimal cytoreduction was associated with improved survival even in stage IVb EMCA patients with extra-abdominal metastases [6].

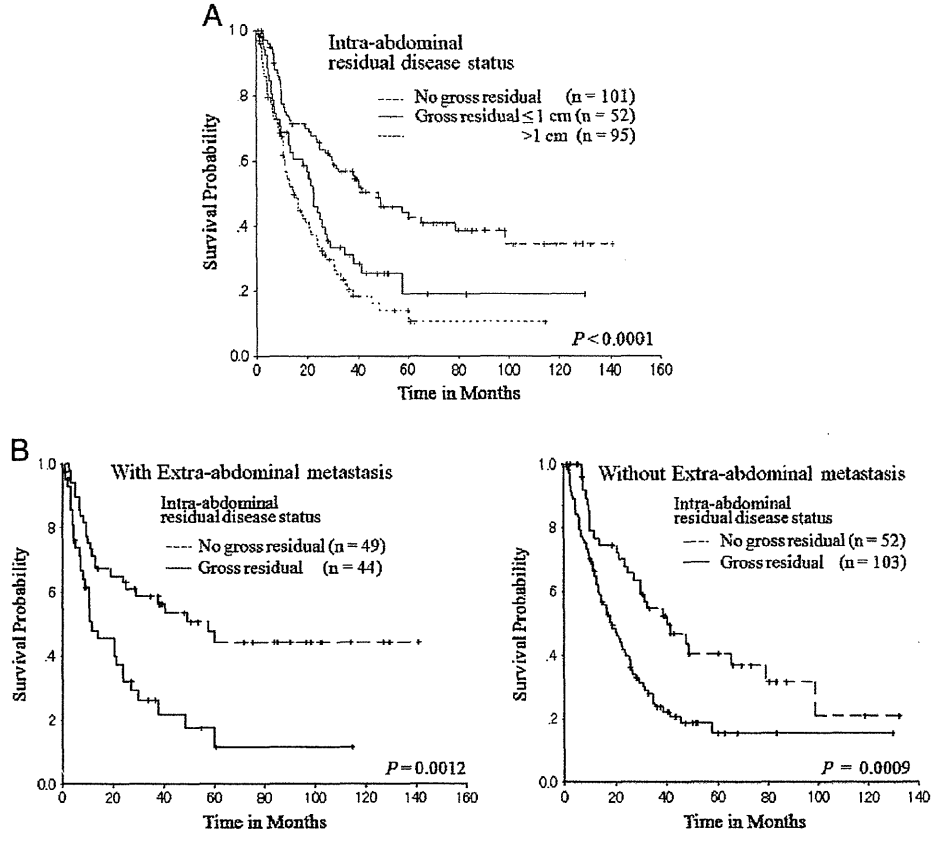

Fig. 2. Kaplan–Meier curves for OS. A: Median OS time according to intra-abdominal residual disease: no residual disease (dashed line), 48 months; ≤1 cm residual disease (solid line), 23 months; > 1 cm residual disease (dotted line), 14 months. B: Median OS time according to intra-abdominal residual disease in patients with (left) and without (right) extra-abdominal metastases. (left): no residual disease (dashed line), 58 months; gross residual disease (solid line), 11 months. (right): no residual disease (dashed line), 40 months; gross residual disease (solid line), 18 months.

The distribution of extra-abdominal disease in this cohort is not identical to that of all stage IVb patients because selection bias for surgery is expected. The majority of patients with extra-abdominal metastases in this cohort had metastases involving only one anatomical region, a good PS, and no symptoms. This group of patients may therefore have had less aggressive disease or a better response to adjuvant chemotherapy than patients with extra-abdominal metastases who did not undergo surgery. We were unable to definitively determine which characteristics were good prognostic factors in patients with extra-abdominal metastases. However, extra-abdominal disease was not associated with poor prognosis in this study. The OS was significantly longer in patients who underwent intra-abdominal cytoreduction than in patients with remaining gross intra-abdominal disease, even in patients with extra-abdominal metastases. We suggest that aggressive surgery should be undertaken to achieve complete macroscopic resection of all intra-abdominal disease if the patient's general condition is good.

The Gynecologic Oncology Group reported that systemic postoperative adjuvant chemotherapy with cisplatin + doxorubicin was associated with improved survival compared with postoperative whole abdominal irradiation [20]. Therapy with paclitaxel + doxorubicin + cisplatin was reported to be superior to doxorubicin + cisplatin [21]. In patients with advanced EMCA, platinum + anthracyclines and taxanes seem to be the most promising agents. Some prospective and retrospective studies of combination adjuvant chemotherapy and radiation for advanced or recurrent EMCA have been conducted [22–24]. However, no studies have focused on adjuvant treatment of stage IVb EMCA.

In the present study, adjuvant therapy was associated with longer OS. Most patients received chemotherapy alone as postoperative treatment, including taxanes + platinum or AP. There were no differences in OS between these two treatment groups. Although it is certain that chemotherapy is an ideal treatment for this systemic disease, we cannot comment on treatment outcomes according to the type of postoperative therapy due to the heterogeneity of treatment schedules in our cohort.

This study has several limitations. First, because it was a retrospective multicenter study, the quality of data may not be uniform. We made a considerable effort to collect uniform data using a case report form to standardize the information collected as much as possible. Second, there were heterogeneous treatment protocols in different institutions. In particular, there may have been a selection bias for the type of treatment initially chosen in patients with distant metastases. Third, the question of whether the improved outcome of patients who undergo optimal cytoreduction is due to the surgery or to the biology and aggressiveness of the tumor is unresolved.

In conclusion, our retrospective study showed that PS, histology/grade, postoperative treatment, and intra-abdominal residual disease were independent predictors of survival in patients with stage IVb EMCA who underwent primary cytoreductive surgery. Cytoreductive surgery and postoperative therapy may prolong survival time in some patients with stage IVb EMCA, particularly those with relatively favorable prognostic factors, even in the presence of extra-abdominal metastases.

Conflict of interest statement

The authors have no conflicts of interest.

