Figure 2. Androgen-dependent regulation of ST3Gal II in LNCap cells. (A) LNCap, PC3, and PNT2 cells were treated with or without testosterone (0–1000 nM) for 120 h, by refeeding with fresh medium with or without testosterone at 72 h. The quantitative real-time PCR analyses of ST3Gal II mRNA were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. **P<0.001. (B) LNCap cells were treated with or without testosterone (0–100 nM) and simultaneously with or without 10 μM bicalutamide for 120 h, by refeeding with fresh medium with or without testosterone and/or bicalutamide at 72 h. The quantitative real-time PCR analyses for ST3Gal II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. **P<0.001. (C) LNCap cells were incubated in charcoal-stripped serum (CSS) for 48 h and then treated with 100 nM testosterone for the indicated times. The quantitative real-time PCR analyses for ST3Gal II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. *P<0.05, **P<0.001.
doi:10.1371/journal.pone.0031234.g002

Therefore, the regulation of ST3Gal I transcription may be different from that of ST3Gal II. We thereafter examined whether the expression of ST3Gal I, like ST3Gal II, was controlled by testosterone in LNCap cells. In this experiment, the LNCap cells were treated with testosterone and incubated for 120 h. The quantitative real-time PCR analyses showed that testosterone treatment resulted in increased expression of ST3Gal I in LNCap cells (Fig. 5A), although the expression of ST3Gal VI, the sialyltransferase required for the synthesis of sialyl paragloboside, was not induced by testosterone treatment (Figure S4). Furthermore, the testosterone-mediated induction of ST3Gal I in LNCap cells was suppressed by bicalutamide (Fig. 5B). To ensure that there were no androgens present in the cell culture media, LNCap cells were incubated in charcoal-stripped serum for 48 h. The basal level of ST3Gal I was not significantly different between the LNCap cells cultured with 10% FBS and charcoal-stripped serum (Fig. 5C). Then, the LNCap cells were treated with 100 nM testosterone, and the time-course of the changes following testosterone treatment was evaluated. The expression of ST3Gal I increased 72 h after testosterone treatment and remained elevated for more than 120 h in LNCap cells (Fig. 5C). In PC3 and PNT2 cells, no significant increase in the expression of ST3Gal I was detected after testosterone treatment (Figure S5).

Next, we examined whether the regulation of ST3Gal I was epigenetic in LNCap cells, as was the case for ST3Gal II. The LNCap cells were treated with 5-azadC and incubated for 120 h (Fig. 5D). The quantitative real-time PCR analyses showed that the expression of ST3Gal I was up-regulated by 5-azadC treatment. Next, LNCap cells were treated with TSA, and incubated for 48 h (Fig. 5E). The quantitative real-time PCR analyses showed that the expression of ST3Gal I was up-regulated by TSA treatment. Thus, the regulation of ST3Gal I, like the regulation of ST3Gal II, may be epigenetic and androgen-dependent in LNCap cells. It was previously reported that the p1 promoter of the human ST3Gal I gene is necessary for the active transcription of the gene [31]. The ST3Gal I promoter sequences are publically available, and, like ST3Gal II, we identified a CpG island in the ST3Gal I p1 promoter using the Methyl Primer Express Software program, version 1.0 (Applied Biosystems, Foster City, CA) (Figure S6A). The methylation at the CpG island in the ST3Gal I promoter was not detected in LNCap cells or in PC3 or DU145 cells by the MSP analysis (Figure S6B), thus suggesting that the methylation of a genome region other than the CpG island may affect the expression of ST3Gal I in LNCap cells. Thus, the methylation status of the CpG islands which affect the gene expression levels are different between ST3Gal I and II.

RelB is required for androgen-dependent regulation of ST3Gal I and II in LNCap cells

We next examined whether RelB was required for the testosterone-mediated induction of ST3Gal I and II. LNCap cells

