of DISS (Fig. 2B). We then inoculated either of these VASH2-knocked down cancer cells subcutaneously into nude mice and observed a significant reduction in tumor growth in terms of both tumor volume and weight (Fig. 2C and D).

Peritoneal dissemination often occurs in serous adenocarcinoma of the ovary and is a sign of poor prognosis. We injected cancer cells into the peritoneal cavity as a model of peritoneal dissemination and observed that there was a significant decrease in the number of disseminated tumors in mice injected with SKOV-3 or DISS cells that had been transfected with sh-VASH2 (Fig. 3A and C). In addition, DISS cells caused the accumulation of a bloody ascites, but it was almost completely abrogated by sh-VASH2 (Fig. 3E). As the result, the survival period was prolonged in mice that had been injected with either SKOV-3 or DISS sh-VASH2 cells (Fig. 3B and D).

We further examined an orthotopic mouse model. Again we observed a significant reduction in tumor growth of SKOV-3 or DISS cells that had been transfected with sh-VASH2 (Fig. 4).

We previously reported that VASH1 inhibits angiogenesis whereas VASH2 promotes it (16). We therefore examined the vasculature in the tumors and found a significant decrease in angiogenesis in tumors derived from either SKOV-3 or DISS sh-VASH2 cells (Fig. 5A and C). The decrease of tumor angiogenesis resulted in the significant increase of cancer cell apoptosis but no changes in cancer cell proliferation *in vivo* (Supplementary Fig. S2). We further investigated the vascular composition of endothelial cells and mural cells. The association of mural cells with endothelial cells was not significantly altered in either type of tumor (Fig. 5B and D). We did not observe any differences in the extent of mural cell coverage in tumor vessels (Fig. 5B).

Expression of VASH2 in cancers increases tumor growth via the promotion of tumor angiogenesis

To further confirm proangiogenic function of VASH2 in tumors, we carried out a gain-of-function experiment by introducing the VASH2 gene in cancer cells. VASH2 can be expressed in bone marrow–derived CD11b$^+$ mononuclear cells (16). To avoid the possible involvement of recipient VASH2, we planned to use *VASH2* ($-/-$) mice. As our *VASH2* ($-/-$) mice were on a C57BL6 background, we examined various tumorigenic C57BL6 murine tumor cells and found that EL-4 and MLTC-1 did not express endogenous VASH2 (Supplementary Fig. S2). We introduced the human *VASH2* gene into EL-4 and established stable clones (Fig. 6A). The introduction of the *VASH2* gene slightly but significantly inhibited the *in vitro* proliferation of these tumor cells (Fig. 6B). We then inoculated these cells subcutaneously into *VASH2* ($-/-$) mice. Parental EL-4 cells are tumorigenic, indicating that they have sufficient angiogenic activity despite of the lack of VASH2 expression. Even though the *VASH2* gene slightly decreased the proliferation of tumor cells, we observed a significant intensification of tumor growth in VASH2 transfectants (Fig. 6C). Then, we examined the vasculature in the tumors. As expected, tumors of mock transfectants contained a certain quantity of tumor vessels. Nevertheless, we observed that there was a significant increase in tumor angiogenesis, as evidenced by the increased vascular luminal area, in the VASH2 transfectants (Fig. 6D). We further observed that the association of mural cell with endothelial cells was not significantly altered in the EL-4 transfectants (Fig. 6E).

VASH2 affects both endothelial cells and cancer cells to promote angiogenesis

VASH1 exhibits its antiangiogenic activity by inhibiting migration and proliferation of endothelial cells (12). As a