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Original contribution

PIK3CA overexpression is a possible prognostic factor for favorable survival in ovarian clear cell carcinoma☆

Azusa Abe MD, Takeo Minaguchi MD, PhD*, Hiroyuki Ochi MD, PhD, Mamiko Onuki MD, PhD, Satoshi Okada MD, PhD, Koji Matsumoto MD, PhD, Toyomi Satoh MD, PhD, Akinori Oki MD, PhD, Hiroyuki Yoshikawa MD, PhD

Department of Obstetrics and Gynecology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki, 305–8575 Japan

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Keywords:
PIK3CA;
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Mutation;
Amplification;
Survival

Summary Dysregulated signaling on the PI3-kinase/Akt cascade is reportedly associated with early stage and favorable prognosis in some kinds of malignancies including breast cancer, endometrial cancer, and colorectal cancer. PIK3CA, a catalytic subunit of PI3-kinase, is known to be activated in ovarian clear cell carcinoma (CCC), which is categorized as type I ovarian cancer. The aim of this study was to investigate the clinical significance of PIK3CA overexpression in the disease. We performed immunohistochemical analyses of PIK3CA, PTEN, p-Akt, p27 and p53 expressions in primary ovarian clear cell carcinomas from 62 Japanese patients. Genetic analyses of *PIK3CA* mutation and amplification were further conducted. PIK3CA was overexpressed in 45 tumors (73%), PTEN expression was negative in 3 (5%), and p53 was positive in 8 (13%). Overexpressed PIK3CA was found to be associated with p-Akt overexpression ($P = .007$). PIK3CA overexpression tended to be observed in more of stage I disease (73% versus 47%, $P = .07$) and was associated with absence of residual tumor at the initial surgery (96% versus 71%, $P = .01$). Furthermore, survival analyses revealed that PIK3CA overexpression correlated with improved overall survival ($P = .03$). Subsequent genetic analyses demonstrated that PIK3CA overexpression correlated with the presence of mutation or amplification of the *PIK3CA* gene in tumors ($P = .009$). Our observations suggest that the subgroup of ovarian clear cell carcinomas harboring activated PIK3CA seems to have better prognosis possibly due to more indolent biological property compared to tumors without PIK3CA activation. PIK3CA may serve as a biomarker for good prognosis and a possible therapeutic target in this lethal subtype of ovarian cancer.

1. Introduction

Recent morphologic and genetic studies have led to the paradigm for the pathogenesis and origin of epithelial ovarian cancer based on a dualistic model of carcinogenesis that divides epithelial ovarian cancer into two categories designated types I and II [1]. Type I tumors comprise low-grade serous and endometrioid, clear cell, and mucinous carcinomas. They are generally indolent, present in stage I, and characterized by specific mutations, including *KRAS*, *BRAF*, *CTNNB1*, *PTEN*, *PIK3CA*, and *ARID1A*. In contrast, type II tumors comprise high-grade serous and endometrioid, and undifferentiated carcinomas. They are highly aggressive, present in advanced stage, and harbor frequent *p53* mutations. Abnormal signaling

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* Corresponding author.
E-mail address: minaguchit@md.tsukuba.ac.jp (T. Minaguchi).

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on the phosphatidylinositol 3′ (PI3)-kinase/Akt pathway is reportedly associated with early stage and favorable prognosis in some kinds of malignancies including breast, endometrial, and colorectal cancers [2-8]. *PIK3CA*, a catalytic subunit of PI3-kinase, is known to be mutated or amplified in ovarian clear cell carcinoma (CCC), type I ovarian cancer [9,10]. Ovarian CCC is one of the most lethal types of ovarian cancer, being often resistant to platinum-based chemotherapy unlike the more common high-grade serous carcinoma [11,12]. However, the impact of *PIK3CA* aberration on patient prognosis in ovarian CCC has yet to be elucidated. Aiming to identify targets for molecular therapies and to potentially individualize therapies, we evaluated expressions of PIK3CA, PTEN, p-Akt, p27, and p53 in primary ovarian CCCs and correlated them to clinicopathological parameters and patient survival. We further investigated the mechanisms of PIK3CA overexpression by examining mutations and amplifications. Our findings provide significant implications for the management of the disease.

2. Materials and methods

2.1. Patients and specimens

The Ethical Committee of the University of Tsukuba Hospital approved the study protocol. All patients diagnosed with CCC of the ovary, treated in the Department of Gynecology and Obstetrics at the University of Tsukuba Hospital between 1988 and 2009, were identified through our database. A total of 62 Japanese patients with tumors of pure clear cell histology or mixed histology with clear cell component greater than 50% were included in the study (Table 1). All patients provided written informed consent. A median follow-up duration, excluding those who died, was 45 months (range, 7-266 months). All patients were adequately staged based on the criteria of International Federation of Gynecology and Obstetrics (FIGO, 1988). Patients generally underwent initial surgery, followed by 6 to 8 cycles of adjuvant chemotherapy. Seven patients had residual tumor at the initial surgery (median diameter of maximal residual tumor, 10 mm; range, 5-20 mm). Neoadjuvant chemotherapy followed by interval debulking surgery was selected for 4 patients with massive ascites or stage IV disease. TC regimen consisting of paclitaxel (175 mg/m^2) and carboplatin (AUC 6) was mostly administered.

Table 1 Patient characteristics

Characteristic	Number (n = 62)
Median age (range)	53.5 (30–81)
FIGO stage	
I	41
Ia	10
Ic	31
II	8
IIb	1
IIc	7
III	9
IIIb	2
IIIc	7
IV	4
Histotype	
Pure type	59
Mixed type	3
Serous	1
Mucinous	1
Endometrioid	1
Endometriosis	
Present	34
Absent	28
Adenomyosis	
Present	24
Absent	38
Treatment	
Surgery	62
Lymphadenectomy	49
Lymph node sampling	3
Chemotherapy	59
Platinum	58
Taxane	48
CPT-11	13

2.2. Immunohistochemistry

Surgical specimens were fixed in 10% formalin and embedded in paraffin; 4-μm sections from ovarian tumors were prepared and deparaffinized, and antigen retrieval was done by microwaving. Endogenous peroxidase activity was blocked with 0.3% H_2O_2. After blocking with normal serum, sections were incubated with monoclonal antibodies against PIK3CA (Cell Signaling, Danvers, MA), PTEN (Cascade, Winchester, MA), p-Akt (Cell Signaling), p27 (BD Pharmingen, Franklin Lakes, NJ), and p53 (Dako, Glostrup, Denmark). Sections were further incubated in biotinylated IgG, followed by avidin-biotin-peroxidase complex (VECTASTAIN Elite ABC Kit, Vector Laboratories, Burlingame, CA). Immunoreactions were visualized using diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. The corresponding normal tissue provided an internal positive control, and negative controls without addition of primary antibody showed low background staining.

