Figure 4. Representative EMT related genes also over-expressed in surgically resected esophageal tumor samples obtained from identical cases. (A) Expression patterns of 2 representative EMT regulators (*ZEB1* and *ZEB2*), 8 typical EMT markers including fibronectin (*FN*), vimentin (*VIM*), 3 collagens (*COL1A2*, *COL3A1*, and *COL14A1*), *FBN1*, *MYH11*, and *ACTC1*, and 2 EMT-related myogenic transcription factors (*MEOX2* and *MEF2C*). (B) Quantitative real-time RT-PCR results of *ZEB1*, *ZEB2*, *FN*, *and VIM*. Closed box: surgical sample; Open box: biopsy sample.
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margin from the surrounding normal tissue. Thus, we obtained surgical samples from a margin (periphery) of the tumor. For the needle biopsy samples, tumor portions (2 mm X 2 mm) were obtained under endoscopy from a margin of the tumor by exclusion of any central necrotic lesions. If the samples were severely contaminated by necrotic lesions, those samples were excluded by quantifying and qualifying RNA. If the samples contained extensive normal lesions, we excluded such samples by

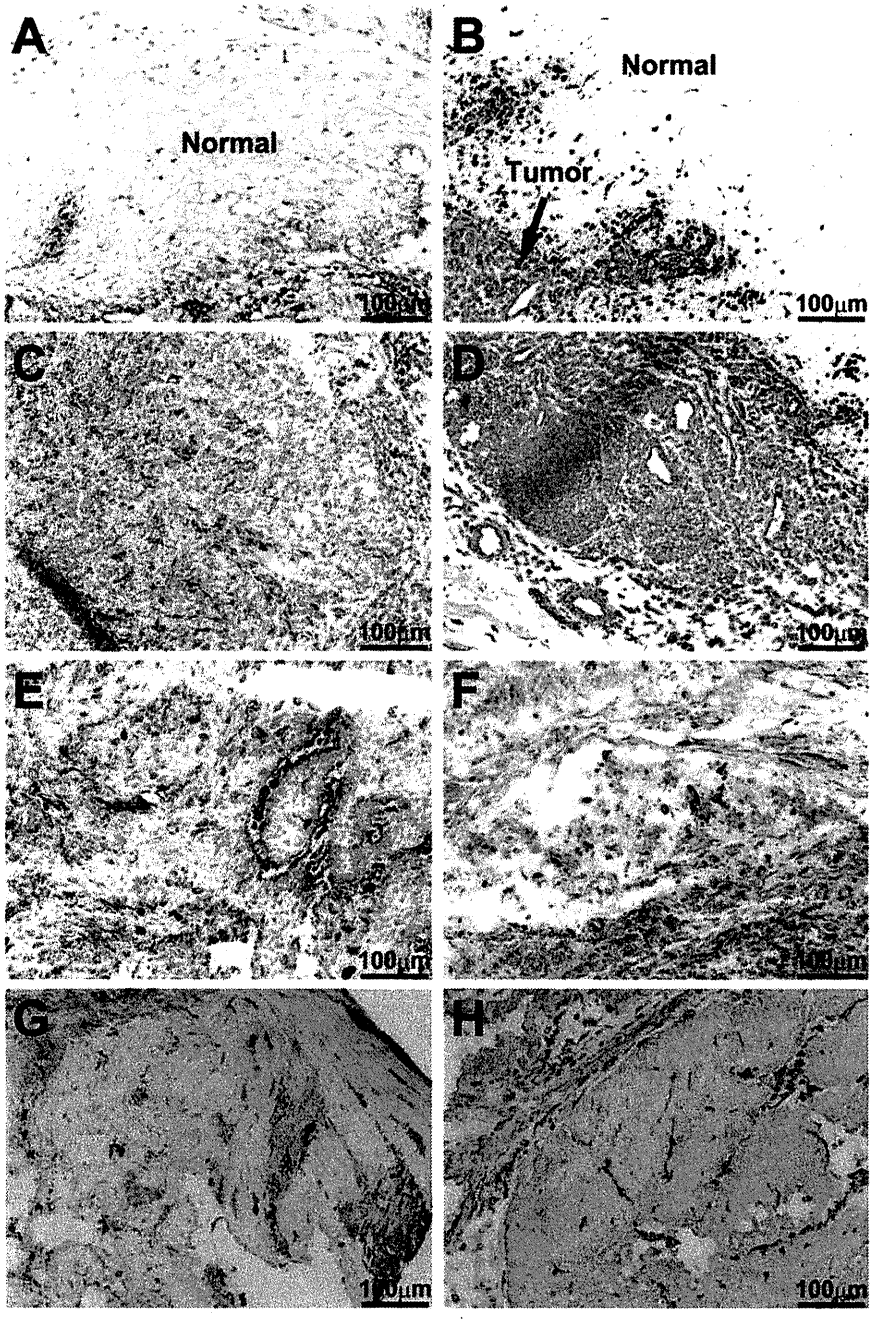


Figure 5. Immunohistochemistry (IHC) of a typical EMT marker vimentin in biopsy and surgically resected tissues. IHC of vimentin in an additional surgical sample, which contained normal portions, showed that normal esophageal epithelial cells were not stained, but invasive tumor cells were (A, B). In 3 out of 5 pairs of biopsy and surgical samples, over-expression of vimentin was observed in the surgical samples (biopsy: C, E, G; surgical: D, F, H).
doi:10.1371/journal.pone.0018196.g005

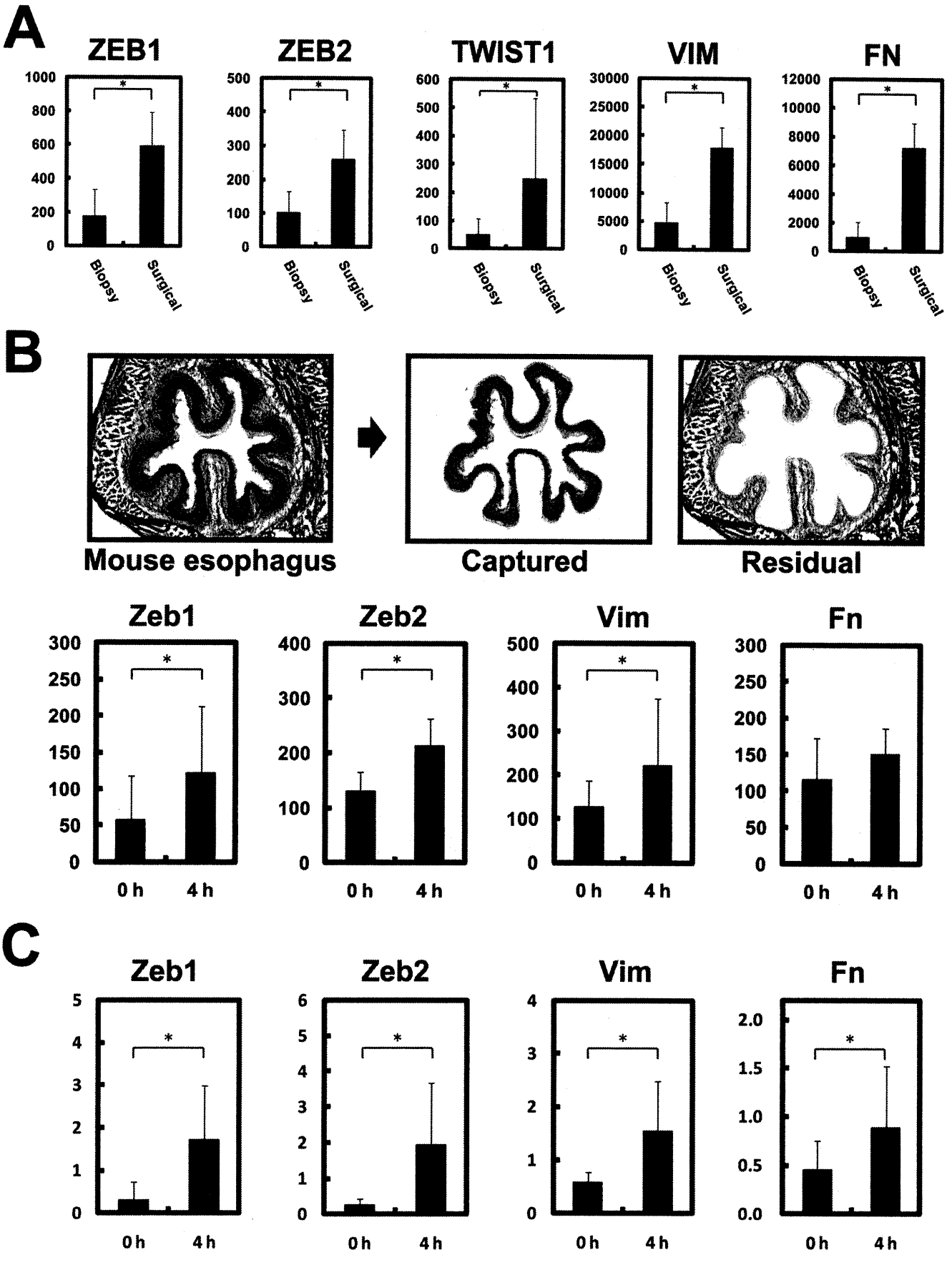

A
ZEB1
ZEB2
TWIST1
VIM
FN
Biopsy
Surgical
B
Mouse esophagus
Captured
Residual
Zeb1
Zeb2
Vim
Fn
0 h
4 h
C
Zeb1
Zeb2
Vim
Fn
0 h
4 h

Figure 6. Over-expression of EMT regulators and markers in surgically resected normal tissues. (A) Over-expression of EMT-regulators (*ZEB1*, *ZEB2*, and *TWIST1*) and EMT-markers (*VIM* and *FN*) in surgically resected normal esophagus mucosa. (B) Induction of mouse *Zeb1*, *Zeb2*, *Vim*, and *Fn* under ischemic condition. After resection of mouse esophagus, we placed it on PBS for 0 or 4 hours at room temperature (under an ischemic condition), immediately made frozen sections, captured the epithelial cell layer (upper) by laser microdissection, amplified mRNA by TALPAT [24–28], and obtained expression profiles using Mouse Expression Array 430 2.0 (Affymetrix, Santa Clara, CA). Experiments were performed on 3 mice. The *Zeb1*, *Zeb2*, *Vim*, and *Fn* genes are induced 4 hours after resection (Lower). *$P<0.05$. (C) Quantitative real time RT-PCR of *Zeb1*, *Zeb2*, *Vim*, and *Fn*. Over-expression of *Zeb1*, *Zeb2*, *Vim*, and *Fn*, shown by microarray, was confirmed.
doi:10.1371/journal.pone.0018196.g006

the expression profile-based scoring method using normal and/or tumor specific genes (in preparation).