Figure 4. Knockdown of VASH2 inhibited orthotopic tumor growth. A, sh-clone2 or mock transfectant cells (1 × 10^5 cells) established from SKOV-3 were orthotopically inoculated into nude mice. Thirty-two days after the inoculation, the mice were sacrificed; and then the tumor size was determined. Mean and SDs are shown (*N* = 5). *, *P* < 0.05 versus mock. B, sh-clone5 or mock transfectant cells (1 × 10^5 cells) established from DISS were orthotopically inoculated into nude mice. Thirty-two days after the inoculation, the mice were sacrificed; and then the tumor size was determined. Mean and SDs are shown (*N* = 5). *, *P* < 0.05 versus mock.
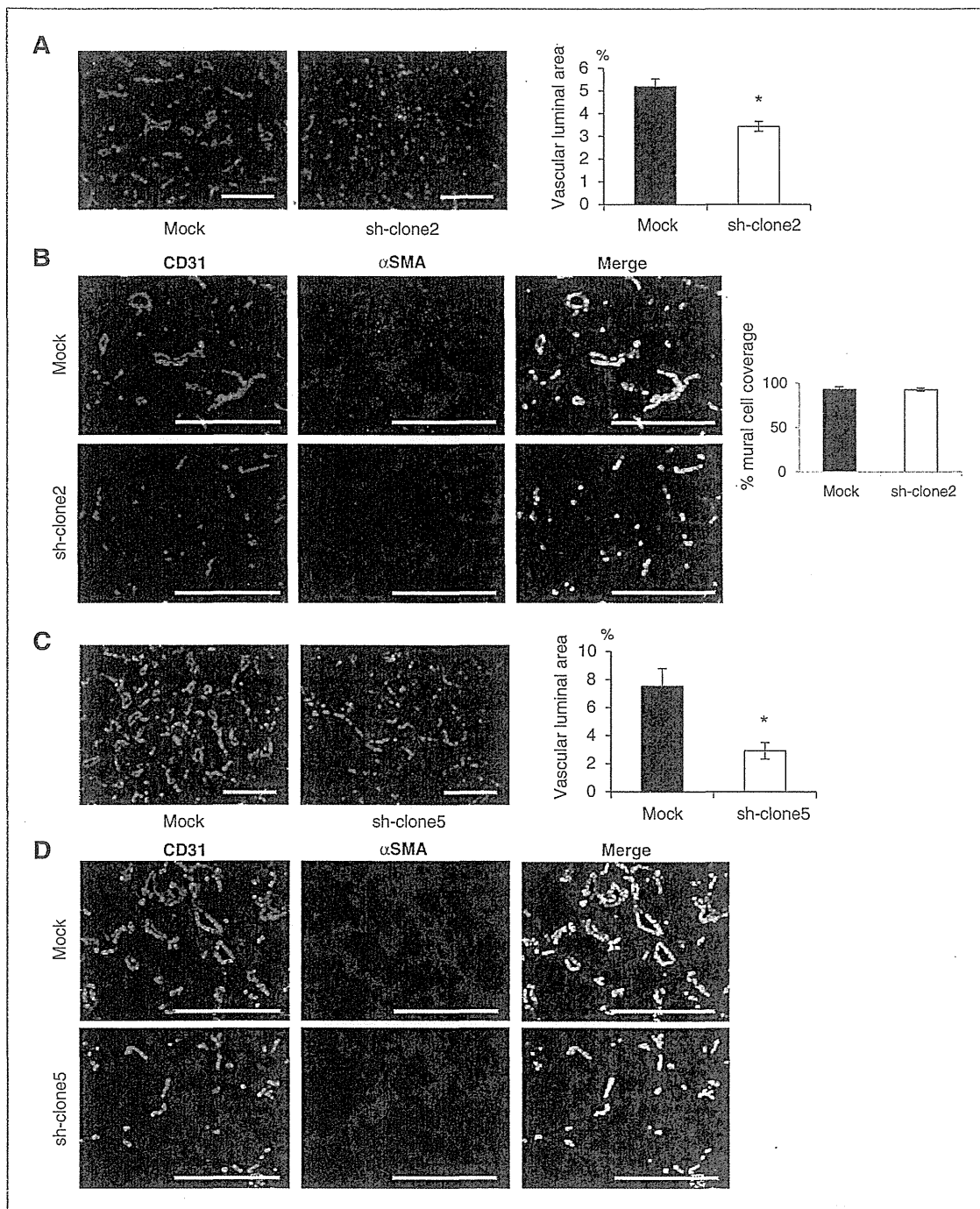
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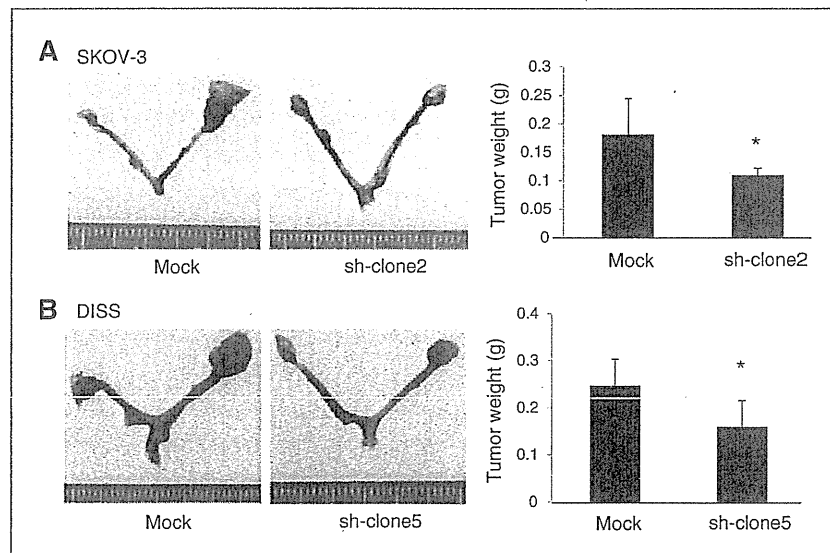
Figure 5. Knockdown of VASH2 inhibited tumor angiogenesis. A, sections of tumors formed by sh-clone2 or mock transfectants established from SKOV-3 were immunostained with anti-CD31. Bar, 300 μm. The vascular luminal area was calculated on the basis of area in 5 different fields. Mean and SDs are shown (N = 3). *, P < 0.01 versus mock. B, sections of tumors generated from sh-clone2 or mock transfectant cells established from SKOV-3 were immunostained with anti-CD31 and anti-αSMA. Bar, 300 μm. C, sections of tumors formed by sh-clone5 or mock transfectant cells established from DISS were immunostained with anti-CD31. Bar, 300 μm. The vascular luminal area was calculated on the basis of 5 different fields. Mean and SDs are shown (N = 4). *, P < 0.01 versus mock. D, sections of tumors formed by sh-clone5 or mock transfectant cells established from DISS were immunostained with anti-CD31 and anti-αSMA. Bar, 300 μm.
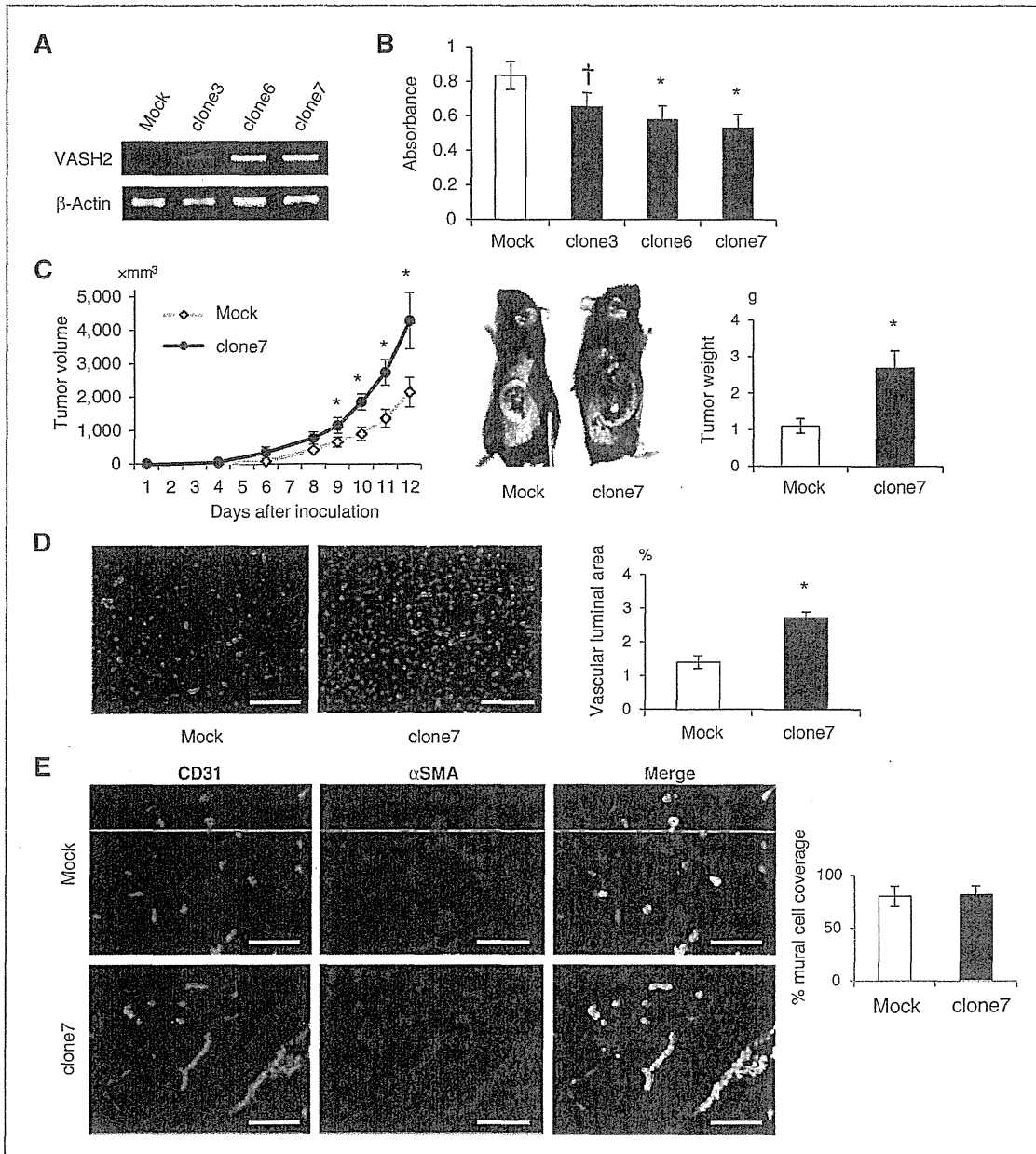
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Figure 6. Stable transfection of human VASH2 gene in VASH2-negative EL-4 cells accelerated tumor growth and tumor angiogenesis *in vivo*. A, human VASH2 stable transfectants (clone3, clone6, and clone7) were established from EL-4 cells. The expression of VASH2 was determined by RT-PCR. B, proliferation of clone3, clone6, and clone7 was compared with that of the mock transfectant under the same cell culture conditions. Mean and SDs are shown ($N = 3$). ﹡, $P < 0.01$; †, $P < 0.05$ versus mock. C, clone7 or mock cells established from EL-4 were inoculated subcutaneously into VASH2 (−/−) mice, and the serial tumor growth was compared. Mean and SDs are shown ($N = 6$). ﹡, $P < 0.01$ versus mock. Thirteen days after the inoculation, photographs were taken; and then the tumor weight was measured. Mean and SDs are shown ($N = 6$). ﹡, $P < 0.01$ versus mock. D, sections of tumors formed by clone7 or mock transfectant cells established from EL-4 were immunostained with anti-CD31. Bar, 300 μm. The vascular luminal area was calculated on the basis of 5 different fields. Mean and SDs are shown ($N = 3$). ﹡, $P < 0.01$ versus mock. E, sections of tumors from clone7 or mock transfectant cells established from EL-4 were immunostained with anti-CD31 and anti-αSMA. Bar, 100 μm.

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homolog of VASH1, VASH2 may exhibit its proangiogenic effect by acting on endothelial cells as well. Here, we showed that CM from VASH2 transfectants stimulated the migration but not the proliferation of endothelial cells when compared with CM from mock transfectants (Fig. 7A). Moreover, those VASH2 transfectants as well as 2 human ovarian cancer cell lines SKOV-3 and DISS expressed SVBP (15) that plays an essential role in vasohibin secretion (Supplementary Fig. S3). These results suggest that VASH2 is secreted from cancer cells and affects on endothelial cells as a paracrine manner.

We intended to verify the mechanism of expression of VASH2 in cancer cells. We first confirmed that SKOV-3 and DISS expressed more VASH2 mRNA than HOECs (Fig. 7B). This increased expression of VASH2 in serous ovarian adenocarcinoma cells was constitutive, as no stimulus, including hypoxia, induced its expression (data not shown). To understand the mechanism of this sustained increase in VASH2 expression, we examined the involvement of microRNAs because a database for microRNA

targets prediction and functional annotations (http://mirdb.org/miRDB/)/indicates VASH2 to have the highest rank as a target of miR-200b. Indeed, there are multiple binding sites of miR-200bc/429 in the 3'-untranslated region of human VASH2 mRNA (Fig. 7C). As expected, the expression of miR-200b was apparently low in SKOV-3 and DISS (Fig. 1B). Pre-miR-200b decreased the expression of VASH2 in SKOV-3 cells (Fig. 7D). Moreover, we observed an inverse correlation between VASH2 expression and miR-200b expression in human ovarian cancer tissue (Fig. 7E). These results suggest that the decreased expression of miR-200b was responsible for the upregulation of VASH2 in serous ovarian adenocarcinoma cells.