2.3. Immunohistochemical scoring

Semiquantitative analyses of immunohistochemical (IHC) staining was performed as follows. Blinded for clinical and pathologic parameters, the intensity of staining in tumor cells was scored independently by 2 investigators (A.A. and T.M.). When readings differed, slides were re-reviewed, and a consensus score was applied. The group assigned 3 showed increased staining intensity; the group assigned 2 showed equivalent staining intensity to the corresponding normal

tissue; the group assigned 1 had decreased or no trace of staining (Fig. 1). Cases with a PTEN score of 1 were defined as negative to exclude false positivity. For p53, negative or positive staining of <10% of tumor cells was considered negative, and positive staining of >10% of tumor cells was positive. For p27, subcellular localization was evaluated

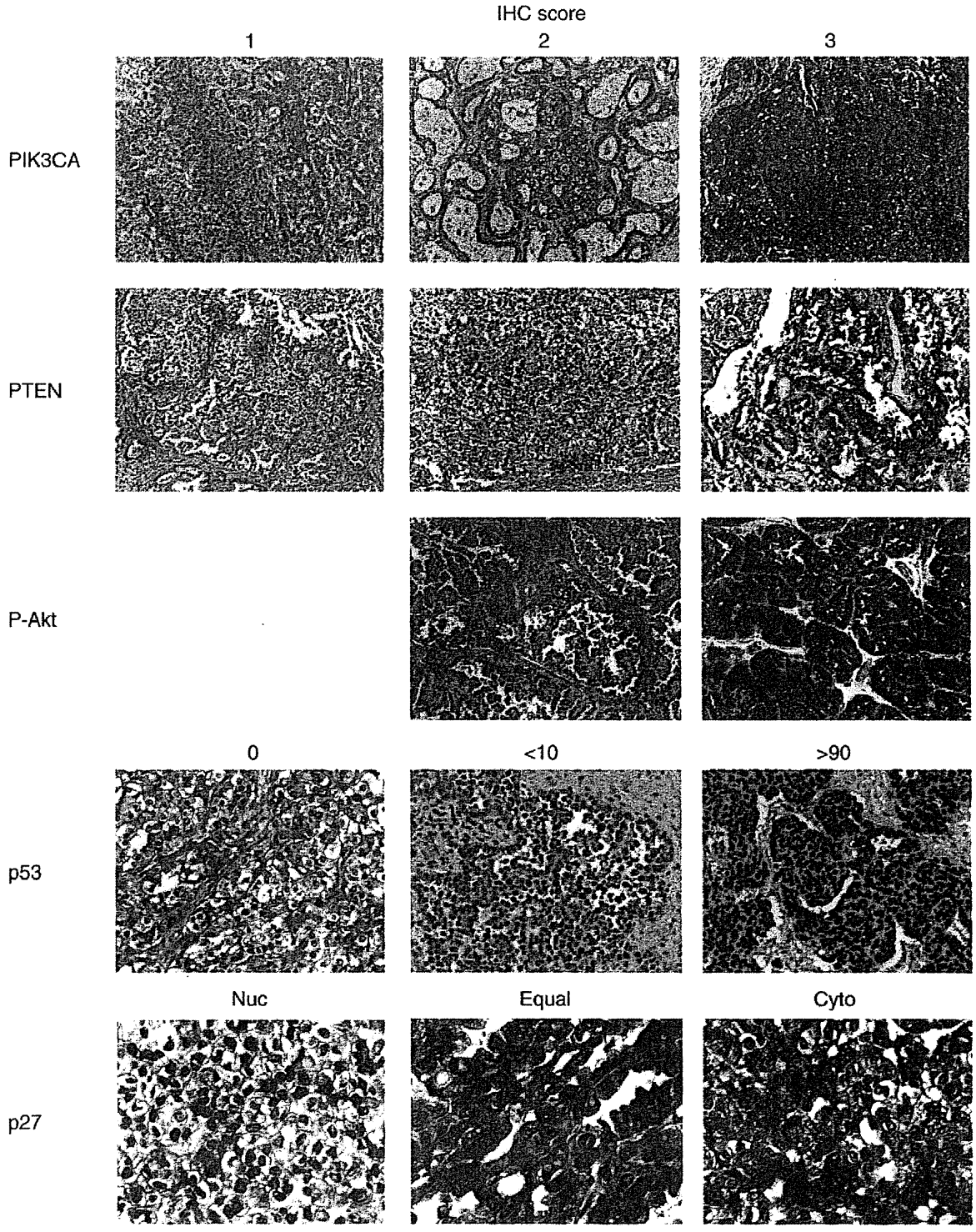

Fig. 1 IHC staining patterns of PIK3CA, PTEN, p-Akt, p53, and p27 in ovarian clear cell carcinomas. PIK3CA, PTEN, and p-Akt stainings for IHC scores 1 to 3 are shown. There was no case of IHC score 1 for p-Akt. For p53, negative and positive stainings in indicated percentages of tumor cells are demonstrated. For p27, nuclear dominant, equal, and cytoplasmic dominant localized stainings are shown. PIK3CA, PTEN, p-Akt, and p53, original magnification ×100; p27, original magnification ×400.

according to dominant or equal staining at nucleus/cytoplasm. For normal control, normal ovarian surface epithelia and normal endometria from 10 cases, respectively, were examined, and cases >90% were scored as 2 for PIK3CA, p-Akt, and PTEN; 100% were negative for p53, and >75% were dominantly nuclear for p27.