The overall process of an esophageal cancer operation requires much time. Therefore, surgical samples were excised from a margin of the tumor by trained pathologists after exposure for 4–7 hours under an ischemic condition, and were immediately frozen at −80°C until use. On the contrary, needle biopsy samples resected under endoscopy were immediately frozen at −80°C until use. Clinicopathological information is given in Tables S3, S4, S5.

Laser Microdissection followed by RNA Extraction and Amplification

Cryostat sections (8μm) of frozen mouse esophageal samples were laser-microdissected with the mmi CellCut system (MMI Inc., Rockledge, FL). Total RNA was isolated by suspending the cells in an ISOGEN lysis buffer (Nippon Gene, Toyama, Japan) followed by precipitation with isopropanol. RNA was amplified by an efficient method of high-fidelity mRNA amplification, called TALPAT (T7 RNA polymerase promoter-attached, adaptor ligation-mediated, and PCR amplification followed by *in vitro* T7-transcription) [24–28].

Microarray Analysis

Gene expression profiles were obtained from 166 samples: tumor sets (different cases) of independent 35 and 20 biopsy samples and 66 surgical samples, another tumor set (identical case) of 18 biopsy samples and 18 surgical samples, a normal set of 4 biopsy samples and 5 surgical samples. Total RNAs extracted from the bulk tissue samples were biotin-labeled and hybridized to high-

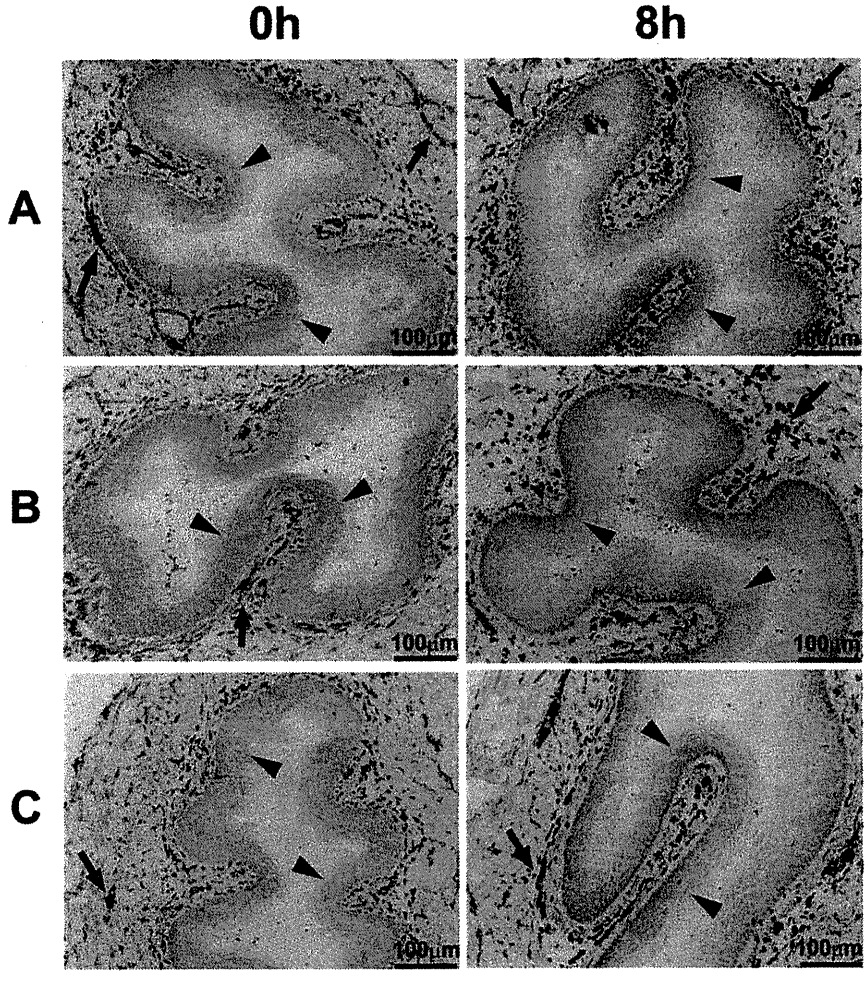
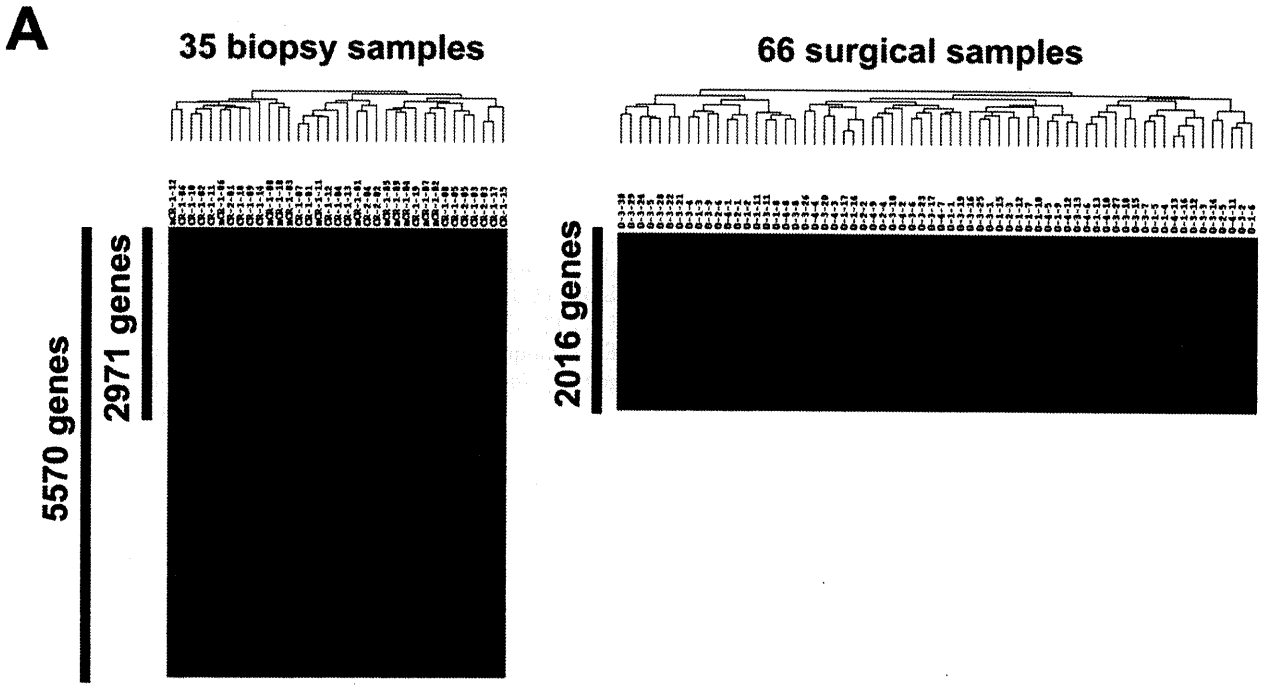
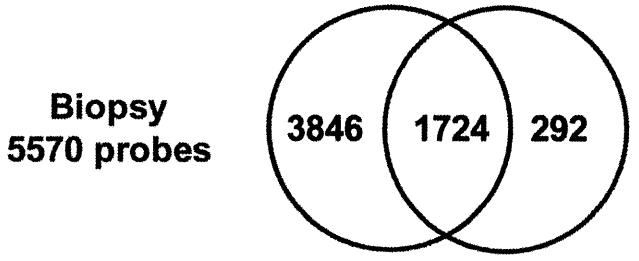


Figure 7. Immunohistochemistry (IHC) of a typical EMT marker vimentin in mouse esophagus. After resection of mouse esophagus, we placed it on PBS for 0, 4, 8 hours at room temperature (under an ischemic condition), immediately made frozen sections, and IHC of vimentin was performed under more sensitive conditions compared with Figure 5. Experiments were performed on 3 mice (A–C). Over-expression of vimentin in mouse esophageal epithelium was not observed even after 8 hours of exposure under an ischemic condition. Arrow: vimentin-positive smooth muscle, arrow head: mouse stratified esophageal epithelial cell layers.
doi:10.1371/journal.pone.0018196.g007



B

U95Av2 microarray data	1. > 1000 signal intensity in > 10% sample	1. > 1000 signal intensity in > 10% sample 2. > 3 fold change from Ave. intensity in > 10% sample
Biopsy 35 samples	6551	5570 (85%)
Surgical 66 samples	4797	2016 (42%)