Discussion

Here, we showed for the first time that VASH2 was preferentially expressed in serous adenocarcinoma among human ovarian cancers. Moreover, specific knockdown of VASH2 from cell lines of human serous adenocarcinoma remarkably attenuated the growth of inoculated tumor cells
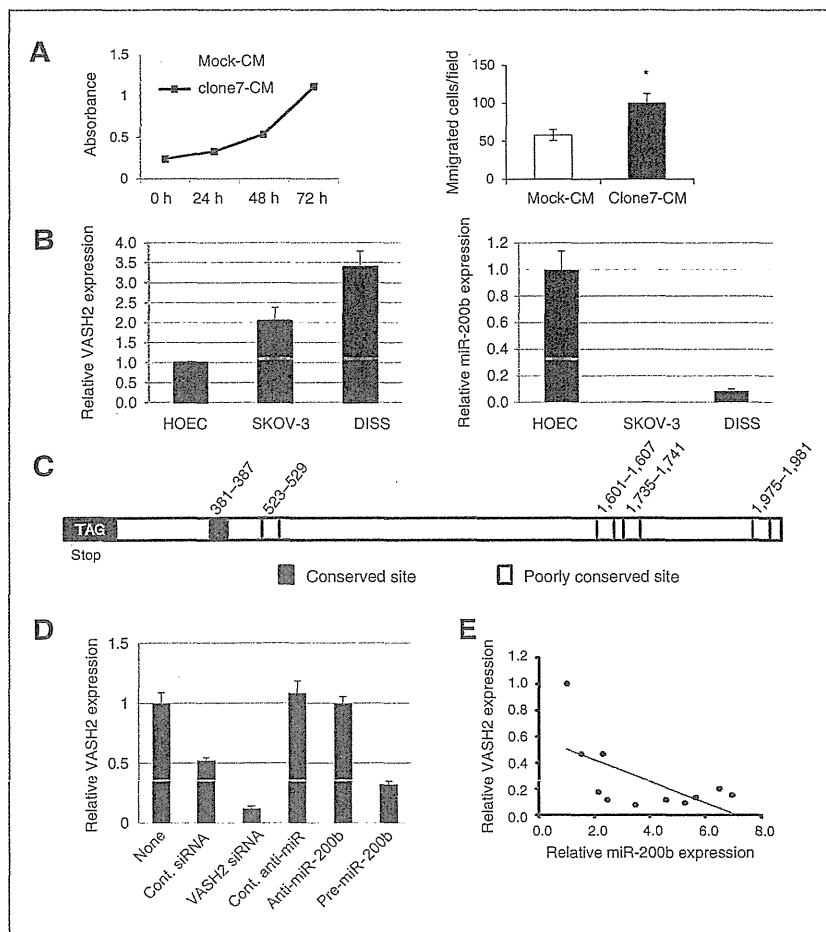

Figure 7. VASH2 stimulated migration of endothelial cells (EC) and its expression was repressed by mir-200b. A, CM from cultures of clone7 or mock transfectant cells established from EL-4 were tested for the proliferation (on the left) and migration (on the right) of ECs as described in Materials and Methods. Mean and SDs are shown ($N = 3$). *, $P < 0.01$ versus mock CM. B, expression of VASH2 and miR200b in HOEC, SKOV-3, and DISS cells was determined by conducting quantitative RT-PCR. Expression of VASH2 and miR200b in SKOV-3 and DISS was compared with that in HOECs. Means and SDs are shown ($N = 3$). C, possible binding sites of miR200bc/429 in the 3'-untranslated region of human VASH2 mRNA are shown. D, expression of VASH2 in SKOV-3 after the indicated treatment as described in Materials and Methods was determined by quantitative RT-PCR. Mean and SDs are shown ($N = 3$). Cont., control. E, total RNA was extracted from human ovarian cancer tissues, and the expression of VASH2 mRNA and miR-200b was determined by quantitative RT-PCR. $N = 11$, $R = -0.6131$, $P = 0.0448$.

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and their peritoneal dissemination as well as tumor angiogenesis. In contrast, transfection of VASH2-negative tumor cells with the VASH2 gene augmented tumor angiogenesis and tumor growth when inoculated into mice. Collectively, our data suggest that VASH2 was responsible for promoting tumor angiogenesis in serous ovarian adenocarcinoma. Whereas several splicing variants of VASH2 are registered in the database, the significance and functional differences among those variants are presently obscure.

Serous adenocarcinoma is the most common histologic subtype of ovarian cancers. As mentioned earlier, because recurrence after the first-line chemotherapy is frequent, several targets have been considered for the treatment of ovarian cancers and one of them is angiogenesis (4). Anti-angiogenic therapy is now approved for several cancers ranging from colon, lung, breast, and kidney; and drugs targeting VEGF signals are in clinical use (30). However, the benefit of such drugs may not last long, as patients will encounter progression of cancers because of the compensatory production of angiogenic factors other than VEGF or recruitment of bone marrow–derived angiogenic cells. Therefore, alternative targets for antiangiogenic therapy are now extensively being investigated (31). Here, we propose that VASH2 can be a candidate target for the treatment of serous adenocarcinoma in light of its stimulating effect on angiogenesis.

We have reported the proangiogenic activity of VASH2 (16), but its precise function has been unclear. Here, we showed that CM from human VASH2 transfectants stimulated the migration of endothelial cells. Ovarian cancer cells express SVBP, a secretory chaperone of vasohibins (15). These results suggest that VASH2 is secreted from the cancer cells and acts on neighboring endothelial cells to stimulate angiogenesis in a paracrine manner. VASH1 inhibits angiogenesis, whereas VASH2 stimulates it. Most plausible mechanism is that these 2 factors share a putative vasohibin receptor and one acts as an agonist whereas the other acts as an antagonist. This hypothesis is currently under investigation.

In terms of the expression of VASH2, we showed that miR-200b repressed the expression of VASH2 in ovarian cancer cells. microRNAs represent a category of small noncoding RNAs that are involved in the regulation of gene expression by translational repression and/or degradation of target mRNAs (32). The expression of VASH2 could not be induced by any of the stimuli tested (data not shown). Moreover, when murine embryonic stem cells form embryoid bodies, the expression of VASH1 is initially low but steeply induced when vessels are formed, whereas that of

VASH2 is initially high in embryonic stem cells and gradually decreases during their differentiation (Abe and Sato, unpublished observations). We think that this pattern of VASH2 expression fits with the microRNA-mediated gene repression. miR-200b belongs to the miR-200 family of microRNAs. This family comprises 5 members (miR-200a, 200b, 200c, 141, and 429), and they are downregulated in cancer cells due to aberrant epigenetic gene silencing and play a critical role in the suppression of epithelial-to-mesenchymal transition (EMT) by targeting and repressing the expression of key molecules such as ZEB1 and ZEB that are involved in EMT (33). It is reported that miR-200 family members are frequently dysregulated in human ovarian cancers (34–37). In this context, it would be interesting to see whether or not VASH2 is involved in the EMT of cancer cells besides its role in angiogenesis.

In summary, VASH2 was expressed in certain ovarian cancers, and it promoted tumor growth and peritoneal dissemination of tumor cells by stimulating tumor angiogenesis. Knockdown of VASH2 significantly attenuated the tumor growth and peritoneal dissemination, which indicates that VASH2 would be a molecular target for the treatment of ovarian cancer. It is important to see whether this role of VASH2 is played in cancers from other organs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: Y. Takahashi, Y. Sato
Development of methodology: Y. Takahashi, T. Moriya
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Takahashi, T. Koyanagi, N. Kanomata, T. Moriya
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Takahashi, Y. Suzuki, T. Moriya
Writing, review, and/or revision of the manuscript: Y. Takahashi, T. Koyanagi, Y. Sato
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Saga, M. Suzuki
Study supervision: Y. Sato

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ORIGINAL ARTICLE

Clinical outcome of pelvic exenteration in patients with advanced or recurrent uterine cervical cancer

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Abstract

Background Pelvic exenteration has attained an important role in the treatment of advanced or recurrent cervical cancer for obtaining a complete cure or longer disease-free survival. The purpose of this study was to evaluate patients undergoing pelvic exenteration and to determine the clinical features associated with outcome and survival.

Methods We retrospectively analyzed the records of 12 patients who underwent pelvic exenteration for uterine cervical cancer between July 2002 and August 2011.

Results Two patients had primary stage IVA cervical adenocarcinoma and 10 patients had recurrent cervical cancer. Eight patients underwent anterior pelvic exenteration, 3 patients underwent total pelvic exenteration, and 1 patient underwent posterior pelvic exenteration. With a median duration of follow-up of 22 months (range 3–116 months), 5 patients were alive without recurrence. Of 5 patients with no evidence of disease, 4 were recurrent or residual tumor, all of whom had common factors, such as a tumor size ≤30 mm, negative surgical margins, complete resection, and no lymph node involvement. The 5-year overall survival rate for 12 patients was 42.2 %. Ileus was the most common complication (42 %) and post-operative intestinal anastomosis leaks developed in 3 patients, but no ureteral anastomosis leaks occurred.

Conclusions Pelvic exenteration is a feasible surgical procedure in advanced and/or recurrent cervical cancer patients with no associated post-operative mortality, and the only therapeutic option for complete cure or long-term survival; however, post-operative complications frequently occur.