2.4. DNA extraction

Genomic DNA was extracted from tumor areas of formalin-fixed, paraffin-embedded ovarian tissue with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

2.5. Mutation analysis

Polymerase chain reaction (PCR) amplifications targeting exons 9 and 20 of *PIK3CA* were performed using 4 pairs of pseudogene exclusionary primers as follows: exon 9, GAT TGG TTC TTT CCT GTC TCT G and CCA TTT TAG CAC TTA CCT GTG AC (first pair) and CTG AAA TCA CTG AGC AGG AGA A and CCA CAA ATA TCA ATT TAC AAC CAT TG (second pair); and exon 20, TGG GGT AAA GGG AAT CAA AAG and ATT CCA GAG CCA AGC ATC AT (first pair) and ACA GCA TGC CAA TCT CTT CA and CCT ATG CAA TCG GTC TTT GC (second pair). PCR was conducted in 40 μL reaction volumes containing 200 ng of genomic DNA, 200 μmol/L of each deoxynucleotide triphosphate, 1× PCR Buffer II (Applied Biosystems, Foster City, CA), 1.5 mmol/L $MgCl_2$, 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), and 1 μmol/L of each primer. PCR amplifications were performed in a GeneAmp PCR System 9700 thermocycler with denaturation at 95°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 53°C to 59°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. DNA purification was performed using a Mini Elute Gel Extraction Kit (Qiagen), and the products were submitted to Operon Biotechnologies (Tokyo, Japan) for direct sequencing.

2.6. Quantitative real-time PCR

PIK3CA amplification was analyzed in an ABI 7700 Sequence Detection System (Applied Biosystems) as described elsewhere [13]. Real-time PCR was conducted in 50-μL reaction volumes containing 1 × Power SYBR Green PCR Master Mix (Applied Biosystems) and 300 nmol/L forward and reverse primers. A cycle threshold (C_T) value in the linear range of amplification was selected for each sample in triplicate and normalized to the glucokinase (*GCK*) as the reference gene. *PIK3CA* dosage was determined using the $2^{-\Delta\Delta CT}$ method. The normalized value (ΔC_T) for each tumor sample was then compared with the mean ΔC_T of five normal ovaries to produce a fold-change ratio and multiplied by 2 to generate a copy number. The cutoff line for amplification was set at 3 gene copies.

2.7. Statistical analysis

Differences in proportions were evaluated by the Fisher's exact test. Kaplan-Meier survival curves were calculated and compared statistically using the log-rank test. The Cox proportional hazard model was used for the univariate and multivariate analyses.

3. Results

The IHC results are summarized in Table 2. All the 3 patients with negative PTEN showed coexistent PIK3CA overexpression. PIK3CA overexpression was significantly associated with p-Akt overexpression (P = .007), which in turn correlated with dominant or equal cytoplasmic p27 localization without statistical significance (P = .11; Table 2). Analysis of associations between IHC results and clinicopathological variables (Table 3) showed that tumors overexpressing PIK3CA tended to include more stage I disease than tumors without PIK3CA overexpression (P =

Table 2 Results of IHC evaluation and correlation with phospho-Akt expression

Expression	Number (n = 62)	P-Akt overexpression		*P*
		(+) n = 46	(−) n = 16	
Overexpressed PIK3CA (IHC score = 3)	45 (73%)	38 (83%)	7 (44%)	.007
Negative PTEN (IHC score = 1)	3 (5%)	3 (7%)	0 (0%)	.56
Overexpressed p-Akt (IHC score = 3)	46 (74%)	-	-	-
Positive p53	8 (13%)	6 (13%)	2 (13%)	1.0
p27 localization				
Cytoplasmic	12 (19%)	41 (89%)	11 (69%)	.11
Equal	40 (65%)			
Nuclear	10 (16%)	5 (11%)	5 (31%)	

Table 3 Relationship between IHC results and clinicopathological features

Clinicopathological features	PIK3CA overexpression		*P*	PTEN expression		*P*	p53 expression		*P*	p-Akt overexpression		*P*	p27 localization		*P*
	(+) n = 45	(−) n = 17		Negative n = 3	Positive n = 59		Positive n = 8	Negative n = 54		(+) n = 46	(−) n = 16		Cyto/Equal n = 52	Nuc n = 10	
Age ≥60	13 (29%)	2 (12%)	.20	1 (33%)	14 (24%)	1.0	0 (0%)	15 (28%)	.18	12 (26%)	3 (19%)	.74	14 (27%)	1 (10%)	.43
FIGO stage I	33 (73%)	8 (47%)	.07	3 (100%)	38 (64%)	.54	5 (63%)	36 (67%)	1.0	32 (70%)	9 (56%)	.37	33 (63%)	8 (80%)	.47
FIGO stage III/IV	7 (16%)	6 (35%)	.16	0 (0%)	13 (22%)	1.0	3 (38%)	10 (19%)	.35	7 (15%)	6 (38%)	.08	11 (21%)	2 (20%)	1.0
Lymph node metastasis	6 (13%)	3 (18%)	.70	0 (0%)	9 (15%)	1.0	2 (25%)	7 (13%)	.33	5 (11%)	4 (25%)	.22	8 (15%)	1 (10%)	1.0
Endometriosis	24 (53%)	10 (59%)	.78	1 (33%)	33 (56%)	.58	7 (88%)	27 (50%)	.06	22 (48%)	12 (75%)	.08	28 (54%)	6 (60%)	1.0
Adenomyosis	16 (36%)	8 (47%)	.56	1 (33%)	23 (39%)	1.0	5 (63%)	19 (35%)	.24	16 (35%)	8 (50%)	.37	18 (35%)	6 (60%)	.17
Residual tumor present	2 (4%)	5 (29%)	.01	0 (0%)	7 (12%)	1.0	1 (13%)	6 (11%)	1.0	4 (9%)	3 (19%)	.36	6 (12%)	1 (10%)	1.0
Lymphadenectomy	35 (78%)	14 (82%)	1.0	3 (100%)	46 (78%)	1.0	8 (100%)	41 (76%)	.19	37 (80%)	12 (75%)	.73	41 (79%)	8 (80%)	1.0
Adjuvant chemotherapy	43 (96%)	16 (94%)	1.0	3 (100%)	56 (95%)	1.0	8 (100%)	51 (94%)	1.0	44 (96%)	15 (94%)	1.0	51 (98%)	8 (80%)	.07

.07), whereas tumors positive for p53 did not show any such trend. Moreover, PIK3CA overexpression was significantly associated with absence of residual tumor at the initial surgery ($P = .01$). Even in stage II-IV disease only, PIK3CA overexpression showed the same tendency (83% versus 44%, $P = .16$; data not shown).