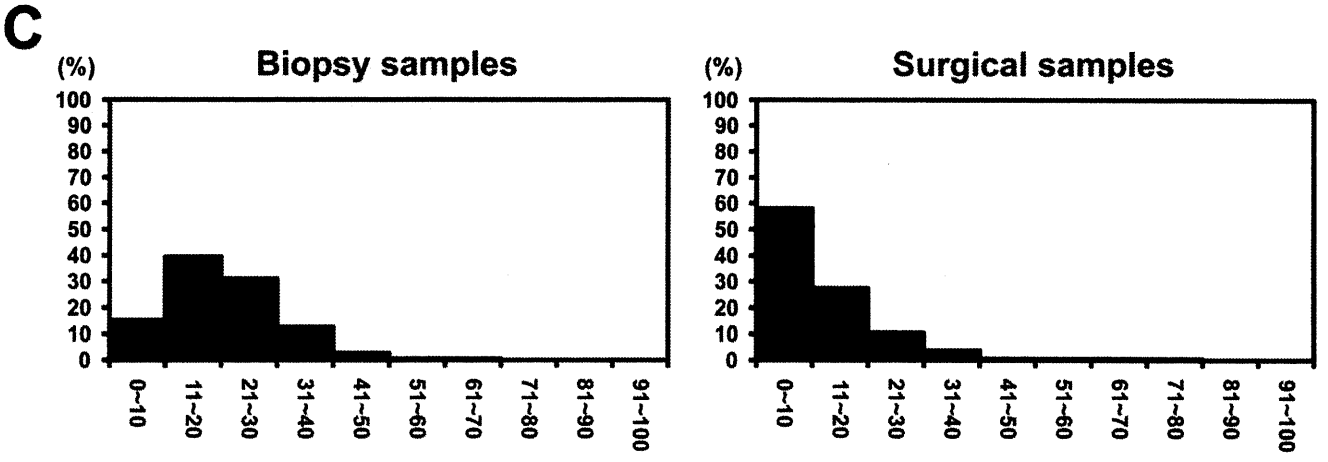


Figure 8. Artificially induced EMT prevents microarray-based subgroup identification. (A) Unsupervised clustering of 35 biopsy and 66 surgically resected esophageal tumor samples with 5,570 and 2,016 processed genes, respectively. A sample cluster with 2,971 genes appears only in the biopsy samples. (B) Comparison of the number of processed genes for unsupervised clustering between biopsy and surgical samples. The number of processed genes and commonly selected genes is indicated. (C) Frequency distribution for percentage of samples of finally processed-gene sets. Each distribution of 5, 570 genes in biopsy samples (Left) and 2,016 genes in surgical samples (Right) is indicated.
doi:10.1371/journal.pone.0018196.g008

density oligonucleotide microarrays (Human Genome U95Av2 or U133PLUS2.0 Array, Affymetrix, Santa Clara, CA, USA) in accordance with the manufacturer's instructions. For laser-captured mouse esophageal epithelial cell layers, Mouse Genome 430 2.0 Array was used. The scanned data of the arrays were processed by Affymetrix Microarray Suite version 4.0 or 5.0, which scaled the average intensity of all the genes on each array to a target signal of 1,000 to reliably compare variable multiple arrays. All the microarray data have been deposited in a MIAME compliant database, GEO; the accession number SuperSeries GSE22954.

Gene Selection from Microarray Data and Hierarchical Clustering

Hierarchical clustering is widely used as one of the unsupervised learning methods. Hierarchical clustering of microarray data was performed by the use of GeneSpring (Agilent Technologies Ltd., CA, USA), Microsoft EXCEL, and Cluster & TreeView software [29].For unsupervised clustering (Figures 1A and 8A), we first selected genes with a signal intensity of more than 1,000 in more than 10% of the samples, and from these genes, we finally selected more than 3-fold changed genes by comparing the average signal intensity of each gene in more than 10% of the samples. For overexpressed genes in the surgical or biopsy samples, we first selected genes by u-test ($p<0.01$), permutation test, and 2- or 3-fold change between the average signal intensities of the two sets of samples, and from the first selected genes we finally selected genes with more than 1,000 in average signal intensity.

Semi-quantitative and Quantitative RT-PCR

Total RNA was isolated by suspending the cells in Isogen lysis buffer (Nippon Gene, Toyama, Japan) followed by precipitation with isopropanol. RT-PCR was carried out using primer sets designed for detecting the 3′ side of cDNA of each human gene: for *IL8*, 5′- TGCCAAGGAGTGCTAAAG -3′ and 5′- CTCCACAACCCTCTGCAC-3′, for *CXCR4*, 5′-TGTATGTCTCGTGGTAGGAC-3′ and 5′-AGACTGTACACTGTAGGTGC-3′, for CXCL9, 5′-ACAAAGAAAATATTTCAAATTACAAGG-3′ and 5′-GGGAACGGTGAAGTACTAAGC-3′, for *PDGFRB*, 5′-ACTGCCCAGACCTAGCAGTG-3′ and 5′-CAGGGAAGTAAGGTGCCAAC-3′, for CCL5, 5′-CCCCGTGCCCACATCAAGGAGTATTT-3′ and 5′-CGTCCAGCCTGGGGAAGGTTTTTGTA-3′, for *TLR2*, 5′-CCAGCAGGAACATCTGCTAT-3′ and 5′-TCCAGGTAGGTCTTGGTGTT-3′, for *ZEB1*, 5′-CGTCTCTTTCAGCATCACCA-3′ and 5′-ATGGGAGACACCAAACCAAC-3′, for *ZEB2*, 5′-CATGACGTTGATCATTTGGGC-3′ and 5′-CGAGCATGGTCATTTTCAAAAG-3′, for *FN*, 5′-CGGGGGAAATAATTCCTGTG-3′ and 5′-CCTTGCAGGCAATCTCTTTG-3′, for *VIM*, 5′-GCTTTCAAGTGCCTTTCTGC-3′ and 5′-GTTGGTTGGATACTTGCTGG-3′, and for *ACTB* (β-actin), 5′-TCATCACCATTGGCAATGAG-3′ and 5′-CACTGTGTTGGCGTACAGGT-3′. Primer sets for detecting each mouse gene were also designed: for *Zeb1*, 5′-TAACATTTATACTTGCCTCC-3′ and 5′-GCTAAGGGAATGAGTTATGG-3′, for *Zeb2*, 5′-ACCAAATCAGACCACGAGGA-3′ and 5′-GCCCCTTCTGTCCCTCTCTA-3′, for *Fn*, 5′-CCGTGGGATGTTTTGAGACT-3′ and 5′-GGCAAAAGAAAGCAGAGGTG-3′, for *Vim*, 5′-ACGGTTGAGACCAGAGATGG-3′ and 5′-CGTCTTTTGGGGTGTCAGTT-3′ , and for *ActB*, 5′-GCTCTTTTCCAGCCTTCCTT-3′ and 5′-GTACTTGCGCTCAGGAGGAG-3′. For semi-quantitative RT-PCR, we showed data within linear range by performing 25–35 cycles of PCR. Quantitative real-time PCR was performed by a Bio-Rad iCycler with iQ Syber Green Supermix (Bio-Rad, Hercules, CA, USA) as directed by the manufacturer. The value of 1/2N (N: the number of PCR cycles corresponding to the onset of the linear amplification of each gene product) was calculated as a relative mRNA expression level of each gene normalized to *ACTB*. The data from 2 independent analyses for each sample were averaged.

Immunohistochemistry

For immunohistochemical staining of frozen sections of human and murine esophagus, specimens that were embedded in a TissueTek OCT medium (VWR Scientific Products, West Chester, CA) and stocked at −80°C until use were cut into 8μm sections, which were then left for 30 min at room temperature followed by fixing in 4% paraformaldehyde for 20 min at room temperature. Endogenous peroxidase activity was inhibited with 3% H_2O_2 in methanol for 30 min. Blocking was carried out with Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Sections were incubated for 60 min at room temperature with diluted mouse monoclonal antibody directed against human vimentin (N1521, DAKO, Carpinteria, CA) or rabbit polyclonal antibody directed against mouse vimentin (#3932, Cell Signaling Technology Japan, Tokyo, Japan). After washing sections with PBS, biotinylated secondary antibodies were applied for 30 min at room temperature. Detection was carried out by using Vectastain ABC Elite Kit (Vector Laboratories) and the DAB system (DAKO, Tokyo), and the sections were counter-stained with 1% Methyl Green. (Sigma, Saint Luis, MO)

Supporting Information

Figure S1 Schema of artificial factors during surgical resection and sample transportation. Biopsy samples are small, much fresher, with low contamination of normal portions compared to surgical samples, whereas some artificial factors such as ischemia, hypoxia, hyponutrition, and cold stress possibly occur during surgical resection and sample transportation.
(TIF)

Figure S2 Expression levels of *ZEB1* and *ZEB2* in two sets of biopsy and surgical samples (different and identical cases). Over-expression of both genes is observed in surgically resected esophageal tumors, except *ZEB2* in the different cases. $*P<0.05$.
(TIF)

Figure S3 Expression levels of *TWIST1* in two sets of biopsy and surgical samples (different and identical cases). Over-expression of *TWIST1* is observed in surgically resected esophageal tumors. $*P<0.05$.
(TIF)

Figure S4 Schema of crosstalk between Hh and EMT signal pathways in esophageal cancers. The primary transcriptional factor GLI1 and an EMT regulator TWIST1 regulate another EMT regulator ZEB2, which activates any gene including membrane type receptors (*PDGFRA*, *EDNRA*, *CXCR4*, *VEGFR2*, and *TRKB*) [9].
(TIF)

Figure S5 Expression levels of *HIF1A*, *HIF1B*, *HIF2A*, and *LOXL2* in two sets of biopsy and surgical samples (different and identical cases). Over-expression of *HIF1A* and its target *LOXL2* is observed only in surgically resected esophageal tumors (different cases).
(TIF)

Figure S6 Expression levels of *NFKB1* and *TGFBR2* in two sets of biopsy and surgically resected tumor samples (different and identical cases) and in biopsy and surgically resected non-cancerous tissues (normal). Over-expression of *NFKB1* and *TGFBR2* is observed in all the sets of surgically resected samples. $*P<0.05$.
(TIF)

Table S1 219 up-regulated genes in 66 surgically resected esophageal tumors.
(DOC)

Table S2 716 up-regulated genes in 18 surgically resected esophageal tumors.
(DOC)

Table S3 Clinicopathological information of biopsy samples from different cases with esophageal squamous cell carcinoma.
(DOC)

Table S4 Clinicopathological information of surgical samples from different cases with esophageal squamous cell carcinoma.
(DOC)

Table S5 Clinicopathological information of biopsy and surgical samples from different cases with esophageal squamous cell carcinoma.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: KA KM MM A. Ohtsu TY HS. Performed the experiments: KA TN AA. Analyzed the data: KA HS. Contributed reagents/materials/analysis tools: KM HI YT A. Ochiai NH HD MM A. Ohtsu. Wrote the paper: KA HS.