Keywords Pelvic exenteration · Uterine cervical cancer · Positron emission tomography/computed tomography · Urinary diversion · Complications

Introduction

Cervical cancer is the fifth most common cancer among women in Japan; the mortality from cervical cancer in 2010 was 4.1 per 100,000 of the female population [1]. Radiotherapy and surgery are the cornerstones of management for patients with cervical cancer. Indeed, radiotherapy or concurrent chemoradiotherapy (CCRT) is recommended for patients who are at high risk for recurrence following radical hysterectomy or for patients with advanced stage disease [2]. Despite the clinical advantage of CCRT for cervical cancer, recurrence rates are 50–70 % for patients with locally advanced disease (The International Federation of Gynecology and Obstetrics (FIGO) IIB, III, and IVA stage) [3]. Treatment options in patients with locally recurrent cervical cancer are limited. In fact, approximately 25 % of patients with recurrences outside the irradiated field respond to chemotherapy while only 5 % of patients respond to chemotherapy if the tumor recurs within the irradiated field [4].

Pelvic exenteration (PE) was initially introduced as a palliative procedure in the treatment of advanced pelvic cancer [5]. Of note, the operative mortality rate was as high as 23 % [5]. Due to improvements in reconstructive procedures, surgical techniques, patient selection, and

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peri- and post-operative care, the operative mortality rate has decreased dramatically [6, 7]. Currently, PE has attained an important role in the treatment of advanced or recurrent cervical cancer for obtaining a complete cure or longer disease-free survival.

We performed PEs on 16 patients with uterine cervical cancer, uterine sarcoma, or vulvar cancer between July 2002 and August 2011. In the current study, 12 patients with cervical cancer who underwent PE at a single institution in Japan were reviewed. The purpose of this study was to describe the incidence and severity of complications associated with PE, and to define which patients were more likely to benefit from PE.

Materials and methods

We retrospectively studied the medical records of 12 patients who underwent PE for uterine cervical cancer between July 2002 and August 2011 at the Tohoku University Hospital. The medical records were reviewed and information was gathered with respect to age at the time of surgery, the histologic features of the primary cancer, prior treatment(s), FIGO stage, extent of disease, method of urinary and stool diversion, operative time, blood loss, tumor size, tumor residual, tumor margin status, lymph node metastasis, complications, and present disease status. The survival times of patients alive or lost to follow-up were censored in June 2012.

The selection criteria for PE were central recurrence; age (<70 years); no gross pelvic side-wall involvement; no para-aortic lymph node enlargement; no distant metastases; and good performance status. An informed consent, including the rationale for the procedure and a statement that the procedure could be terminated intra-operatively without completing the resection, was obtained in every case. The diagnosis of recurrent tumor was confirmed by pathologic examinations of a biopsy specimen from each patient, but we did not perform surgical explorations, such as open or laparoscopic biopsies.

All surgical procedure was performed by gynecologic oncologists in collaboration with urologists and general surgeons. Total pelvic exenteration (TPE) involves removal of the reproductive tract, bladder, portions of the ureters, and rectosigmoid colon. Anterior pelvic exenteration (APE) is removal of the reproductive tract, bladder, and portions of the ureters, while posterior pelvic exenteration (PPE) is removal of the reproductive tract and rectosigmoid colon. Pelvic lymphadenectomy is performed for primary stage IVA patient who undergo PE. The recurrent patients after CCRT receive selective biopsy for lymph nodes with suspected metastasis. Intra-operative radiation therapy was not administered to any patient.

All statistical analyses were performed with StatFlex 6.0 (Artec, Inc., Osaka, Japan). Survival probabilities were estimated using the Kaplan–Meier method, and statistical significance was determined by the log-rank test.

Results

Patient characteristics and surgical data of the 12 patients are presented in Table 1. The median age at the time of surgery was 46 years (range 34–63 years). Of the 12 patients, 2 had primary cervical adenocarcinoma (stage IVA) and 10 had recurrent cervical cancer (squamous cell carcinoma, $n = 6$; and adenocarcinoma, $n = 4$). All 10 patients with recurrences had received radiotherapy, 6 of whom underwent hysterectomies before PE.

The median tumor size at the time of PE was 32.5 mm (range 15–82 mm). The operative procedures were APE ($n = 8$), TPE ($n = 3$), and PPE ($n = 1$). The median operative time was 491.5 min (range 266–683 min) and the estimated blood loss was 2537.5 g (range 1565–5572 g). Eight of 12 patients had no macroscopic residual tumor after PE, and as a result the surgical margins had no malignant cells microscopically in 8 cases. The resected specimens from nine patients contained lymph nodes. Of the nine patients, three had positive lymph node metastases and the histopathologic diagnoses were adenocarcinomas. The median hospital stay post-PE was 65.5 days (range 16–103 days).

The surgical outcomes and complications are summarized in Table 2. Ileus was the most common complication, occurring in 5 patients (42 %). Post-operative leaks of intestinal anastomoses developed in 3 patients (25 %). Two patients (17 %) required re-laparotomies because of ileus, a wound infection, or peritonitis. In contrast, no post-operative leaks of ureteral anastomoses were documented. There were no peri-operative deaths and no cardiovascular or thromboembolic events. Two patients (17 %) had no major post-operative complications.

The types of urinary reconstructive procedures and leakages are summarized in Table 3. Before performing PE, 10 patients received pelvic radiation therapy. Only one patient (no. 88) did not require urinary diversion because a PPE was performed. The methods of urinary diversion were ileal conduits ($n = 4$); ureterocutaneostomy ($n = 3$); transverse colon conduits ($n = 3$); and sigmoid colon conduit ($n = 1$). Three patients with ureterocutaneostomies did not require intestinal anastomoses. No patients had ureteral anastomosis leakages. Two patients had ileoileal anastomosis leaks in the ileal conduit using the ileum within the radiation field.

With a median duration of follow-up of 22 months (range 3–116 months), 5 patients were alive without

Table 1 Backgrounds and characteristics

Case	Age	Stage	Histology	Status	Prior treatment	Site of recurrence	PET/CT	Tumor size (mm)	Exent type	Operation hours (min)	Blood loss (g)	Tumor residuals	Margin status	Positive lymph nodes	Length of hospital stay after PE (days)	Survival Period after PE (months)	Progression free period after PE (months)	Disease status
1	63	IB2	SCC	Relapse	Surgery, CCRT	Vaginal stump	(−)	50	TPE	677	3205	None	(−)	(−)	90	3	2	DOD
2	41	IIB	SCC	Relapse	CCRT, Chemotherapy	Uterus	(−)	28	APE	395	2650	None	(−)	(−)	84	116	116	NED
3	45	IB2	AC	Relapse	Surgery	Vaginal stump	(−)	35	APE	490	2600	None	(−)	(+)	100	54	44	DOD
4	41	IVA	AC	Primary	None		(−)	82	APE	502	5572	None	(−)	(+)	103	106	106	NED
5	49	IIIA	SCC	Relapse	CCRT	Uterus	(+)	15	APE	425	1910	None	(−)	(−)	47	99	99	NED
6	34	IIB	SCC	Relapse	CCRT, chemotherapy	Uterus, pelvic lymph nodes	(+)	39	APE	266	1565	None	(−)	Not removed	23	7	2	DOD
7	60	IIB	AC	Relapse	Surgery, CCRT	Vaginal stump	(+)	38	APE	470	1700	<1 cm	(+)	(+)	88	21	10	DOD
8	56	IIIB	SCC	Relapse	CCRT, chemotherapy	Uterus	(+)	25	PPE	342	1780	<1 cm	(+)	Not removed	100	18	5	DOD
9	42	IIB	SCC	Relapse	NAC, surgery, RT, chemotherapy	Vaginal stump	(−)	50	TPE	591	2755	>2 cm	(+)	(−)	32	24	24	AWD
10	47	IVA	AC	Primary	Residual tumor after CCRT		(+)	20	APE	493	1330	None	(−)	(−)	16	23	23	NED
11	36	IB2	AC	Relapse	Surgery, RT, chemotherapy	Vaginal stump, bladder	(+)	25	TPE	683	2475	<1 cm	(+)	Not removed	43	12	4	DOD
12	52	IB2	AC	Relapse	Surgery, CCRT, chemotherapy	Vaginal stump	(+)	30	APE	662	4517	None	(−)	(−)	20	14	14	NED

SCC squamous cell carcinoma, *AC* adenocarcinoma, *CCRT* concurrent chemo-radiation therapy, *NAC* neoadjuvant chemotherapy, *RT* radiation therapy, *PET/CT* positron emission tomography/computed tomography, *PE* pelvic exenteration, *TPE* total pelvic exenteration, *APE* anterior pelvic exenteration, *PPE* posterior pelvic exenteration, *DOD* dead of disease, *AWD* alive with disease, *NED* no evidence of disease

recurrences, 1 was alive with disease, and 6 died of disease at the time the study was concluded. We calculated the predictable overall survival (OS) and progression-free survival (PFS) after undergoing PE for the 12 patients. As shown in Fig. 1, the 5-year OS rate for all patients was 42.2 %. We performed univariate analysis on the previously-described patient prognostic factors; however, none of the factors were statistically significant.