Subsequently, we carried out survival analyses according to IHC evaluations (Fig. 2). Patients with tumors overexpressing PIK3CA showed significantly better overall survival than those without PIK3CA overexpression ($P = .031$; Fig. 2A), whereas patients with tumors positive for p53 showed a trend toward worse overall survival ($P = .092$; Fig. 2C). Cytoplasmic or equal localization of p27, another important tumor suppressor regulating cell cycle, also showed a trend toward worse overall survival ($P = .10$; Fig. 2E). Notably, even in the subset of advanced-stage (II-IV) disease, PIK3CA overexpression was associated with a trend toward better overall survival ($P = .089$; Fig. 2G). Interestingly, when patients were subclassified with PIK3CA and p53 status, survival for patients with overexpressed PIK3CA and negative p53 was the best and survival for patients with not-overexpressed PIK3CA and positive p53 was the worst ($P = .007$; Fig. 2H). Univariate analysis was further performed for various favorable

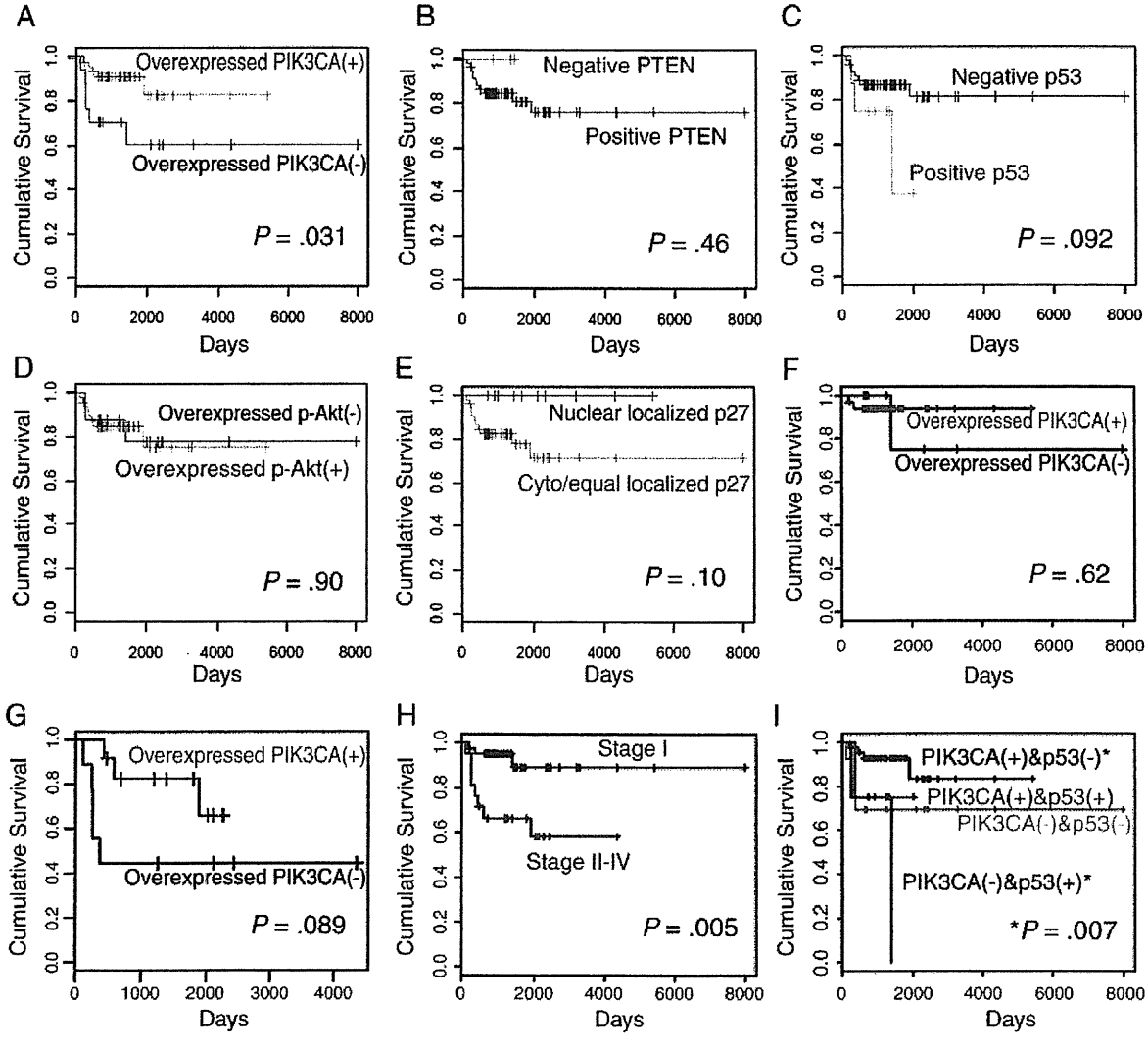

Fig. 2 Kaplan-Meier curves for overall survival according to protein expression levels in ovarian clear cell carcinomas. A, Patients with overexpressed PIK3CA (n = 45) versus without overexpressed PIK3CA (n = 17). B, Patients with negative PTEN (n = 3) versus positive PTEN (n = 59). C, Patients with negative p53 (n = 54) versus positive p53 (n = 8). D, Patients with overexpressed p-Akt (n = 46) versus without overexpressed p-Akt (n = 16). E, Patients with nuclear localized p27 (n = 10) versus cytoplasmic/equal localized p27 (n = 52). F, Patients with overexpressed PIK3CA (n = 33) versus without overexpressed PIK3CA (n = 8) in stage I disease. G, Patients with overexpressed PIK3CA (n = 12) versus without overexpressed PIK3CA (n = 9) in advanced disease (stage II-IV). H, The whole group of patients in stage I disease (n = 41) versus stage II-IV disease (n = 21). I, Patients with overexpressed PIK3CA and negative p53 (n = 41), overexpressed PIK3CA and positive p53 (n = 4), not-overexpressed PIK3CA and negative p53 (n = 13), and not-overexpressed PIK3CA and positive p53 (n = 4).