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Serous Adenocarcinoma of the Uterine Cervix: A Clinicopathological Study of 12 Cases and a Review of the Literature

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Key Words
Clinicopathological characteristics · Serous adenocarcinoma · Uterine cervix

Abstract
Background/Aims: To determine the clinicopathological characteristics and potentially associated outcomes in patients diagnosed with serous adenocarcinoma of the uterine cervix. ***Methods:*** The records of surgically-treated patients with pathological stage pT1b–2b serous adenocarcinoma were reviewed. ***Results:*** Of 12 patients with serous adenocarcinoma who underwent radical hysterectomy, five had pT1b1N0 disease, two pT1b1N1, two pT1b2N0, and three pT2bN1. The 5-year overall survival rate for patients with or without parametrial involvement (pT2b vs. pT1b) was 0 and 89%, respectively. The 3-year recurrence-free survival rate for those with or without parametrial involvement was 33 and 89%, respectively. Four patients suffered recurrence, namely one of those who had pT1b (1/9, 11%) and 3 of those who had pT2b disease (100%). The sites of recurrence of pT2b disease were outside the pelvis in all 3 patients. Of these, 2 (67%) had peritoneal spread and 1 distant node metastasis. ***Conclusion:*** While patients with pathological stage pT1b disease may have a relatively favorable outcome after radical surgery, those with more advanced disease have a poor prognosis because of extra-pelvic recurrence.

Introduction

Serous adenocarcinoma of the uterine cervix (SACC) is a very rare tumor, while this histological subtype is common in the ovary, Fallopian tube, and peritoneum. Probably for this reason, details of the clinicopathological features of SACC are largely unknown [1, 2]. Reports in the literature are on very limited numbers of patients, with the largest to date including 17 patients [3], this being the only report concerning the clinicopathological factors and prognosis of more than 10 cases. Of these 17 patients, the prognosis of 9 was made after hysterectomy. The present retrospective study assessed the clinicopathological features and prognosis of 12 patients with SACC who underwent hysterectomy.

Material and Methods

We reviewed the medical records and pathological specimens obtained from all patients with primary cervical carcinoma treated and diagnosed at the Gynecology Division and Pathology Section of the National Cancer Center Hospital, Tokyo, Japan, between 1985 and 2005. All definitive diagnoses were made based on excised specimens.

This study included patients who had had a lesion fulfilling the histological criteria for serous adenocarcinoma according to the criteria of the World Health Organization International Histological Classification of Tumors and who had undergone a pri-

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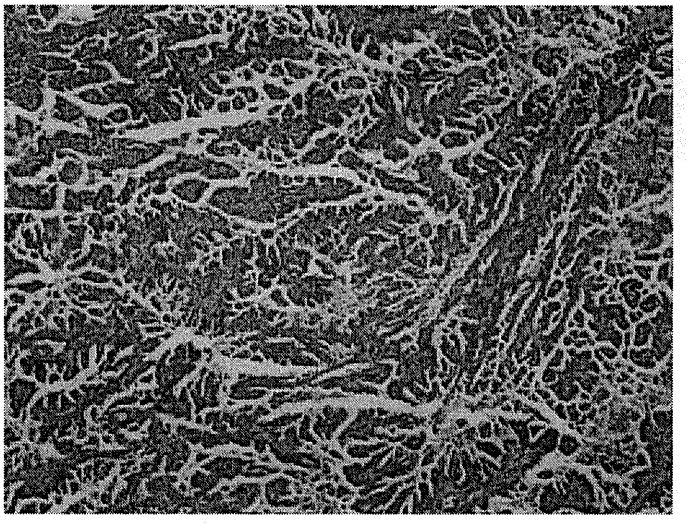

Fig. 1. Papillary serous adenocarcinoma. The tumor shows slender papillae with fibrovascular cores lined by small cells. HE stain, ×40.

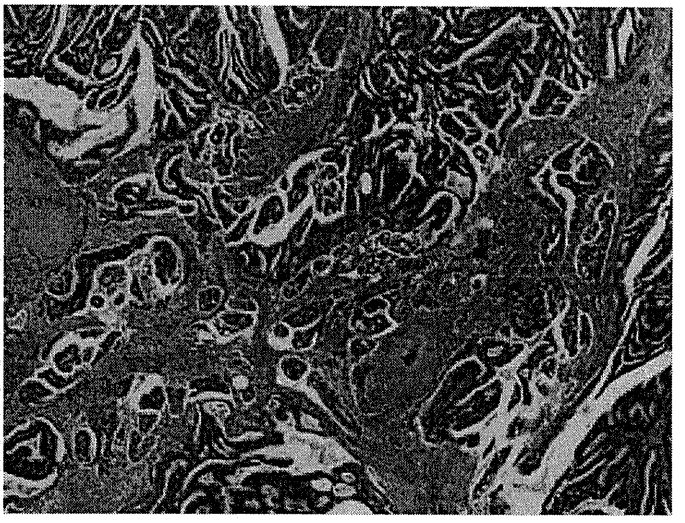

Fig. 2. Papillary serous adenocarcinoma invading cervical stroma, with clusters of tumor cells. HE stain, ×100.

mary hysterectomy. The diagnosis of serous adenocarcinoma was made only when an invasive cervical adenocarcinoma exhibited a prominent papillary structure and/or slit-like glandular spaces, and usually moderate to marked cytologic atypia (fig. 1, 2) without either intra- or extra-cytoplasmic mucin. At least 10% of the tumor area had to be of papillary serous type for inclusion in this study.

For this study, 2 gynecologic pathologists re-examined all surgically removed pathological specimens. Postoperative pathological classification was carried out according to the 2002 revision of the International Union against Cancer (UICC) TNM classification of malignant tumors.

Radical hysterectomy has long been a standard treatment option for the patients with FIGO stage IB–IIB disease in our institute. In patients with pelvic lymph node metastasis or parametrium involvement proven by pathological examination following surgery, adjuvant irradiation to the whole pelvis was administered.

Following primary treatment, asymptomatic patients underwent pelvic examination, Pap smear, ultrasound, and serial determination of tumor markers (CA125, CA19-9 and carcinoembryonic antigen) every 4–6 months. Symptomatic patients underwent the appropriate examination where indicated using chest radiography, computed tomography (CT) and magnetic resonance imaging (MRI).

Follow-up continued until January 2009. Recurrence-free and overall survival curves were calculated using the Kaplan-Meier method and were compared by non-parametric survival analysis (log-rank test). A p value of <0.05 was considered statistically significant. JMP software (version 5.0.1; SAS Institute Inc., Cary, N.C., USA) was used for statistical analysis.

Results

Patient Characteristics

Twelve patients met the study criteria. Their characteristics are summarized in table 1. Their median age was 52 years (range 30–74) and median follow-up time including death was 55 months (range 5–127). No patient was lost to follow-up. Baseline evaluation consisted of a complete gynecologic examination that included PAP smears, colposcopy and biopsy, laboratory studies inclusive of pretreatment CA125, CA19-9 and carcinoembryonic antigen as well as diagnostic imaging (CT, ultrasound, and MRI) at the initial visit. Eleven patients (92%) presented with abnormal genital bleeding as the primary symptom, with no other symptoms. The remaining patient (8%) was asymptomatic and was diagnosed based on abnormal cervical cytology. Nine patients had stage pT1b disease (seven with pT1b1 and two with pT1b2), and three had stage pT2b. All patients underwent radical hysterectomy with bilateral salpingo-oophorectomy and pelvic lymphadenectomy. All tumors were completely removed. Adjuvant radiotherapy or chemotherapy was administered to the 4 patients in whom lymph node metastasis or parametrial invasion was proven by pathological examination of resected specimens. Three of these 4 patients received adjuvant radiotherapy to the whole pelvis, for a total dose of 50 Gy, and 1 patient was treated with chemotherapy (cisplatin, doxorubicin and cyclophosphamide). In this case, the primary care doctor selected not radio-

Table 1. Clinicopathological characteristics of the 12 patients with SACC

Patient No.	Age	Pathological stage	Growth pattern	Grade	Histologic type	Depth of invasion, mm (depth ratio)	Length mm	LVSI	Ovarian metastasis	Adjuvant therapy	Recurrent site	Recurrence months	Status (months)
1	44	pT1b1 N0	Endophytic	2	pure[a]	16 (<3/3)	25	+	–	none	NA	NA	NED (54)
2	46	pT1b1 N0	Exophytic	3	pure	5 (<1/3)	40	–	–	none	NA	NA	NED (106)
3	47	pT1b1 N0	Endophytic	3	mixed[b]	9 (<3/3)	21	–	–	none	NA	NA	NED (65)
4	30	pT1b1 N0	Endophytic	2	mixed	12 (<3/3)	35	+	–	none	NA	NA	NED (127)
5	61	pT1b1 N0	Exophytic	2	mixed	6 (<1/2)	31	+	–	none	NA	NA	NED (62)
6	51	pT1b1 N1	Exophytic	3	pure	10 (<1/3)	27	+	+	chemotherapy	lung	2	DOD (5)
7	53	pT1b1 N1	Exophytic	3	pure	15 (<2/3)	30	+	–	radiotherapy	NA	NA	NED (48)
8	68	pT1b2 N0	Exophytic	2	mixed	40 (<3/3)	55	+	–	none	NA	NA	NED (28)
9	50	pT1b2 N0	Endophytic	2	mixed	20 (<3/3)	40	–	–	none	NA	NA	NED (45)
10	51	pT2b N1	Endophytic	2	pure	21 (<3/3)	23	+	–	radiotherapy	peritoneum	43	DOD (51)
11	74	pT2b N1	Endophytic	3	pure	15 (3/>3)	80	+	+	radiotherapy	peritoneum	35	DOD (40)
12	52	pT2b N1	Endophytic	3	pure	17 (3/>3)	20	–	–	none	PALN	12	DOD (28)

SACC = Serous adenocarcinoma of the uterine cervix; LVSI = lymph-vascular space involvement; NA = not applicable; PALN = para-aortic lymph node; NED = no evidence of disease; DOD = dead of disease. + = positive; – = negative.