Discussion

Pelvic exenteration was initially introduced in 1948 as a palliative procedure for patients with advanced pelvic cancer [5]. With the advent of surgical diversion techniques, advances in post-operative management, thromboprophylaxis, and the use of prophylactic antibiotics, the associated operative mortality has improved. In the most recently published studies, the operative mortality rate has been reduced to 0–2 % [8–10]. Therefore, the exact surgical indications for PE have gradually changed over time, and PE is currently considered a safe and feasible procedure for select patients.

To select the appropriate candidates for PE, pre-operative imaging is the most important diagnostic tool for assessment. Computed tomography (CT) scans and/or magnetic resonance imaging system (MRIs) have not been reported in sufficient numbers as imaging methods before performing PEs to assess efficacy as therapeutic modalities and in the pre-operative evaluation of lesions [11]. In fact, most of the patients in our series had previously undergone pelvic surgery and/or radiation therapy, thus it was difficult to distinguish between post-radiation pelvic fibrosis and recurrent lower genital tract cancers using CT scans and/or MRIs as imaging modalities. We performed positron emission tomography/CT (PET/CT) scans to identify the recurrent tumors in six patients who had surgery after 2004. All of the patients with central disease detected by PET/CT had histopathologic confirmation of the surgical specimens. These six patients underwent CT and/or MRI prior to PET/CT; uterine relapse was not detected in two patients by CT scan and 3 patients by MRI. These results, as well as the results in previous reports [11, 12] indicate that PET/CT is the most useful modality with which to determine eligibility for PE.

Factors such as positive node status, tumor size, side wall fixation, histologic type, and margin status, have been shown to be associated with prognosis in patients with advanced cervical cancer [7, 8, 13–19]. In our series, 5

Table 2 Surgical outcome and complications ($n = 12$)

	Patients
Early and late operative complications	
Ileus	5 (42 %)
Insufficiency of the intestinal anastomosis	3 (25 %)
Re-laparotomy	2 (17 %)
Wound infection	2 (17 %)
No complication	2 (17 %)
Pelvic abscess	1 (8 %)
Infectious lymphocele	1 (8 %)
Infection of urinary tract	1 (8 %)
Severe appetite loss	1 (8 %)
Cardiovascular and/or thromboembolic events	0 (0 %)
Insufficiency of the ureteral anastomosis	0 (0 %)
Secondary bleeding	0 (0 %)
Operative mortality	0 (0 %)

Table 3 Types of urinary reconstructive procedures and leak

RT radiation therapy, PE pelvic exenteration, TPE total pelvic exenteration, APE anterior pelvic exenteration, PPE posterior pelvic exenteration

[a] No intestinal anastomosis

[b] No ureteral anastomosis

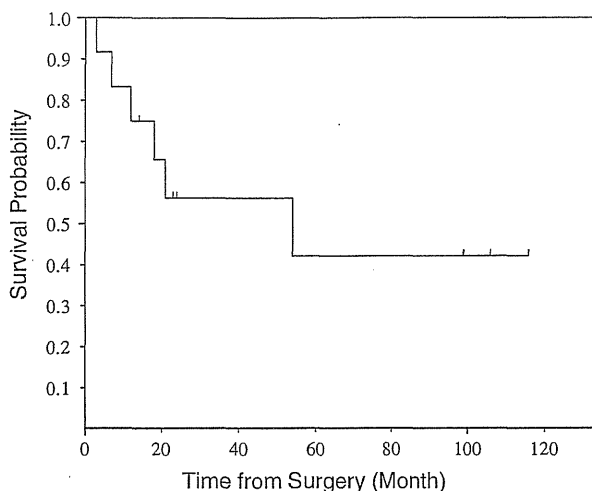| Case	Exent type	Method of urinary diversion	RT before PE	Leak of intestinal anastomosis	Leak of ureteral anastomosis
1	TPE	Sigmoid colon conduit	+	−	−
2	APE	Ileal conduit	+	+	−
3	APE	Ileal conduit	−	−	−
4	APE	Ileal conduit	−	−	−
5	APE	Ureterocutaneostomy	+	−[a]	−[b]
6	APE	Ureterocutaneostomy	+	−[a]	−[b]
7	APE	Ileal conduit	+	+	−
8	PPE	No urinary diversion	+	−[a]	−[b]
9	TPE	Ureterocutaneostomy	+	−[a]	−[b]
10	APE	Transverse colon conduit	+	−	−
11	TPE	Transverse colon conduit	+	−	−
12	APE	Transverse colon conduit	+	−	−

Fig. 1 Overall survival for the entire patients

patients (41.7 %) had no evidence of disease after PE (nos. 2, 4, 5, 10, and 12). Moreover, 2 patients (nos. 2 and 5) had long-term survival >8 years in spite of recurrence. Of the 5 patients with no evidence of disease, 4 (nos. 2, 5, 10, and 12) were treated for recurrences or residual tumor. All 4 patients had common factors tumor size ≤30 mm, negative surgical margins, complete resection, and no lymph node involvement. Although the number of patients was too small to demonstrate a statistical difference, these factors are thought to be important in selecting candidates for PE. In contrast, patient no. 4 had long-term survival, despite a bulky tumor (>80 mm), positive lymph nodes, and cervical adenocarcinoma. Patient no. 4 was diagnosed with FIGO stage IVA cervical adenocarcinoma and underwent PE primarily. The therapeutic strategy for stage IVA cervical cancer remains controversial. Surgical resection for patients with stage IVA cervical cancer is not recommended in the United States and Japan [2, 20]. In contrast, half of the patients with stage IVA undergo PE primarily in Germany [17]. Marnitz et al. [17] reported that the overall cumulative survival after PE was 52.5 % in the primary treatment group and tumor-free resection margin was significantly correlated with a good prognosis. Our cases also achieved tumor-free surgical margins; therefore, PE may be an alternative to primary chemoradiation if the tumor is considered to be completely resectable.

PE, in some situations, is associated with severe complications. Intestinal anastomosis leaks cause peritonitis and inevitably lead to re-laparotomies, resulting in lengthy hospital stays. In our series, insufficiency of the intestinal anastomosis occurred in 3 of 8 cases (37.5 %), which is higher than previous reports (19.1–29.8 %) [21, 22]. All three patients with intestinal leakages had irradiated small intestines with normal appearances. On the basis of these results, we used a transverse colonic conduit for urinary

diversion in the current three patients, and had no post-operative intestinal leakages at the time the study was concluded. We deem transverse colonic conduits to be suitable in patients with previous radiation therapy.

In conclusion, PE is a feasible surgical procedure, especially in select patients with recurrent tumors ≤30 mm in size, negative surgical margins, and no lymph node involvement, and is a valuable option for cure or long-term survival, although post-operative complications remain high. Intra-operative procedures, such as urinary diversion, affect complications during the early post-operative period and will continue to be revised to further reduce the complication rate. Cooperation with general surgeons and/ or urologists, intensive post-operative management, and patient selection are the cornerstones to improve survival and quality of life in patients with advanced and/or recurrent cervical cancer.

Conflict of interest The authors have no conflicts of interest to declare.

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Original Articles

Efficacy of neoadjuvant chemotherapy followed by radical hysterectomy in locally advanced non-squamous carcinoma of the uterine cervix: a retrospective multicenter study of Tohoku Gynecologic Cancer Unit

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Summary

Objective: Radical hysterectomy (RH) is a standard treatment for locally advanced non-squamous cell carcinoma (N-SCC) of the uterine cervix, but there have been no reports on whether neoadjuvant chemotherapy (NAC) followed by radical hysterectomy could improve the outcome of patients with this disease. *Materials and Methods:* This multicenter retrospective study enrolled 77 patients with Stage IB2 to IIB N-SCC of the uterine cervix. Of these, 27 patients were treated with NAC prior to radical hysterectomy (NAC group) and 50 with RH alone (RH group). The two-year recurrence-free survival (RFS) rate, progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Clinical parameters such as clinical stage, histological type, and post-operative treatment were also examined between the groups. *Results:* While the two-year RFS rates were 81.5% and 70.0% in NAC and RH groups, respectively ($p = 0.27$) and the median PFS was 51 months and 35 months in NAC and RH groups, respectively ($p = 0.35$), the median OS was 58 months and 48 months in NAC and RH groups, respectively, which was significant ($p = 0.0014$). The median OS of patients with mucinous adenocarcinoma in NAC group was significantly higher than that in RH group: 58 months versus 37 months ($p = 0.03$). *Conclusion:* NAC prior to RH may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma.

Key words: Uterine cervical carcinoma; Non-squamous cell carcinoma; Neoadjuvant chemotherapy; Radical hysterectomy; Outcome.