Table 4 Univariate and multivariate analyses of favorable prognostic factors for overall survival

Favorable factor	*P* (HR, CI)	
	Univariate	Multivariate
Overexpressed PIK3CA (IHC score = 3 *vs* 1-2)	0.04 (0.3, 0.09-0.9)	0.3 (0.5, 0.1-1.8)
Negative PTEN (IHC score = 1 *vs* 2-3)	1 (3.8×10^{-8}, 0-∞)	
Negative p53 (*vs* positive)	0.1 (0.3, 0.09-1.3)	
Nuclear localized p27 (*vs* Cytoplasmic/equal)	1 (3.8×10^{-9}, 0-∞)	
Age <60 (*vs* ≥60)	0.6 (0.7, 0.2-2.6)	
FIGO stage I/II (*vs* III/IV)	0.001 (0.1, 0.04-0.5)	0.2 (0.3, 0.06-1.7)
Residual tumor absent (*vs* present)	8.1×10^{-5} (0.07, 0.02-0.26)	0.1 (0.2, 0.03-1.4)
Endometriosis present (*vs* absent)	0.1 (0.4, 0.1-1.3)	
Adenomyosis present (*vs* absent)	0.3 (0.5, 0.1-1.9)	

Abbreviations: HR, hazard ratio; CI, 95% confidence interval.

prognostic factors. PIK3CA overexpression, FIGO stage I/II, and absent residual tumor at the initial surgery were found to be significant indicators of improved overall survival (P = .04, .001, and 8.1×10^{-5}, respectively; Table 4). Multivariate analysis was subsequently conducted using those three factors, none of which were found significant (Table 4).

Next we searched for *PIK3CA* mutation and amplification using DNAs from the 62 archival specimens. Direct sequencing was successful only in 41 specimens even after repeatedly optimizing the conditions of DNA extraction and PCR amplification, probably due to DNA fragmentation by formalin fixation. *PIK3CA* was mutated in 14 (34%) and amplified in 7 (17%) of the 41 samples (Table 5). All of the found mutations except N1044S were the most frequent three aberrations of *PIK3CA* in human cancers (Table 5) [14]. Presence of *PIK3CA* mutation or amplification was found to be associated significantly with PIK3CA overexpression observed in immunohistochemistry (61% versus 10%, P = .009; Table 5).

Table 5 *PIK3CA* mutation, amplification, and expression in ovarian clear cell carcinoma specimens

Case	Nucleotide	Amino acid	Gene copy No.	Amplification	Expression
1	1624G>A	E542K	1.0	N	Elevated
2	1624G>A	E542K	1.8	N	Elevated
3	1633G>A	E545K	1.1	N	Elevated
4	1633G>A	E545K	1.2	N	Elevated
5	1633G>A	E545K	2.9	N	Elevated
6	1633G>A	E545K	1.8	N	Elevated
7	1633G>A	E545K	1.9	N	Elevated
8	1633G>A	E545K	1.9	N	Elevated
9	3131A>G	N1044S	2.3	N	Elevated
10	3140A>G	H1047R	1.9	N	Elevated
11	3140A>G	H1047R	1.3	N	Elevated
12	3140A>G	H1047R	1.5	N	Elevated
13	3140A>G	H1047R	0.7	N	N
14	3140A>G	H1047R	3.9	Amplified	Elevated
15	WT	-	3.0	Amplified	Elevated
16	WT	-	5.1	Amplified	Elevated
17	WT	-	13.4	Amplified	Elevated
18	WT	-	3.1	Amplified	Elevated
19	WT	-	3.4	Amplified	Elevated
20	WT	-	3.3	Amplified	Elevated
21	WT	-	2.4	N	Elevated
22	WT	-	1.8	N	Elevated
23	WT	-	2.2	N	Elevated
24	WT	-	0.9	N	Elevated
25	WT	-	1.6	N	Elevated
26	WT	-	1.5	N	Elevated
27	WT	-	1.5	N	Elevated
28	WT	-	1.1	N	Elevated
29	WT	-	1.5	N	Elevated
30	WT	-	1.8	N	Elevated
31	WT	-	2.1	N	Elevated
32	WT	-	1.8	N	Elevated
33	WT	-	0.9	N	N
34	WT	-	1.8	N	N
35	WT	-	1.5	N	N
36	WT	-	0.8	N	N
37	WT	-	1.9	N	N
38	WT	-	1.6	N	N
39	WT	-	1.0	N	N
40	WT	-	1.5	N	N
41	WT	-	1.4	N	N

Abbreviations: WT, wild type; N, not amplified/not elevated.

4. Discussion

Our IHC analyses demonstrated that PIK3CA overexpression is a frequent event and both negative PTEN and accumulated p53 are rare events in ovarian CCC (Table 2), keeping in line with the published finding where mutations at hot spots of *PIK3CA*, *p53*, and *PTEN* were detected in 33%, 15%, and 5% of ovarian CCCs, respectively [10]. Genetic analyses further showed that PIK3CA overexpression was associated with the presence of mutation or amplification of *PIK3CA*, which was found in 49% of the examined specimens (Table 5), being consistent with the published observation that *PIK3CA* amplification or mutation was detected in up to 60% of ovarian CCCs [9]. These findings indicate that major molecular mechanisms for immunohistochemically observed PIK3CA overexpression were mutation and amplification. However, the mechanism by which those activating mutations result in elevated expression of PIK3CA is at present unknown. It is possible that activated PIK3CA may be unlikely to be degraded by cytoplasmic proteasome because of interaction with proteins localized to

the plasma membrane, that is, p85 and Ras [15]. Meanwhile, the mechanism for PIK3CA overexpression in tumors without mutation nor amplification (Table 5) may be transcriptional activation by transcription factors such as FOXO3a and others [16].