[a] Only serous adenocarcinoma was observed; [b] another pathological subtype (endocervical and endometrioid) was observed.

therapy but chemotherapy because he thought that chemotherapy was suitable for cervical serous adenocarcinoma at that time. For 7 patients without lymph node metastasis or parametrial invasion as evaluated histopathologically, no adjuvant therapy was performed. The remaining patient with pT2bN1 disease elected not to receive adjuvant therapy.

Pathological Features

There were large macroscopic tumors (20–80 mm), located in the uterine cervix. Five tumors showed an exophytic pattern and 7 an endophytic one. In 5, another pathological subtype of uterine cervical adenocarcinoma was observed. Three cases had endometrioid adenocarcinoma accounting for 30–60% of the tumor, whereas the other 2 had endocervical-type mucinous adenocarcinoma accounting for 50–70% of the tumor. These 12 cases were classified into 3 cytologic grades according to Zhou's criteria [3]. Six were grade 2, with moderate nuclear pleomorphism, small nucleoli, and moderate amounts of cytoplasm. The other 6 were grade 3, with marked nuclear pleomorphism and prominent nucleoli. All tumors, regardless of grade, had >10 mitotic figures per 10 high-power fields. Psammoma body was present in 1 of the grade 2 cases.

The patients were excluded if they had a history of previous primary serous carcinoma of the ovary, Fallopian tube, endometrium, or peritoneum. There were no serous adenocarcinoma lesions of the Fallopian tube or peritoneum. In 9 cases, tumor extent was confined to the uterine cervix, but 1 case had microscopic ovarian metastasis, and in 1 other, the primary tumor had started to invade the endometrium. Another case had both microscopic ovarian metastasis and myometrial invasion, but the size of the cervical tumor was 80 mm. These ovarian metastatic lesions were extremely small (1 and 6 mm) compared with the cervical mass and were present not in the parenchyma but on the surface of the ovary. In order to distinguish between primary cervical cancer and metastasis from serous ovarian cancer, 2 gynecologic pathologists re-examined all surgically removed pathological specimens.

Survival

Four (33%) patients suffered tumor recurrence after a median of 23 months following initial surgery (range 2–41 months). Two of these patients presented with symptoms of dyspnea caused by pleural effusions, and back problems related to peritoneal recurrence. All 4 of the patients with recurrent tumor died at a median of 9 months after the onset of recurrence despite intensive multimodal therapy (systemic chemotherapy, radiation and surgery).

For all 12 patients, the 5-year overall survival rate was 62% and 3-year recurrence-free survival (RFS) was 74%. The 5-year overall survival rate for patients with or without parametrial involvement (pT2b vs. pT1b) was 0 and 89%, respectively (fig. 3). This was statistically significant ($p = 0.01$). The 3-year RFS rate for patients with or without parametrial involvement was 33 and 89%, respectively, which was also statistically significant ($p = 0.01$).

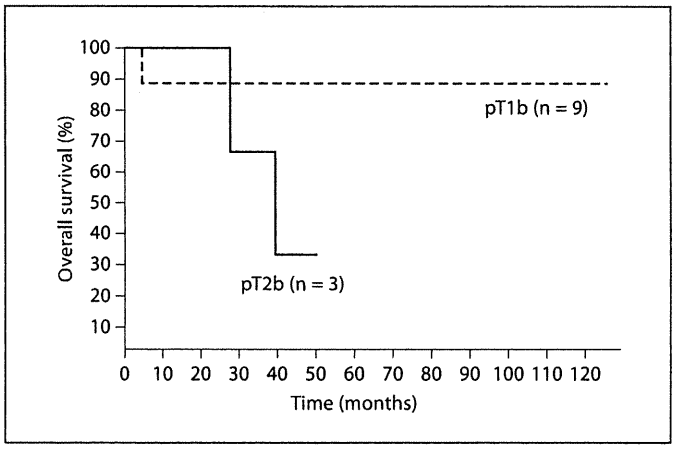

Fig. 3. Overall survival for 12 patients with SACC stratified by clinical stage.

Three-year RFS for patients with lymph node metastasis was 40 compared to 100% for those without. There was a significant difference in RFS between these 2 groups ($p = 0.005$).

The average number of resected lymph nodes was 28 (range 18–48). Pelvic lymph node metastasis was found in the surgically resected specimens from 1 (12.5 %) of the 8 patients with no recurrence, but in all 4 of those whose cancer recurred. All patients with 2 or more positive pelvic lymph node metastases suffered recurrence. Neither the one patient with only 1 positive pelvic lymph node metastasis nor any of the patients without metastasis suffered recurrence.

Recurrence Site

The initial sites of recurrence were located outside the pelvis in all 4 patients. The most frequent site of distant metastasis was the peritoneum (2/4, 50%), followed by the lung (1/4, 25%) and para-aortic lymph nodes (1/4, 25%).

Discussion

SACC is a very rare tumor. No large-scale multicenter study has been performed and the optimal primary therapeutic approach to SACC has not been determined. Several cases have been reported, but only very limited clinical data on SACC are available. The existing reports mostly provided information on the morphologic features and the behavior of this entity, but no accurate staging and assessment of lymph node metastasis based on reviewing surgical specimens for half the patients. Using 'serous adenocarcinoma' and 'uterine cervix' as key words, we conducted a Medline search of articles on SACC published in English from 1984 to 2008, and extracted papers describing accurate surgical staging, sites of recurrence and outcomes.

The literature provides information on a total of 25 patients including those in the present study. Twenty-one (84%) out of these 25 patients underwent radical hysterectomy [3–8]. The clinical characteristics of all 25 patients are summarized in table 2. The pathological stages were one case of pT1a, nineteen of pT1b, two of pT2a and three of pT2b. One patient with pT2a disease was categorized as advanced stage because of the presence of para-aortic lymph node metastasis. Recurrence occurred in 9 patients (three with pT1b disease, two with pT2a, three with pT2b, and one whose status was not mentioned), of whom 7 died of the disease. The recurrence rate in early (pT1 and pT2aM0) and advanced stage (pT2b and pT2aM1) was 23.8% (5/21) and 100% (4/4), respectively. Advanced stage was associated with poor prognosis.

Fregnani et al. [9] reported that the recurrence rate of patients with early-stage adenocarcinoma (FIGO stages IB and IIA) was 11.4% (4/35) and the 5-year RFS rate was 87.9%. Grisaru et al. [10] reported that the 5-year RFS rate of FIGO stage IA–IB patients with common-type adenocarcinoma (mucinous or endometrioid adenocarcinoma) was 90%. In both studies, all patients had undergone radical hysterectomy with or without adjuvant therapy. Kasamatsu et al. [11] reported that the recurrence rate for early-stage (pT1b–2a) patients with common-type adenocarcinoma (mucinous or endometrioid adenocarcinoma) was 16.7% (17/102) and their 3-year RFS rate was 91% and 100% for pT1b and pT1a, respectively. Although the recurrence rate in early-stage patients in the present review of the literature seems high (23.8%), 2 patients whose cancer recurred had not been treated with radical hysterectomy, but only with simple hysterectomy.

A radical hysterectomy with or without adjuvant therapy for early-stage SACC appeared to be associated with a favorable prognosis almost identical with common-type cervical adenocarcinoma. We suggest that the biological behavior of early-stage SACC is similar to common-type adenocarcinoma. On the other hand, all patients with advanced-stage SACC suffered recurrence, despite radical hysterectomy. In our institute, Kasamatsu et al. [11] reported that the 3-year RFS rate for patients with pT2b common-type adenocarcinoma (mucinous or endometrioid adenocarcinoma) who underwent radical

Table 2. Outcome and patterns of recurrence in 25 patients with SACC who underwent surgery: survey of the literature

	Postsurgical stage (n)	Age (mean)	Surgery (n)	Adjuvant therapy (n)	Lymph node metastasis (n)	Recurrence site (n)	Status (n)
Gilks et al. 1992 [4]	pT1b[a]	32	RH	Radiotherapy	Negative	None	NED
Shintaku et al. 1993 [5]	pT2a (1)	66	SH	Radiotherapy	Positive	Peritoneum	DOD
Rose et al. 1993 [6]	pT1a (1)	30	RH	None	Negative	None	NED
Zhou et al. 1998 [3]	pT1b (9)	Not mentioned	RH (8) SH (1)	Not mentioned	Not mentioned	Distant node (7) Peritoneum (3) Lung (2) Liver (1) Skin (1)	DOD (1) AWD (1) NED (7)
Kaplan et al. 1998 [7]	pT1b (1)	39	SH	Chemotherapy and radiotherapy	Positive	Peritoneum	DOD
Batistatou et al. 2000 [8]	pT2a[b] (1)	63	RH	Chemotherapy and radiotherapy	Positive	Lung, mediastinum and loco-regional	DOD
Present study	pT1b (9)	30–68 (50)	RH (9)	Chemotherapy (1) Radiotherapy (1)	Positive (2) Negative (7)	Lung (1)	DOD (1) NED (8)
	pT2b (3)	51–74 (57)	RH (3)	Radiotherapy (2)	Positive (3)	Peritoneum (2) PALN (1)	DOD (3)

SACC = Serous adenocarcinoma of the uterine cervix; RH = radical hysterectomy; SH = simple hysterectomy; PALN = para-aortic lymph node; NED = no evidence of disease; DOD = dead of disease; AWD = alive with disease.

[a] This patient is also included in the report by Zhou et al. [3]. [b] This patient has para-aortic lymph node metastasis.

hysterectomy was 38%. Patients with advanced-stage SACC may have more aggressive tumor behavior than those with common-type adenocarcinoma.

All patients whose tumor recurred had extra-pelvic metastasis in the present review of the literature. Of the 5 distant sites of recurrence, the most frequently reported was distant lymph node (8/21, 38%), followed by peritoneal spread (7/21, 33%), and then lung (4/21, 19%). In our institute, among the 123 patients with common-type adenocarcinoma who underwent radical hysterectomy, 27 (22%) suffered tumor recurrence [11]. Of these, the initial failure sites were inside the pelvis in 10 patients (38%), outside in 15 (58%) and both in 1 patient (4%). Of all distant failure sites, the most frequent were distant nodes (48%) and peritoneal spread (48%), followed by lung (8%). The spread pattern of the initial failure site in patients with SACC or common-type adenocarcinoma is therefore similar in that extra-pelvic metastasis is more frequent, in particular, peritoneal spread and distant node metastasis. Based on these findings, checking for extra-pelvic metastasis should be considered a priority issue for improving the survival of patients with SACC.