Introduction

Radical hysterectomy and radiotherapy are a traditional therapeutic modality for invasive carcinoma of the uterine cervix in Japan. Since some observations showed that chemo-radiotherapy with cisplatin offered the advantage of clinical outcome in locally advanced carcinoma of the uterine cervix, chemotherapy has become the treatment of preference of uterine cervical carcinoma [1-7]. The Italian multicenter randomized study, which enrolled patients with locally advanced Stage IB2 to IIB squamous cell carcinoma of the uterine cervix, showed that NAC prior to RH improved the patient outcome as compared to conventional radiation therapy alone [8]. Combination of docetaxel and carboplatin in the neoadjuvant setting for patients with advanced or recurrent uterine cervical malignancy showed complete or partial response in all of patients with uterine cervical adenocarcinoma, suggesting that the combination may be quite promising for treatment of uterine cervical adenocarcinoma [9]. However, there is no evidence that NAC improves the outcome of

patients with uterine cervical adenocarcinoma. The aim of this multicenter study was to retrospectively evaluate whether NAC can improve the outcome of patients with locally advanced N-SCC of the uterine cervix.

Materials and Methods

This study enrolled 77 patients with Stage IB2 to IIB N-SCC of the uterine cervix who underwent RH at the institutions belonging to the Tohoku Gynecologic Cancer Unit (TGCU) between January 1996 and December 2008. Of these, 27 patients were treated with NAC prior to RH (NAC group), and 50 patients were treated with RH alone (RH group). The two-year recurrence-free survival (RFS) rate, progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Clinical parameters, such as: clinical stage, histological type, and postoperative treatment were also examined between the groups.

The PFS and OS in the two groups were calculated by the Kaplan-Meier method, and the statistical significance of differences in the cumulative curves between the two groups was evaluated by log-rank test. Categorical variables comparisons were conduced by two-tailed Chi square or Mann-Whitney U test where appropriate. A result was deemed significant at $p < 0.05$.

Results

Patient characteristics

The median age was 49 and 45 years in NAC and RH groups, respectively. Eleven (40.7%) and 29 (58.0%) patients had Stage IB2 disease in NAC and RH groups, respectively, and 16 (59.3%) and 21 (42.0%) patients had Stage II disease NAC and RH groups, respectively. In regard to the histological type, 13 patients had mucinous adenocarcinoma, four had endometrioid adenocarcinoma, three had clear cell carcinoma, and seven had adenosquamous carcinoma in the NAC group, while 27 patients had mucinous adenocarcinoma, nine had endometrioid adenocarcinoma, two had clear cell carcinoma, nine had adenosquamous carcinoma, and three had other types in RH group. Of the 27 patients in NAC group and 50 in RH group, 19 (70.4%) and 40 (80.0%) underwent any postoperative treatments, respectively (Table 1).

NAC regimens and number of cycles

Because this was a retrospective and multicenter study, the combination of anti-cancer agents utilized was heterogeneous as shown in Table 2. Of the 27 patients in NAC group, eight received DC: seven patients received two cycles and one patient received three cycles. Five patients received cisplatin alone. Four patients received MEP: one patient received one cycle, two patients received two cycles, and one patient received three cycles. Three patients received TC of two cycles. Three patients received FCAP: one patient received one cycle and two patients received three cycles. Other four patients received cisplatin/CPT-11 of two cycles, cisplatin/Adriamycin of two cycles, cisplatin/mitomycin C of three cycles, and carboplatin/actinomycin D of three cycles, respectively.

Comparison of clinical outcome between NAC and RH groups

The two-year RFS rate was 81.5% in NAC group and 70.0% in RH group ($p = 0.27$, Table 3). The median PFS was 51 months (range, 14-157 months) in NAC group and 35 months (range, 4-157 months) in RH group ($p = 0.35$, Table 3). On the other hand, the median OS was 58 months (range, 15-157 months) in NAC group and 48 months (range, 9-157 months) in RH group, which was significant ($p = 0.0014$, Table 3 and Figure 1A).

Comparison of clinical outcome according to clinical parameters

There were no significant differences in the median PFS and OS between NAC and RH groups according to stage, histological type and adjuvant therapy, except mucinous adenocarcinoma (Table 4). While the median PFS of patients with mucinous adenocarcinoma was 58 months (range, 8-124 months) in NAC group and 33 months (range, 4-125 months) in RH group ($p = 0.34$), the

Table 1. — Patient characteristics.

Variable	NAC (n = 27)	RH (n = 50)	p value
Median age in years [range]	49 [30-63]	45 [25-76]	$p = 0.85$*
Stage			
IB2	11 (40.7)	29 (58.0)	$p = 0.15$**
II			
IIA	0	6 (12.0)	
IIB	16 (59.3)	15 (30.0)	
Histological type			
Adenocarcinoma			
mucinous	13 (48.1)	27 (54.0)	$p = 0.98$**
endometrioid	4 (14.9)	9 (18.0)	
clear cell	3 (11.1)	2 (4.0)	
Adenosquamous carcinoma	7 (25.9)	9 (18.0)	
Others	0	3 (6.0)	
Adjuvant therapy			
administered	8 (29.7)	10 (20.0)	$p = 0.34$**
not administered			
Chemotherapy	9 (33.3)	16 (32.0)	
Chemoradiation therapy	5 (18.5)	14 (28.0)	
Radiotherapy	5 (18.5)	10 (20.0)	

*Mann-Whitney U test, **Chi-square test, numbers of parenthesis represent %.

Table 2. — List of NAC regimens.

Regimen	No. of patients
DC (Docetaxel 70 mg/m², carboplatin AUC6 day 1 q21 days)	8
Cisplatin alone (total 200 mg/body for 3 days)	5
MEP (MMC 10 mg/m² day 1, etoposide100 mg/m² days 1,3,5, cisplatin 50 mg/m² day 1, q28 days)	4
TC (Paclitacel 175 mg/m², carboplatin AUC6 day 1 q21 days)	3
FCAP (5-FU 200 mg/body, CPM100 mg/body, cisplatin 20 mg/m² days 1-7, ADM 35 mg/m² day 7)	3
Cisplatin/CPT-11 (cisplatin 70 mg/m² day 1, CPT-11 70 mg/m² days 1,8 q21 days)	1
Cisplatin/ADM (cisplatin 100 mg/body, ADR 40 mg/body days 1,2 q21 days)	1
Cisplatin/MMC (cisplatin 50 mg/body, MMC 4 mg/body day 1 q21 days)	1
Carboplatin/Actinomycin D (Carboplatin 300 mg/body, Actinomycin D 1.5 mg/body day 1 q21 days)	1

MMC: mitomycin C; CPM: cyclophosphamide; ADM: adriamycin.

Table 3. — Comparison of the clinical outcome between the two groups.

	NAC (n = 27)	RH (n = 50)	p value
Two-year RFS rate	81.5% (22/27)	70.0% (35/50)	$p = 0.27$
Median PFS (range)	51 months (14-157)	35 months (4-157)	$p = 0.35$
Median OS (range)	58 months (15-157)	48 months (9-157)	$p = 0.0014$

RFS: recurrence free survival; PFS: progression-free survival; OS: overall survival.

median OS of those with mucinous adenocarcinoma was 58 months (range, 24-124 months) in NAC group and 37 months (range, 9-125 months) in RH group, which was significant ($p = 0.03$) (Table 4 and Figure 1B).

Clinical outcome according to therapeutic modality after NAC and radical surgery

The outcome of patients who underwent chemotherapy or chemoradiotherapy or radiotherapy after NAC and RH were compared. As shown in Table 5, chemotherapy after NAC and surgery prolonged PFS and OS, and increased

Table 4. — *Comparison of the clinical outcome according to clinical parameters.*

Clinical parameters	Median PFS			Median OS		
	NAC	RH	*p* value	NAC	RH	*p* value
Stage						
IB2	64 (11-157)	37 (9-157)	0.26	64 (15-157)	54 (12-157)	0.26
II	33 (4-124)	45 (4-92)	0.45	39 (16-124)	45 (9-92)	0.40
Histological type						
Adenocarcinoma						
mucinous	58 (8-124)	33 (4-125)	0.34	58 (24-124)	37 (9-125)	0.03
endometrioid	31 (10-97)	70 (14-97)	0.29	31 (10-97)	70 (20-97)	0.49
clear cell	22 (4-108)	64 (12-106)	0.89	22 (16-108)	83 (60-106)	0.41
Adenosquamous	36 (12-157)	45 (12-157)	0.11	43 (21-157)	46 (12-157)	0.31
Adjuvant therapy						
administered	77 (25-157)	31 (5-157)	0.18	77 (35-157)	32 (12-157)	0.24
not administered	33 (4-124)	47 (4-106)	0.66	39 (16-124)	51 (9-106)	0.61

Numbers show months: Parenthesis means range. PFS: progression-free survival; OS: overall survival.