Subsequent association analyses demonstrated that tumors overexpressing PIK3CA tended to include more stage I disease compared to tumors without PIK3CA overexpression, while tumors positive for p53 did not show any such trend (Table 3). Moreover, PIK3CA overexpression was associated with absence of residual tumor at the initial surgery, whereas positive p53 was not. These findings suggest that tumors with PIK3CA activation may have a more indolent biological property such as to invade and disseminate more slowly compared to tumors without PIK3CA activation.

Further survival analyses revealed that PIK3CA overexpression correlated with better overall survival, while positive p53 showed a trend toward worse overall survival (Fig. 2A and C). Notably, PIK3CA overexpression correlated with a trend toward better overall survival even in the subset of advanced disease in contrast with stage I disease (Fig. 2F and 2G). Subsequent univariate and multivariate analyses showed that the significant favorable prognostic factors, that is, PIK3CA overexpression, early FIGO stage, and no residual tumor at the initial surgery, were not independent but mutually related (Table 4). In addition, PIK3CA overexpression was associated with p-Akt overexpression, and p-Akt overexpression was in turn associated with cytoplasmic or equal subcellular localization of p27 (Table 2), being consistent with the signaling mechanism of the PI3-kinase/Akt pathway. In contrast to PIK3CA, however, p-Akt did not show any prognostic impact on overall survival, and subcellular localization of p27 showed prognostic impact rather contrary to PIK3CA (Fig. 2D and E). These findings indicate that the observed PIK3CA impact on prognosis appears to be attributed to not functions of downstream effectors but rather *PIK3CA* genetic alteration itself, which is thought to represent the biological and clinical characteristics of tumor.

Deregulated signaling on the PI3-kinase/Akt cascade has been reported to be associated with early stage and favorable prognosis in some types of malignancies including breast, endometrial, and colorectal cancers [2-8]. Regarding ovarian cancer, however, prognostic significance of aberrant signaling on the PI3-kinase/Akt pathway has yet to be investigated. Together with the above results on association analyses of clinicopathological factors, our survival analyses suggest that ovarian CCC with PIK3CA activation may have better prognosis possibly due to slower invasion and dissemination compared to CCC without this genetic aberration. Further clinical and molecular studies are warranted to validate our proposal.

Type I ovarian cancers are generally slow growing and localized to the ovary at diagnosis. However, when these tumors spread beyond the ovary, chemotherapeutics that are effective against rapidly proliferating type II tumors are not as effective for type I tumors [1]. Hence, new chemotherapeutic approaches are needed for type I tumors, as which CCC is categorized. The PI3-kinase/Akt pathway is involved in a diverse range of cellular processes contributing to carcinogenesis. Now several molecular therapeutics targeting the components of this pathway are being examined in clinical trials combined with conventional chemotherapeutics in ovarian cancers, for example, bevacizumab, sunitinib, everolimus, temsirolimus, and sorafenib. These targeted agents are reasonably expected to be active against ovarian CCC with activated PI3-kinase/Akt pathway [17,18]. It is now becoming very important in terms of health economics to identify the group of patients more likely to benefit from expensive molecular targeted agents.

In conclusion, we have demonstrated here that PIK3CA overexpression is associated with better overall survival in ovarian CCC and that PIK3CA overexpression was associated with the presence of mutation or amplification of *PIK3CA*. The current data suggest that the subgroup of ovarian CCCs with PIK3CA activation seems to have a favorable outcome possibly due to indolent biological behavior. Our findings raise the possibility that the subgroup of patients with this lethal type of ovarian cancer harboring activated PIK3CA particularly in advanced stage may benefit more from aggressive treatment including the addition of molecular targeted therapeutics to the conventional regimen.

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Original Article

Increased expression of OCIA domain containing 2 during stepwise progression of ovarian mucinous tumor

Chigusa Nagata,[1] Hiromi Kobayashi,[1] Akiko Sakata,[1] Kaishi Satomi,[1] Yuko Minami,[1] Yukio Morishita,[1] Rena Ohara,[2] Hiroyuki Yoshikawa,[2] Yuko Arai,[3] Masato Nishida[3] and Masayuki Noguchi[1]

[1]Department of Pathology, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tsukuba and [3]Department of Obstetrics and Gynecology, Kasumigaura Medical Center, National, Hospital Organization, Ibaraki, Japan

Ovarian cancer immunoreactive antigen domain containing 2 (OCIAD2) has been reported to show cancer-specific expression in early invasive lung adenocarcinoma. OCIAD2 shows high homology with OCIAD1, which was originally immunoscreened from ascites of a patient with ovarian cancer and found to be a tumor-specific protein. Therefore, like OCIAD1, OCIAD2 is expected to show high immunoreactivity in ovarian tumors. In this study, we examined the expression pattern of OCIAD2 in 117 ovarian mucinous tumors, and confirmed that it was more highly expressed in borderline tumor and carcinoma (51/74 cases, 69%) than in adenoma (6/43 cases, 14%). The immunoreactivity of OCIAD2 in borderline tumor and carcinoma was more specific than that of OCIAD1 (adenoma, 21/43 cases, 49%), and more sensitive than that of CEA (borderline tumor and carcinoma, 35/74 cases, 47%). Like OCIAD1, OCIAD2 is a cancer-related protein and its expression level increases during the course of malignant progression and is thought to be a very useful marker for evaluating the malignancy of ovarian mucinous tumors.

Key words: OCIAD2, ovarian mucinous tumor, malignancy

Ovarian carcinoma is still a major cause of death among women in Japan and western countries. Ovarian tumors are classified histologically into various categories such as surface epithelial tumors, sex-cord stromal tumors and germ cell tumors. Among them, surface epithelial tumors constitute the major category and have four subgroups: serous tumor, mucinous tumor, endometrioid tumor and clear cell tumor. These surface epithelial tumors are further subclassified into benign (adenoma), borderline malignancy and malignant (carcinoma) forms. Among the ovarian surface epithelial tumors, the concept of multistep carcinognenesis has been accepted, especially in the development of mucinous carcinoma.[1,2] Based on an increased frequency of KRAS mutation, mucinous adenoma is thought to develop into mucinous carcinoma through a mucinous borderline lesion.[3–6] In terms of prognosis, mucinous borderline tumor is defined as an ovarian tumor of low malignant potential exhibiting epithelial proliferation of mucinous-type cells more pronounced than that seen in its benign counterpart, but without evidence of stromal invasion.