In patients with advanced-stage SACC, pelvic control alone usually does not lead to a favorable outcome because of the high incidence of distant metastasis. Whole-pelvic irradiation is generally selected in many institutes as a post-operative adjuvant therapy.

The largest single study of 17 cases, published by Zhou et al. in 1998 [3], revealed several key features. They reported that there was a bimodal age distribution, with 1 peak occurring before the age of 40 years and the second peak after the age of 65. In the present study, however, the mean age of all patients at the time of diagnosis was 52.2 years (range 30–74) and there are 9 cases in patients between the ages of 40 years and 65 years. From the literature review, the mean age of patients with common uterine cervical adenocarcinoma was 48.4 (±12.9) [12]. Thus, at 51 years (range 24–78) [11], the mean age of the

patients with SACC is similar to common-type adenocarcinoma.

Zhou et al. [3] reported that the presenting symptoms were abnormal vaginal bleeding or discharge (13 patients), and abnormal cervical cytology (4 patients). In the present study, 11 patients (92%) presented with abnormal genital bleeding and 1 (8%) with abnormal cervical cytology.

In summary, we have reported detailed clinicopathological features of 12 cases of SACC and reviewed the literature. Patients with pT1b disease may have a favorable outcome with radical surgery. In contrast, patients with more advanced-stage disease had a poor prognosis with established therapy, because of extra-pelvic recurrence. We need to seek effective systemic therapy for advanced-stage SACC.

Further study is warranted and is necessary to confirm the clinical behavior of SACC and to determine optimal therapy.

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Prognostic Impact of the History of Breast Cancer and of Hormone Therapy in Uterine Carcinosarcoma

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Objective: Recent studies reveal an association between hormone therapy for breast cancer (BC), such as tamoxifen (TAM) and toremifene (TOR), and uterine carcinosarcoma (UCS). The aim of this study was to investigate the characteristics and prognosis of patients with UCS after BC and hormone therapy.

Methods: Between January 1997 and December 2007, we treated 51 patients with UCS. The medical records of these patients were reviewed, and factors that influenced their survival were retrospectively analyzed using univariate and multivariate analyses.

Results: Ten (19.6%) of the 51 patients had a history of BC; 6 (11.8%) had received hormone therapy with TAM or TOR. The characteristics of the patients with UCS were similar regardless of whether they had a history of BC or hormone therapy. On univariate analysis, age greater than 56 years, elevated serum lactate dehydrogenase levels, residual tumors, FIGO (International Federation of Gynecology and Obstetrics) stage higher than stage IIIa, and non–endometrioid carcinomatous components were identified as prognostic factors. On multivariate analysis, in addition to residual tumors, FIGO stage higher than stage IIIa, and non–endometrioid carcinomatous components, a history of BC (relative risk, 0.14), a history of TAM use (relative risk, 15.9), and a history of TOR use (relative risk, 16.9) were also identified as independently significant prognostic factors.

Conclusions: Our data suggest that a history of BC and hormone therapy for BC is a risk factor for developing UCS without obvious impacts on the characteristics of UCS. Both of these factors had statistically significant impacts on the prognosis of patients with UCS. Further studies are necessary to clarify and validate these associations.

Key Words: Uterine carcinosarcoma, Breast cancer, Tamoxifen, Toremifene

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Tamoxifen (TAM) is a selective estrogen receptor modulator and is widely used as an adjuvant in patients with estrogen or progesterone receptor–positive breast cancer (BC). Toremifene (TOR), which has a similar structure to TAM, is now also used as hormone therapy for BC. Patients who receive long-term hormone therapy are well known to show an increased incidence of endometrial adenocarcinoma. Patients undergoing TAM therapy longer than 5 years are estimated to have an increased risk of endometrial adenocarcinoma by about 3- to 7-fold[1–3]; although its incidence is reported to be lower in patients undergoing TOR therapy.[4] In early studies, the prognosis of patients with endometrial adenocarcinoma related to TAM was thought to be worse than for those

unrelated to TAM.[3] However, later studies show a comparable prognosis between patients with diseases related to and unrelated to TAM[5,6]; however, this issue remains controversial.

Recently, the association between TAM and uterine carcinosarcoma (UCS) was demonstrated by a number of case reports and case-control studies.[3,7–14] Uterine carcinosarcoma is a tumor with carcinomatous and sarcomatous components and used to be classified as uterine sarcoma. Uterine carcinosarcoma is now considered one of the aggressive subtypes of endometrial adenocarcinoma, and its etiological features and symptoms are thought to be similar to those of endometrial adenocarcinoma. There are studies examining the prognosis of patients with UCS related to TAM.[5,11–13] However, there is still no consensus about the prognosis of patients with UCS related to TAM. Moreover, the prognosis of patients with UCS subsequent to BC without a history of hormone therapy is still unknown.

In the present study, we investigated the characteristics of patients with UCS and whether a history of BC and hormone therapy (e.g., TAM or TOR) can alter their prognosis.

TABLE 1. Characteristics of the patients with UCS in relation to their history of BC and of hormone therapy

	With a History of BC				
Factors	**Hormone Therapy**	**No Hormone Therapy**	**Subtotal**	**Without a History of BC**	**Total**
No. patients	6	4	10	41	51
Age, y	54–80	56–68	54–80	36–79	36–80
Mean, y	68.5	63.3	66.4	60.7	61.8
Median, y	71.5	64.5	67	62	63
≤56	1	1	2	13	15
>56	5	3	8	28	36
Menstrual status					
Premenopausal	0	0	0	8	8
Postmenopausal	6	4	10	33	43
Serum LDH					
Within normal limits	5	2	7	31	38
>Upper normal limits	1	2	3	10	13
Surgical procedures					
TAH + BSO	3	2	5	17	22
RAH or TAH + BSO + PLA	3	2	5	24	29
Sarcomatous component					
Homologous	4	3	7	27	34
Heterologous	2	1	3	14	17
Carcinomatous component					
Endometrioid	6	3	9	28	37
Non-endometrioid	0	1	1	13	14
Residual tumor					
None	6	2	8	34	42
Any	0	2	2	7	9
FIGO stage					
I	4	1	5	14	19
II	1	0	1	6	7
III	1	2	3	17	20
IV	0	1	1	4	5
Classified FIGO stage					
I–IIIa	5	2	7	32	39
IIIb–IV	1	2	3	9	12

TAH, total abdominal hysterectomy; RAH, radical abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; PLA, pelvic lymphadenectomy.

MATERIALS AND METHODS

Patients

Between January 1997 and December 2007, we treated 51 patients with UCS at the National Cancer Center Hospital, Japan. We reviewed the medical and pathological records of these patients. The data on whether the UCS patients had BC and had undergone hormone therapy, such as TAM and TOR, in addition to other possible prognostic factors of UCS were extracted from their records. According to the Japanese ethical guideline for epidemiologic study, this study was approved by the institutional review board of the National Cancer Center.

Our standard surgical treatment for endometrial cancer, including UCS, consists of total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. We performed radical abdominal hysterectomy in patients with apparent cervical involvement or those with a preoperative diagnosis of cervical carcinoma. Patients with biopsy-proven lymph node metastases also underwent para-aortic lymphadenectomy. In patients with no or superficial myometrial invasion on macroscopic examination of the resected uterus, pelvic lymphadenectomy was omitted and only palpation and sampling of swollen nodes were performed. In patients with extrauterine tumor spread, 6 cycles of postoperative chemotherapy were provided. Paclitaxel/carboplatin combination (TC regimen) and cyclophosphamide/doxorubicin/cisplatin combination (CAP regimen) chemotherapies were administered to 10 and 2 patients, respectively. Other treatment regimens of ifosfamide/cisplatin combination or doxorubicin/dacarbazine combination chemotherapy were administered to 1 patient each. All the patients underwent primary surgical treatment, and no neoadjuvant chemotherapy was performed. Before the start of treatment, written informed consent was obtained from all of the patients.

Statistical Analysis

Patient survival was measured from the day of starting treatment, that is, the day of surgery. Survival curves were determined by the Kaplan-Meier product limit method. Factors influencing survival were analyzed using the log-rank test (univariate) and Cox proportional hazards regression analysis (multivariate). A value of $P < 0.05$ was considered to indicate statistical significance. These analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL). On multivariate analyses, stepwise backward screening was performed with an exclusion $P = 0.05$ to identify independent prognostic factors. Contingency table analysis was performed using Fisher exact test or χ^2 test for trends. Differences in age were examined by nonpaired Student t test.

RESULTS

Associations Between UCS and a History of BC and of Hormone Therapy

The characteristics of the 51 patients with UCS were classified according to their histories of BC and hormone therapy and are summarized in Table 1. Ten (19.6%) of the 51 patients had a history of BC, and 6 patients (11.8%) had

TABLE 2. Characteristics of the patients with UCS and with a history of BC

Case	Age, y	FIGO Stage	BC Laterality	BC to UCS, y	Treatment	Residual Tumor	Carcinomatous Component	Sarcomatous Component	Hormone Therapy, y	Outcome
1	59	Ib	Right	13	TAH + BSO + PLA	No	Endometrioid	Homo	TAM (2)	DOD at 5 mo
2	68	IVb	Left	Synchronous	TAH + BSO + OMT	Yes	Serous	Hetero	No	DOD at 3 mo
3	63	Ib	Left, right	20, 5	TAH + BSO + PLA	No	Endometrioid	Homo	No	NED at 62 mo
4	71	Ic	Unknown	26	TAH + BSO	No	Endometrioid	Homo	TAM (5)	NED at 48 mo
5	80	Ic	Right	4	TAH + BSO	No	Endometrioid	Hetero	TAM (4)	DOD at 7 mo
6	66	IIIb	Right, left	30, 7	TAH + BSO	Yes	Endometrioid	Homo	No	DOD at 35 mo
7	54	Ib	Right	5	TAH + BSO + PLA	No	Endometrioid	Homo	TAM (2)	NED at 63 mo
8	56	IIIa	Right	11	TAH + BSO + PLA	No	Endometrioid	Homo	No	NED at 61 mo
9	75	IIIc	Left	8	TAH + BSO + PLB	No	Endometrioid	Hetero	TOR (5)	DOD at 19 mo
10	72	IIa	Right	7	TAH + BSO + PLA	No	Endometrioid	Homo	TOR (5)	DOD at 22 mo

TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; PLA, pelvic lymphadenectomy; PLB, pelvic lymph node biopsy; OMT, omentectomy; Homo, homologous; Hetero, heterologous; DOD, died of disease; NED, no evidence of disease.

a history of hormone therapy. The median follow-up period of the patients, excluding those who died, was 59 months (range, 12–85 months). Although the patients with a history of hormone therapy were slightly older than those without a history of BC, the difference was not statistically significant (mean age, 68.5 vs 60.7, $P = 0.087$, nonpaired Student t test). The patients with a history of hormone therapy seemed to have earlier stage UCS than those without a history of BC, but this difference was not significant ($P = 0.090$, χ^2 test for trends). The differences in the distributions of the other prognostic factors among the groups were also not statistically significant (P values not shown).