Table 5. — *Clinical outcome according to therapeutic modality after NAC and radical surgery.*

	Chemotherapy (n = 9)	Chemoradiotherapy (n = 5)	Radiotherapy (n = 5)
PFS (months)	42 (10-108)	30 (4-76)	22 (8-97)
OS (months)	42 (19-108)	30 (16-76)	31 (22-97)
Two-year RFS rate	88.9%	60.0%	60.0%

PFS: progression-free survival; OS: overall survival; RFS: recurrence free survival.

the two-year RFS rate compared to chemoradiotherapy or radiotherapy after NAC and surgery, although they did not reach significance.

Discussion

Numerous phase II studies have reported the favorable effects of NAC in the treatment of locally advanced carcinoma of the uterine cervix. The authors have previously reported the efficacy and safety of NAC with cisplatin plus irinotecan in this disease [10]. However, few randomized clinical trials (RCT) have evaluated the effect of NAC in the clinical outcome of patients with this disease. Sardi *et al.* reported a significant improvement of the seven-year survival rate in patients treated by NAC and radical surgery and radiotherapy (65%), as compared with that in those treated by radical surgery and radiotherapy (41%) in a four-arm randomized controlled trial (RCT) (NAC and radical surgery and radiotherapy, radical surgery and radiotherapy, radiotherapy alone, and NAC and radiotherapy) [11]. However, the retrospective study did not show obvious improvement of the five-year survival rate in patients with Stage IB2 carcinoma of the uterine cervix treated by NAC prior to surgery, as compared with that in those treated by surgery alone (80% versus 69%) [12]. These two reports were conducted in patients with SCC of the uterine cervix. Since N-SCC of the uterine cervix has recently increased in Japan, it is an important issue to evaluate the effectiveness of NAC in the outcome of patients with N-SCC of the uterine cervix. Some evidence showed that the outcome of patients with N-SCC of the uterine cervix was poorer than that of

patients with SCC of the uterine cervix [13, 14], because of the higher incidence of lymph node metastases at a relatively early stage of the disease, and a lower sensitivity to radiotherapy in N-SCC of the uterine cervix [15, 16]. Chemotherapy is therefore expected to have a greater beneficial effect on the outcome of patients with N-SCC than radiotherapy or chemoradiation therapy. Because the present study was conducted retrospectively in multicenters, the combination of anti-cancer agents used was heterogeneous. The NAC regimens used in this study invariably included one of platimun derivatives, such as cisplatin and carboplatin, so platimun agents seem favorable for chemotherapy prior to surgery in N-SCC of the uterine cervix.

The two-year RFS rate and the median PFS were better in NAC group than in RH group, which were not significant, whereas the median OS in NAC group was significantly longer than in RH group (p = 0.0014). Furthermore, prognostic analysis in clinical parameters showed that the median OS of patients with mucinous adenocarcinoma in NAC group was significantly longer than in RH group (p = 0.03), although other histological types and postoperative treatment did not significantly affect the prognosis of patients between NAC and RH groups. These results suggest that NAC may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma. Because mucinous adenocarcinoma accounts for approximately 70% out of adenocarcinomas of the uterine cervix, NAC may improve prognosis of patients with N-SCC of the uterine cervix, although NAC should be used individually at the present time.

The present study showed that chemotherapy after NAC and surgery prolonged PFS and OS, compared to chemoradiotherapy or radiotherapy after NAC and surgery, which did not reach significance. Tattersall *et al.* reported that primary chemotherapy followed by radiotherapy significantly decreased the survival rate of patients with uterine cervical carcinoma compared to those who were treated by radiotherapy alone [17]; furthermore, meta-analysis showed that chemotherapy followed by radiotherapy did not improve the survival time
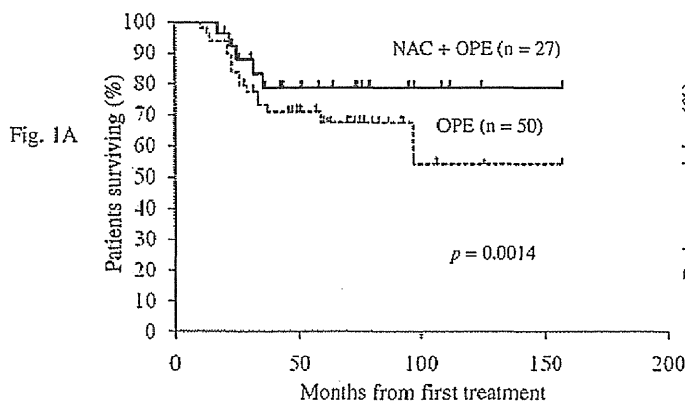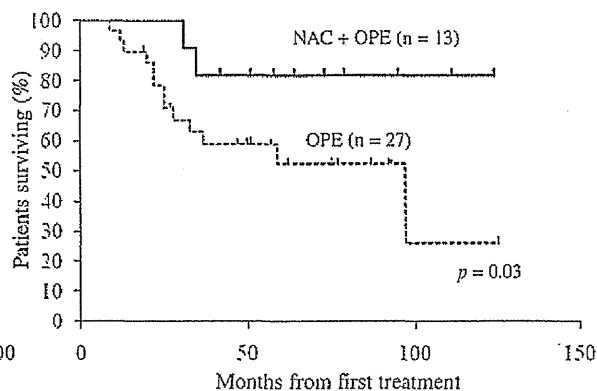
Fig. 1A

Fig. 1B

Figure 1. — 1A: overall survival in all patients who underwent neoadjuvant chemotherapy followed by radical hysterectomoy (NAC) or radical hysterectomy alone (RH). 1B: overall survival in patients with mucinous adenocarcinoma who underwent neoadjuvant chemotherapy followed by radical hysterectomoy (NAC) or radical hysterectomy alone (RH).

in uterine cervical carcinoma [18]. Considering these reports together with the present results, chemoradiotherapy or radiotherapy after NAC and surgery may contribute to unfavorable outcome of the patients with uterine cervical adenocarcinoma compared to chemotherapy after NAC and surgery, although further investigation is necessary to confirm the appropriate therapeutic modality follwing NAC and surgery.

The recent reports demonstrated that taxanes were used effectively in NAC for uterine cervical adenocarcinoma [19, 20]. Most of the institutions joining TGCU had adopted cisplatin-based regimens in the 1990s, and switched to the regimens combining taxanes and platinum derivatives after 2000. Despite the diverse NAC regimens and the small sample size, the authors believe that the present results have provided constructive ideas for the development of new therapeutic strategy for N-SCC of the uterine cervix. An effective chemotherapeutic regimen for N-SCC of the uterine cervix should be urgently integrated in a phase II study and then a RCT that compares a new single NAC and radical surgery with radical surgery alone, is warranted to confirm the present results.

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Hysteroscopic Inspection and Total Curettage Are Insufficient for Discriminating Endometrial Cancer from Atypical Endometrial Hyperplasia

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Endometrial cancer (EC) is the most prevalent gynecologic malignancy in Japan. Atypical endometrial hyperplasia (AEH) is viewed as the premalignant lesion of EC, however it is often difficult to distinguish EC from AEH. The rate of concurrent EC in women diagnosed preoperatively with AEH based on endometrial biopsy was reported as 17-52%. Although hysteroscopic inspection and total curettage are considered as useful methods to make diagnosis of endometrial lesions, there is no report using this combined method to discriminate EC from AEH. The purpose of this study was to examine whether hysteroscopic inspection and total curettage improve the prevalence of EC among women diagnosed preoperatively with AEH. We reviewed 22 patients who underwent hysteroscopic inspection and total curettage and were diagnosed with AEH before undergoing hysterectomy between November 2001 and May 2011. The diagnosis made with the hysterectomy specimens revealed AEH in 10 patients (45.5%), endometrial hyperplasia without atypia in 3 (13.6%), and endometrioid adenocarcinoma, the most common type of EC, in 9 (40.9%). Endometrioid adenocarcinoma included 7 patients without myometrial invasion (31.8%) and 2 patients with superficial myometrial invasion (9.1%). There was no hysteroscopic finding that was specific for EC or AEH. In conclusion, about 41% of women who underwent hysterectomy under a diagnosis of AEH were found to have coexisting adenocarcinoma, although the prevalence of EC among those women was similar to that in earlier reports with endometrial biopsy. Accordingly, we must be careful in planning the therapeutic strategy for women with a preoperative diagnosis of AEH.

Keywords: Atypical endometrial hyperplasia; endometrial cancer; hysteroscopy; myometrial invasion; total curettage
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Endometrial cancer (EC) is the most common gynecologic malignancy in Europe and the United States. EC has also increased in Japan in the last 20 years, and in 2009 moved ahead of cervical cancer as the most prevalent malignant gynecologic disease in Japan (Sakuragi et al. 2010). Endometrioid adenocarcinoma is the most common pathologic type of EC, and adenomatous hyperplasia was shown to be a premalignant lesion of endometrioid adenocarcinoma 50 years ago (Gusberg and Kaplan 1963). Currently, endometrial hyperplasia, atypical endometrial hyperplasia (AEH) and well-differentiated endometrioid adenocarcinoma are viewed as a continuous spectrum of the disease. Kurman et al. (1985) classified hyperplasia as simple hyperplasia without atypia, complex hyperplasia without atypia, simple atypical hyperplasia, and complex atypical hyperplasia with cellular atypia and architectural atypia. They found that the rates of progression to EC from simple hyperplasia without atypia, complex hyperplasia without atypia, simple atypical hyperplasia and complex atypical hyperplasia were 1%, 3%, 8% and 29%, respectively (Kurman et al. 1985). The World Health Organization (WHO) and the International Society of Gynecologic Pathologists (ISGP) now use this classification.