We have previously reported genes that are overexpressed in early invasive adenocarcinomas of the lung in comparison to *in situ* adenocarcinomas, based on cDNA microarray analysis of their gene expression profiles.[7] Among the genes selected, OCIAD2 (ovarian cancer immunoreactive antigen domain containing 2) showed significantly higher expression in early invasive than in *in situ* carcinoma. Immunohistochemically, OCIAD2 was expressed in most invasive pulmonary adenocarcinomas but completely negative in normal lung tissue. OCIAD2 belongs to a family of genes that contain the ovarian carcinoma immunoreactive antigen (OCIA) domain and related eukaryotic sequences, and are especially expressed in ovarian carcinoma. The OCIAD gene family includes OCIAD1 and OCIAD2. OCIAD1, showing high homology with OCIAD2, was originally immunoscreened by Luo *et al.* from ascites of a patient with ovarian cancer and found to be a tumor-specific protein and immunoreactive antigen.[8] They reported that patients who had tumors expressing OCIAD1 might develop an antibody against it. This means that OCIAD1 could be a cancer-specific protein potentially applicable as a marker for detection of carcinoma. Over the past few years, several studies have focused on OCIAD1, and it has become apparent that OCIAD1 overexpression is related to progression of ovarian cancer and plays a role in the formation of metastatic foci by affecting cancer cell adhesion.[9,10] So far, however, the role of OCIAD2 has not been studied. On the basis of the available evidence, the purpose of

Correspondence: Masayuki Noguchi, MD, Department of Pathology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki 305-8575, Japan. Email: nmasayuk@md.tsukuba.ac.jp

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the present study was to analyze the expression pattern of OCIAD2 in ovarian mucinous tumors in comparison with known tumor markers such as CEA and OCIAD1, and to discuss the multistep development of ovarian mucinous tumors.

MATERIALS AND METHODS

Patients and tissue specimens

All the cases studied were taken from samples that had been surgically resected between 1996 and 2009 at Tsukuba University Hospital (Tsukuba, Japan) and between 2004 and 2009 at Kasumigaura Medical Center Hospital (Tsuchiura, Japan). We extracted 40 mucinous borderline tumors, including 30 of the intestinal type and 10 of the endocervical type, and 34 mucinous carcinomas, including 8 of the infiltrative invasion type and 26 of the expansile invasive type. In addition, 43 mucinous adenomas were selected from 842 adenomas that had been resected during the same period in the two hospitals as control cases. The patient age distribution was matched for the borderline tumors and the carcinomas (Fig. 1). All patients had given informed consent to the use of their materials before surgery. The specimens were formalin-fixed and paraffin-embedded, and subjected to hematoxylin-eosin (HE) and immunohistochemical staining. Four independent investigators (CN, HK, AS and KS), including one gynecological pathologist (AS), reviewed the sections and confirmed the diagnoses. In this study, we followed the 2003 WHO classification for tumors of the female genital organs.[11–13] For diagnosis of the mucinous borderline tumors, we mainly examined papillary proliferation and epithelial atypia, as there was no definite stromal invasion to differentiate them from true benign adenomas and real invasive adenocarcinomas.[14–18] Therefore, all of the mucinous borderline tumors were classical (typical) ones, and tumors with microinvasion and intraepithelial carcinoma were excluded from this study. One case was diagnosed as a borderline tumor with the features of pseudomyxoma peritonei. We then selected representative sections for each of the cases. Clinical data on the patients were collected in an anonymised manner (Table 1).

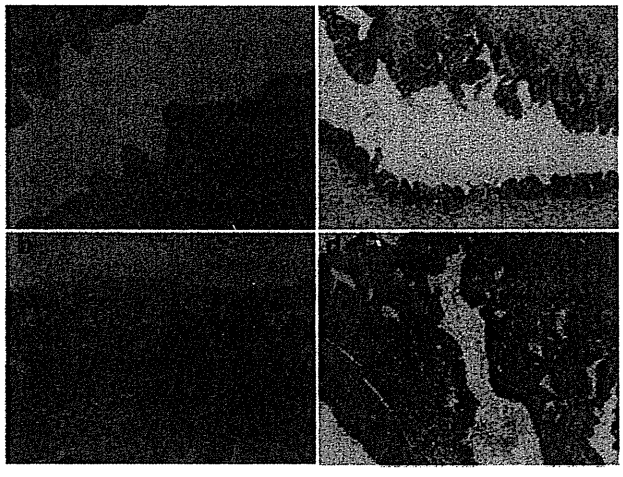

Figure 1 Histological features (HE staining) of mucinous ovarian tumors. Adenoma:1a, borderline lesion (endocervical type);1b, borderline lesion (intestinal type);1c, and adenocarcinoma;1d.

Immunohistochemical staining

Cut sections were deparaffinized and rehydrated, then autoclaved in 10 mmol/L citrate buffer (pH 6.0) at 121°C for

Table 1 Clinicopathological features of the patients

	Mucinous adenoma	Mucinous borderline tumor	Mucinous adenocarcinoma
Patient age (yr)	18–76 (mean 45.7)	17–90 (mean 49.6)	22–77 (mean 54.8)
Laterality			
Right	20	14	14
Left	23	24	20
Unknown	0	2	0
Maximum diameter (cm)	1.5–28 (mean 10.5)	4–40 (mean 13.6)	5–26 (mean 14)
FIGO stage		Ia 34	Ia 15
		Ic 1	Ib 1
		IIb I	Ic 8
		IIIc 2	IIa 1
		IIIb 1	IIb 2
		IV 1	IIc 1
			IIIa 1
			IIIc 5
Follow-up period (months)	1–108	7–156	1–144
Patients' outcome			
NED	43	38	26
Dead	0	1	5
Unknown	0	1	3