Characteristics of the Patients With a History of BC

The detailed characteristics of the 10 patients with a history of BC are shown in Table 2. All of the 10 patients underwent surgery for BC, and 6 patients (60%) received additional hormone therapy (4 patients with TAM and 2 patients with TOR). The durations of TAM treatment ranged from 2 to 5 years and that of TOR was 5 years. The mean intervals between BC and incidence of UCS in patients with and without a history of hormone therapy were 10.5 years (range, 4–26 years) and 15.3 years (range, 0–30 years), respectively. The interval between BC and incidence of UCS in the 2 patients with bilateral BC was calculated from the time of initial BC diagnosis. The difference in the interval between BC and incidence of UCS was not statistically significant ($P = 0.490$, nonpaired Student t test). The mean interval between hormone therapy cessation and incidence of UCS was 6.7 years (range, 0–21 years).

Analysis of Prognostic Factors

We performed both univariate and multivariate analyses to screen for potential prognostic factors of UCS. Table 3 lists the factors analyzed and the results. The prognostic factors found to be significant from the univariate analysis were age greater than 56 years, elevated serum lactate dehydrogenase (LDH) levels, presence of residual tumors, FIGO (International Federation of Gynecology and Obstetrics) stage higher than stage IIIa, and carcinomatous components other than endometrioid adenocarcinoma. Regarding multivariate analysis, all 11 factors were included in the Cox proportional hazards model, and stepwise backward analysis was performed. The results from this analysis revealed 6 independently significant prognostic factors (Table 3). The presence of residual tumors, FIGO stage higher than stage IIIa, and carcinomatous components other than endometrioid adenocarcinoma were again identified as significant prognostic factors. In addition, a history of BC, history of TAM use, and history of TOR use were also identified as independently significant prognostic factors.

Survival of Patients With UCS in Relation to Their History of BC and of Hormone Therapy

Figure 1 shows the survival curves of the patients with UCS. The 51 patients were divided into 3 groups based on their history of BC and of hormone therapy: patients with UCS not related to BC (n = 41), patients with UCS subsequent

TABLE 3. Univariate and multivariate analyses to identify significant prognostic factors for patients with UCS

	Univariate Analysis	Multivariate Analysis		
Factors	***P***	**Risk Ratio**	**95% CI**	***P***
Age (>56 [n = 36] vs ≤56 [n = 15]), y	0.038	—	—	NS
Menstrual status (postmenopause [n = 43] vs premenopause [n = 8])	0.651	—	—	NS
Serum LDH level (>upper normal limit [n = 13] vs within normal limits [n = 38])	<0.001	—	—	NS
Surgical procedures (RAH or TAH + BSO + PLA [n = 29] vs TAH + BSO [n = 22])	0.153	—	—	NS
Residual tumor (any [n = 9] vs none [n = 42])	<0.001	8.942	2.791–28.647	<0.001
FIGO stage (IIIb–IV [n = 12] vs I–IIIa [n = 39])	<0.001	4.116	1.296–13.074	0.016
Sarcomatous component (heterologous [n = 17] vs homologous [n = 34])	0.107	—	—	NS
Carcinomatous component (non-endometrioid [n = 14] vs endometrioid [n = 37])	0.017	2.896	1.088–7.708	0.033
BC (positive [n = 10] vs negative [n = 41])	0.869	0.139	0.022–0.886	0.037
TAM use (positive [n = 4] vs negative [n = 47])	0.932	15.895	1.461–172.913	0.023
TOR (positive [n = 2] vs negative [n = 49])	0.300	16.872	1.676–169.870	0.016

RAH, radical abdominal hysterectomy; TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; PLA, pelvic lymphadenectomy; NS, not significant.

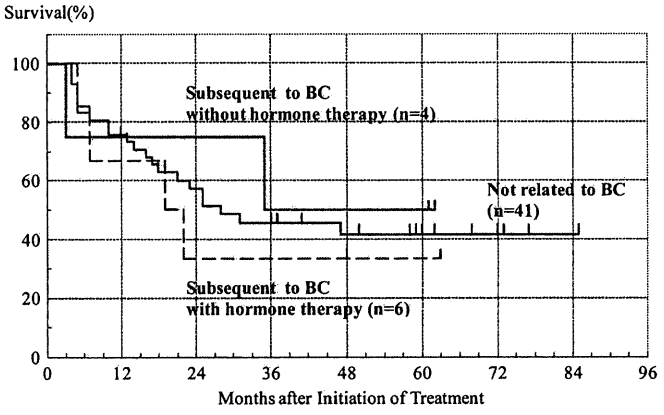

FIGURE 1. Survival curves for patients with UCS with respect to their history of BC and of hormone therapy. The survival rates of patients with UCS unrelated to BC (n = 41), patients with UCS subsequent to BC without hormone therapy (n = 4), and patients with UCS subsequent to BC with hormone therapy (n = 6) are shown in solid black, solid gray, and dotted black lines, respectively.

to BC without hormone therapy (n = 4), and patients with UCS subsequent to BC with hormone therapy (n = 6). Median survival periods and 5-year survival rates of the patients in each of the abovementioned groups were 28 months and 41.8%, 35 months and 50.0%, and 19 months and 33.3%, respectively. The differences in survival rates were not statistically significant on univariate analysis (P = 0.828, log-rank test).

DISCUSSION

In the present study, we investigated the characteristics of patients with UCS and investigated whether a history of BC and/or a history of hormone therapy for BC affected the prognosis of UCS.

We found relatively high incidences of patients with a history of BC (19.6%) and of hormone therapy (11.8%) among those with UCS. A history of pelvic radiation is a well-known risk factor for UCS. Our study population included 2 patients (3.9%) with a history of pelvic radiation (cases 5 and 6; Table 2). Both patients had a history of BC, with one having a history of hormone therapy. Although it is not clear which factors were associated with the development of UCS in these patients, the proportions of patients with histories of BC and hormone therapy were very high. Taking into account the lifetime cumulative incidence of BC that is about 5% in Japanese women,[15] the proportion of surviving female patients with a history of BC and of hormone therapy among the population is probably less than 5%. Thus, our data suggest an etiologic correlation between UCS and hormone therapy consistent with previous reports[3,6,14] and further suggest a similar correlation between UCS and BC itself.

No significant differences in clinical or pathological characteristics were found among UCS patients, without a history of BC, with a history of BC, or with a history of hormone therapy. Kloos et al.[12] suggested that TAM users had more advanced stages of UCS in their series. In contrast, McCluggage et al.[11] and Arenas et al.[13] suggested earlier stages of UCS in their series of TAM users. Our patients with a history of hormone therapy had a relatively early stage of UCS compared with those without BC, although the difference was not statistically significant (P = 0.090, χ^2 test for trends). Meanwhile, the differences in the interval from BC to incidence of UCS (P = 0.490, nonpaired Student t test) and the age of preceding BC (P = 0.353, nonpaired Student t test, data not shown) were not statistically significant between the patients with a history of BC and those with a history of hormone therapy. Taken together, the characteristics of the UCS patients without a history of BC, with a history of BC, and with a history of hormone therapy were not markedly different from each other.

Several prognostic factors of UCS have been reported previously, the most important being the FIGO stage of the tumor.[16,17] The presence of carcinomatous components other than endometrioid adenocarcinoma is also a poor prognostic factor.[18] On the other hand, the prognostic impact of heterologous sarcomatous components is controversial. In the present study, 5 factors were identified as significant prognostic factors using a univariate analysis. Among these, FIGO stage higher than stage IIIa, the presence of residual tumors, and carcinomatous components of non–endometrioid adenocarcinoma remained as independently significant prognostic factors of UCS on a multivariate analysis, whereas age greater than 56 years and elevated serum LDH levels were not independent prognostic factors on the multivariate analysis. We measured serum LDH levels in the routine preoperative systemic evaluations. The serum LDH level is reported to be higher in patients with endometrial cancer compared with healthy controls, but serum LDH level did not correlate with deep myometrial invasion or high histological grade of endometrial cancer.[19] Our data suggest some correlation with prognosis; thus, further assessment of the meaning of elevated serum LDH level is necessary to address its relevance as a predictive measure of UCS. In contrast, the history of TAM or TOR therapy and the history of BC were identified as independently significant prognostic factors on multivariate analysis, but not on univariate analysis. On univariate analysis, even when we analyzed the prognosis by dividing patients into 3 groups, that is, patients without a history of BC (41 patients), with a history of BC (4 patients), and with a history of hormone therapy (6 patients), no significant differences were found (Fig. 1). However, even when we combined the history of TAM or TOR as the history of hormone therapy, the results of multivariate analysis were almost identical. The relative risk of UCS with a history of hormone therapy and the history of BC were 16.410 (95% confidence interval [CI], 2.044–131.746) and 0.138 (95% CI, 0.022–0.867), respectively. As mentioned above, there were no marked differences in the distributions of other characteristics. Thus, the cumulative nonsignificant differences of other prognostic factors, especially FIGO stage higher than stage IIIa, presence of residual tumors, and carcinomatous component of non–endometrioid adenocarcinoma may conceal the significant prognostic impact of the history of BC, TAM, and TOR

on UCS. In previous reports, a case series suggested poor prognosis of patients with a history of TAM,[11–13] and a case-control study of patients with BC suggested poorer prognosis for patients with a history of long-term TAM use among those with endometrial cancer, including UCS.[3] On the other hand, a follow-up study of patients with BC found no prognostic impact of TAM use in patients with UCS subsequent to BC.[5] To our knowledge, there have been no previous studies analyzing the prognostic impact of the history of BC itself and of hormone therapy among patients with UCS, including those patients without preceding BC. We found that the history of BC was a significantly better prognostic factor of UCS, whereas the history of TAM and TOR treatment was a significantly poor prognostic factor of UCS on our multivariate analysis.