In patients diagnosed as AEH with endometrial biopsy, the reported rate of concurrent endometrial adenocarcinoma in hysterectomy specimens is 17-52% (Kurman and Norris 1982; Dunton et al. 1996). The prevalence of EC with

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myometrial invasion was also reported as 8-39% (Kurman and Norris 1982; Merisio et al. 2005). Because of the high rate of concomitant cancer, even if endometrial atypical hyperplasia is diagnosed with endometrial biopsy, complete endometrial curettage is recommended in the Japan Society of Gynecologic Oncology guidelines for treatment of uterine body neoplasms (Nagase et al. 2010). However, only a few studies examined endometrial adenocarcinoma in hysterectomy specimens after diagnosis of AEH with total curettage (Shutter and Wright 2005; Merisio et al. 2005; Trimble et al. 2006), and there is no report using hysteroscopic inspection and total curettage preoperatively. The purpose of this study was to examine whether hysteroscopic inspection and total curettage are sufficient for diagnosing AEH. In this study, we reviewed the pathological diagnosis of hysterectomy specimens in women diagnosed as AEH with hysteroscopic inspection and total curettage. The primary endpoint was the prevalence of endometrial adenocarcinoma. Secondary endpoints were the rate of myometrial invasion and the grade of endometrioid adenocarcinoma.

Patients and Methods

The subjects were 22 patients who were diagnosed with AEH based on hysteroscopic inspection and total curettage, and then underwent total hysterectomy between November 2001 and May 2011 in the Department of Gynecology, Tohoku University Hospital. The indications for hysteroscopic inspection and total curettage in our institute were women diagnosed with AEH by endometrial biopsy; referred with a diagnosis of AEH; clinically suspected to have AEH or endometrial cancer by endometrial cytology or imaging studies, although these diagnoses were not made with endometrial biopsy; or under treatment with medroxyprogesterone acetate (MPA) therapy for AEH or early stage EC. Patients treated with MPA to preserve fertility were excluded from the study.

Hysteroscopic inspection and total curettage were performed in three steps under intravenous anesthesia as a day case admission: 1) insertion of a flexible scope into the uterine cavity for filling with isotonic sodium chloride as a distension medium, and inspection for the presence of an elevated lesion, atypical vessel or necrotic tissue; 2) thorough curettage of the uterine cavity with particular attention to

endometrial lesions; and 3) reinsertion of a hysteroscope to ensure that all endometrial lesions had been removed. Clinical information and hysteroscopic findings were obtained from medical records. The primary doctor made a pathological diagnosis and two other doctors reviewed this decision and established the final diagnosis. A pathological diagnosis of non-atypical hyperplasia in the hysterectomy specimen was regarded as AEH.

Results

Of the 22 patients, the diagnoses made with the hysterectomy specimens were AEH in ten patients (45.5%), endometrial hyperplasia without atypia in three patients (13.6%), and endometrioid adenocarcinoma in nine patients (40.9%). The AEH patients included two patients with simple atypical hyperplasia and eight patients with complex atypical hyperplasia. The three patients diagnosed with endometrial hyperplasia without atypia showed complex hyperplasia without atypia. Endometrioid adenocarcinoma included seven grade 1 (G1) patients without myometrial invasion, one G1 patient with superficial myometrial invasion, and one grade 2 (G2) patient with superficial myometrial invasion, giving a total of seven patients (31.8%) without myometrial invasion and two patients (9.1%) with superficial myometrial invasion (Table 1). Total hysterectomy with bilateral salpingo-oophorectomy was performed in 21 patients, and total hysterectomy with preservation of the ovaries was performed in one patient diagnosed with AEH based on the hysterectomy specimen. No patient has shown recurrence up to May 2012.

Comparison of the patients with endometrioid adenocarcinoma (EC group, $n = 9$) and all other patients (AEH group, $n = 13$) showed no significant differences in age, menstrual status, parity, body mass index (BMI), symptoms, thickness of the endometrium measured by trans-vaginal ultrasonography, and time from diagnosis to hysterectomy (Table 2).

Hysteroscopic findings of an elevated lesion, atypical vessel or necrosis also did not differ significantly between the two groups (Table 3). MRI information was available

Table 1. Diagnosis based on the hysterectomy specimen.

Diagnosis from hysterectomy	No. of patients	(%)
Non-atypical endometrial hyperplasia	3	13.6
Simple hyperplasia	0	
Complex hyperplasia	3	
Atypical endometrial hyperplasia (AEH)	10	45.5
Simple atypical hyperplasia	2	
Complex atypical hyperplasia	8	
Endometrial carcinoma (EC)	9	40.9
Endometrioid adenocarcinoma 1a Grade1	7	
Endometrioid adenocarcinoma 1b Grade1	1	
Endometrioid adenocarcinoma 1b Grade2	1	
Total	22	

Table 2. Comparison of clinical parameters between patients in the EC and AEH groups.

Clinical parameter	EC n = 9	AEH n = 13	P
Age	53.4 (± 9.1)	53.4 (± 8.3)	0.57
Post-menopause	3 (33%)	6 (46%)	0.67
Parity	1.7 (± 1.0)	1.3 (± 1.2)	0.51
BMI	22.8 (± 3.7)	23.4 (± 4.5)	0.54
Abnormal genital bleeding	5 (56%)	10 (77%)	0.38
Endometrial thickness (mm)	20.9 (± 12.6)	14.4 (± 8.5)	0.25
Time from diagnosis to hysterectomy (days)	58 (± 17)	80 (± 122)	0.38

Values are shown as a mean (± s.d.) or the number of patients (%). EC, endometrial cancer; AEH, atypical endometrial hyperplasia; BMI, body mass index.

Table 3. Comparison of hysteroscopic findings between patients in the EC and AEH groups.

Hysteroscopic findings[a]	EC n = 9	AEH n = 13
Elevated lesion	6 (66%)	9 (69%)
Atypical vessel	2 (22%)	2 (15%)
Necrosis	0 (0%)	0 (0%)
No finding	2 (22%)	3 (23%)

Values are shown as the number of patients (%).
[a]Multiple findings were possible; therefore the totals are greater than 100%.
EC, endometrial cancer; AEH, atypical endometrial hyperplasia.

Table 4. Comparison of MRI findings between patients in the EC and AEH groups.

MRI findings[a]	EC group n = 8	AEH group n = 11
Endometrial abnormal signals[b]	4 (50%)	5 (45%)
Laceration of junctional zone	2 (25%)	2 (18%)
No findings	4 (50%)	6 (55%)

Values are shown as the number of patients (%). EC, endometrial cancer; AEH, atypical endometrial hyperplasia; MRI, magnetic resonance imaging.
[a] Multiple findings were possible; therefore the totals are greater than 100%.
[b] Heterogenous intensity or intermediate to low intensity lesion.

for eight patients in the EC group and 11 patients in the AEH group (Table 4). The exceptions were two patients in the AEH group and one patient with superficial myometrial invasion in the EC group. Abnormal endometrial signals (heterogenous intensity or intermediate to low intensity) were observed in 50% of EC patients and 45% of AEH patients. In the grade 1 patient with superficial myometrial invasion, the junctional zone was intact. The association between laceration of the junctional zone and myometrial invasion was unclear, and two EC patients and three AEH patients were incorrectly judged to have laceration of the junctional zone. MRI findings were similar between the two groups. We also analyzed whether hysteroscopic findings were concordant with MRI findings in patients who underwent MRI (n = 19). Endometrial elevated lesions were detected in eight patients (42%) by both hysteroscopy

and MRI, in four patients (21%) by hysteroscopic inspection, and in one patient (5%) by MRI, whereas they were not detected by either method in 6 patients (32%) (data not shown).

Discussion

In this retrospective study, 41% of women who underwent hysterectomy under a diagnosis of AEH were found to have coexisting endometrial adenocarcinoma. These results are similar to those in previous reports (Table 5) (Kurman and Norris 1982; Janicek and Rosenshein 1994; Lambert et al. 1994; Hunter et al. 1994; Liapis et al. 1994; Widra et al. 1995; Dunton et al. 1996; Xie et al. 2002; Agostini et al. 2002; Kimura et al. 2003; Bilgin et al. 2004; Shutter and Wright 2005; Merisio et al. 2005; Garuti et al. 2006; Trimble et al. 2006; Dordević et al. 2007). Sampling errors