Our data suggest that histories of both BC and hormone therapy for BC are important risk factors for UCS, whereas characteristics of the UCS are similar regardless of the presence of these risk factors. Moreover, the history of BC appears to be a good prognostic factor in UCS patients, whereas a history of hormone therapy is a poor prognostic factor. However, because of the rarity of UCS occurrence and UCS related to BC as shown by Lavie et al,[20] our study is based on a small cohort of patients from a single institution. This is a limitation of our study, along with the retrospective nature of our study. Thus, the results from our study need to be validated by future studies to clarify the association between prognosis of UCS and a history of BC and/or of hormone therapy.

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CASE REPORT Open Access

A rare case of recurrent ovarian cancer presenting as a round ligament metastasis

Shinichi Togami*, Tomoyasu Kato, Takateru Oi, Mitsuya Ishikawa, Takashi Onda, Shun-ichi Ikeda and Takahiro Kasamatsu

Abstract

We report a rare case of recurrent ovarian cancer presenting as a round ligament metastasis. A 44-year-old woman presented with a lower abdominal mass. Computed tomography showed a pelvic mass. Primary surgery was performed. A histopathological examination showed an ovarian serous adenocarcinoma of Stage IIIb. The patient received 6 cycles of paclitaxel and carboplatin. Almost 2 years after the initial operation, the patient noticed a left inguinal mass. Computed tomography showed a left inguinal mass, 18 mm in size. An excisional biopsy was performed and the tumor was found to originate in the left round ligament. A histopathological examination showed serous adenocarcinoma and there was no evidence of lymph node tissue. Recurrence of ovarian cancer in the round ligament is extremely rare. This unique case suggests, however, that the round ligament in rare cases may be a recurrence site for ovarian cancer, and that accurate differentiation including confirmation by diagnostic imaging and excisional biopsy, is necessary for a definitive pathological diagnosis.

Keywords: diagnosis, inguinal mass, ovarian cancer, recurrence, round ligament

Background

The majority of women with ovarian cancer present with advanced stage disease. A complete clinical remission after surgical cytoreduction and platinum-based chemotherapy can be achieved in 80-90% of these patients. Despite this, 70-90% of patients will develop recurrent disease [1]. Fifty-five percent of the first relapse cases were found at the pelvis or abdomen [2]. There was a wide variety among the other recurrent sites, such as, retroperitoneal nodes, liver or spleen, brain, and bone [2,3]. We experienced a case with solitary recurrence at the left round ligament. To the best of our knowledge, this is the first report of recurrent ovarian cancer occurring in the round ligament.

Case

A 44-year-old woman presented with a 1.5-year history of progressive enlargement of a lower abdominal mass. On physical examination, a pelvic mass was noted. Transvaginal ultrasound revealed bilateral adnexal masses (left: 80*58 mm, right: 54*40 mm) with solid components in the pelvic cavity. Computed tomography (CT) (Figure 1) and magnetic resonance imaging (MRI) showed bilateral adnexal tumors and dissemination extending from the pelvis to the upper abdomen. There was no evidence of lymphadenopathy. A left mammary tumor was incidentally discovered by CT, and fine-needle aspiration of the breast revealed cells consistent with adenocarcinoma. The serum cancer antigen 125 (CA125) level was elevated to 348 U/ml (normal range, < 35 U/ml). She underwent a total abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy, periaortic lymph node biopsy, splenectomy and left partial mastectomy. On intraoperative examination, her surgeons noted involvement of the omentum, spleen and dissemination into Douglas pouch. There was no dissemination involving the diaphragm, liver, paracolic gutters, uterus or peritoneum surrounding the bilateral round ligaments. After primary debulking surgery, she had microscopic residual disease in the Douglas pouch. The pathologic specimen showed extension of the tumor throughout the fallopian tubes, spleen, and omentum. The body of the uterus and the bilateral round ligaments around were not involved in the tumor. The final pathologic diagnosis of the tumor was the

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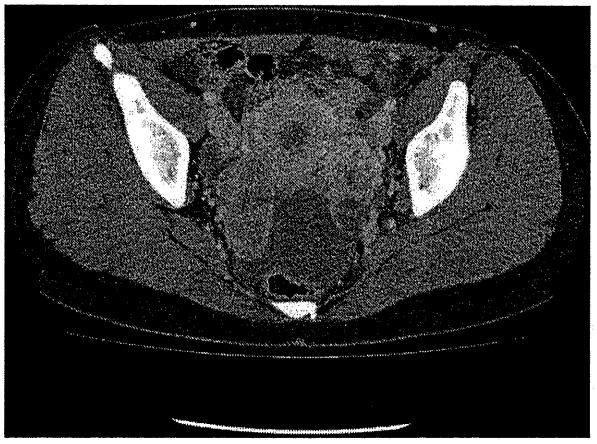

Figure 1 Enhanced axial CT showing bilateral adnexal tumors.

International Federation of Gynecology and Obstetrics (FIGO) Stage IIIb ovarian serous adenocarcinoma and left breast invasive ductal carcinoma.

Initially, the patient received 6 cycles of paclitaxel (175 mg/m2) and carboplatin (AUC = 6) for ovarian cancer in April 2009. In August 2009, her serum CA125 levels declined to 5 U/ml. She then received adjuvant radiotherapy (WB 50Gy/25 f) for breast cancer in October 2009. After that, she received routine follow-up from her gynecologic oncologist.

Almost 2 years after her initial ovarian cancer operation, the patient noticed a left inguinal mass. CT showed a left inguinal mass, 18 mm in size (Figure 2), but no abnormal mass in chest, abdomen or pelvis. This mass was located on the fascia and medially to the femoral vein. The serum CA125 level was within normal limits. A fine-needle aspiration of the left inguinal mass revealed cells consistent with adenocarcinoma, but it was difficult to confirm the location of the primary lesion. She underwent an excision biopsy of the left inguinal mass. An incision was made directly over the mass which was present in the left round ligament. At the proximal site of the tumor, a normally appearing left round ligament was exposed. Then the mass was removed entirely. The pathological diagnosis of the tumor was serous adenocarcinoma, which was similar to the prior ovarian cancer, and there was no evidence of lymph node tissue (Figure 3).

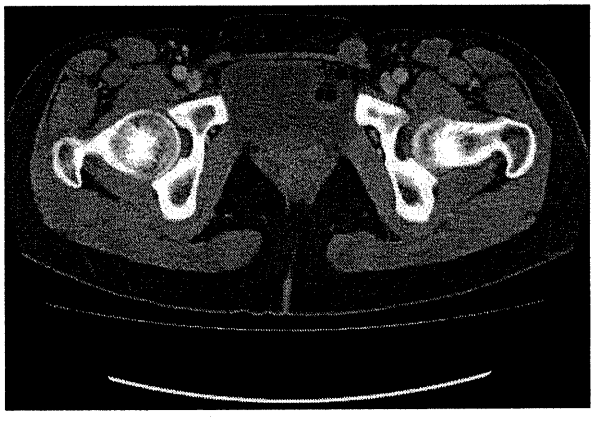

Figure 2 Enhanced axial CT shows a left inguinal mass, 18 mm in size (arrow).

Discussion

The majority of patients with ovarian cancer responds well to the initial treatment, but most of them will develop recurrent disease [4]. Recurrent disease involves most frequently the pelvis or the abdomen [2,3]. This case demonstrates a most unusual recurrence for ovarian cancer, presenting as a round ligament metastasis. The round ligament extends from the uterus, through the inguinal canal, and ends in the region of the mons pubis and labia majora. Embryologically, this is the female equivalent of gubernaculums testis and is predominantly composed of smooth muscle fibers, connective tissue, blood vessels, and nerves with a mesothelial coating [5].

Because round ligament recurrence of ovarian cancer is very rare, we performed a MEDLINE search of the English language literature, but no example could be found. Some unusual tumors involving the round ligament have been reported in the literature: dermoid cyst, endometriosis, mesothelial cyst, leiomyoma and leiomyosarcoma [6-8]. These tumors of the round ligament are very rare developmental disorders, which have been reported as case reports. Especially, leiomyoma is the most common tumor associated with the round ligament [9]. Patil et al. [9] reported the clinicopathological

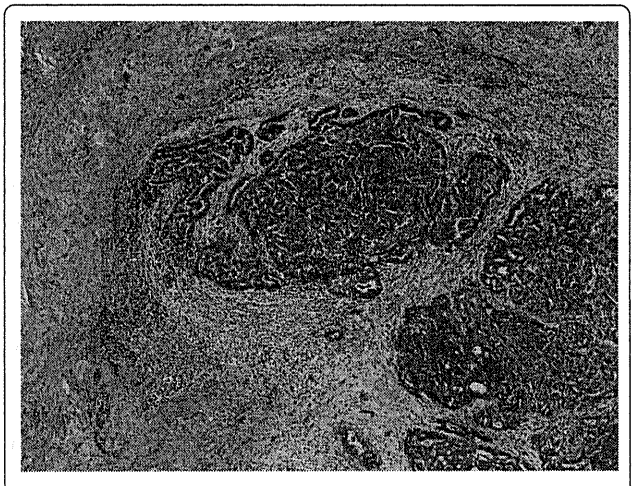

Figure 3 The histopathological appearance of the round ligament recurrence: Serous adenocarcinoma exists in the context of fibrous connective tissue, including small blood vessels. (H&E, ×40).