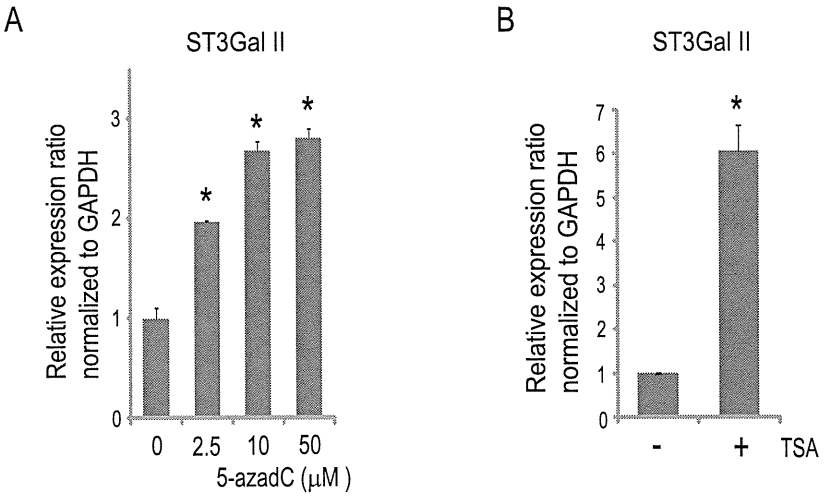

Figure 3. Epigenetic regulation of ST3Gal II in LNCap cells. (A) LNCap cells were treated with 5-aza-2′-deoxycytidine (5-azadC) (0–50 μM) for 120 h, by refeeding with fresh medium with or without 5-azadC at 72 h. The quantitative real-time PCR analyses for ST3Gal II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. *P<0.05. (B) LNCap cells were treated with 5 μM trichostatin A (TSA) for 48 h. The quantitative real-time PCR analyses for ST3Gal II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. *P<0.05.
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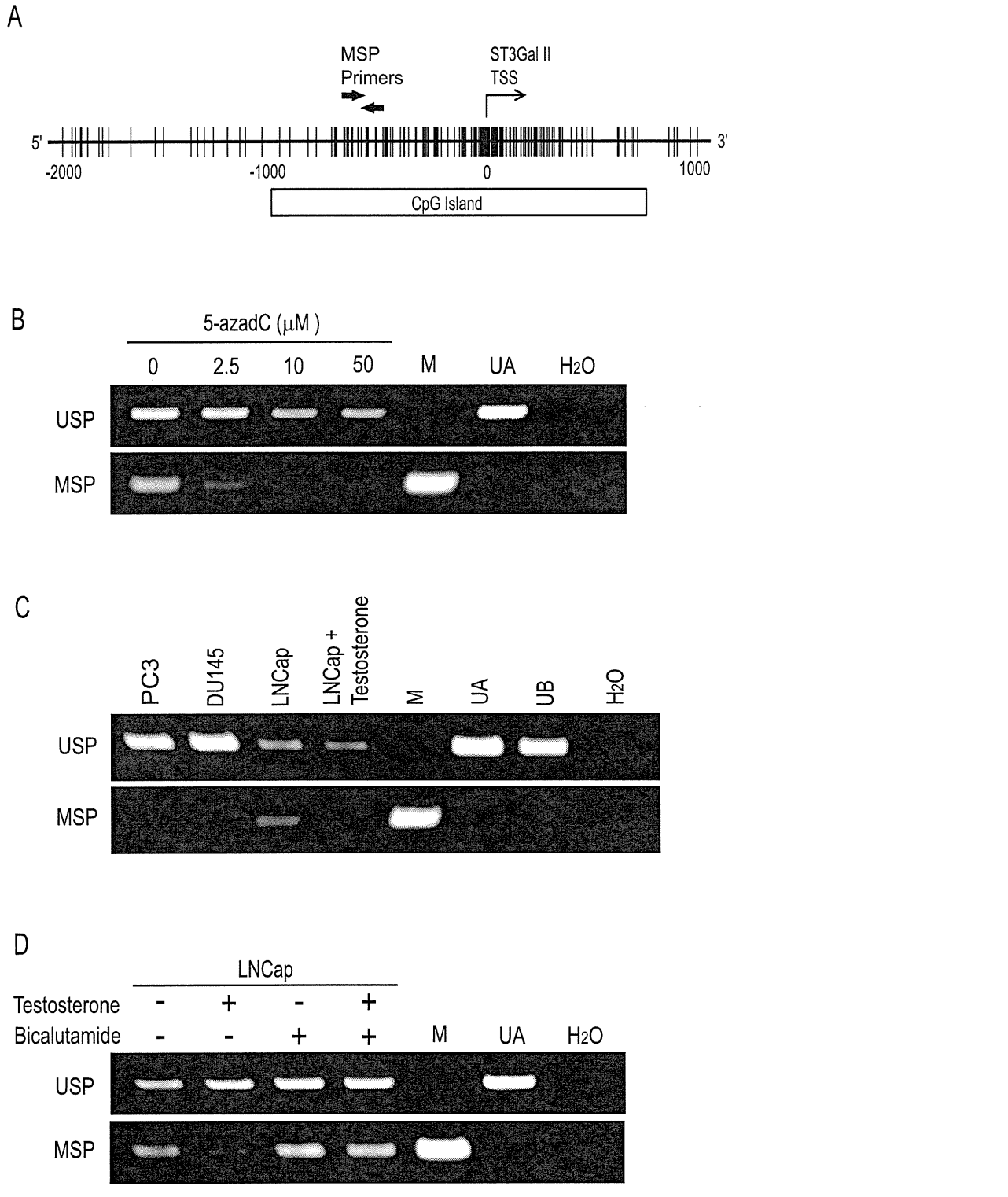

Figure 4. Control of DNA methylation at the CpG island in the ST3Gal II promoter in prostate cancer cells. (A) The CpG island in the ST3Gal II p1 promoter and the location of the MSP primers. The vertical bars represent CpG sites and TSS represents the transcriptional start site. (B–D) The MSP analyses of the CpG island of ST3Gal II. DNA was isolated from LNCap cells treated with 5-azadC (0–50 μM) for 120 h (B), castration-resistant prostate cancer cell lines (PC3 and DU145) or LNCap cells treated with or without 100 nM testosterone for 120 h (C) or LNCap cells treated with or without 100 nM testosterone simultaneously with or without 10 μM bicalutamide for 120 h (D). Then, the DNA was treated with sodium bisulfite, and finally amplified with primers specific for the unmethylated (USP) or the methylated (MSP) form of the CpG island in the ST3Gal II promoter (M, methylated control; UA, unmethylated control A; UB, unmethylated control B). The MSP analyses were repeated 3 times with the same results, and a representative image is shown in the figures.
doi:10.1371/journal.pone.0031234.g004

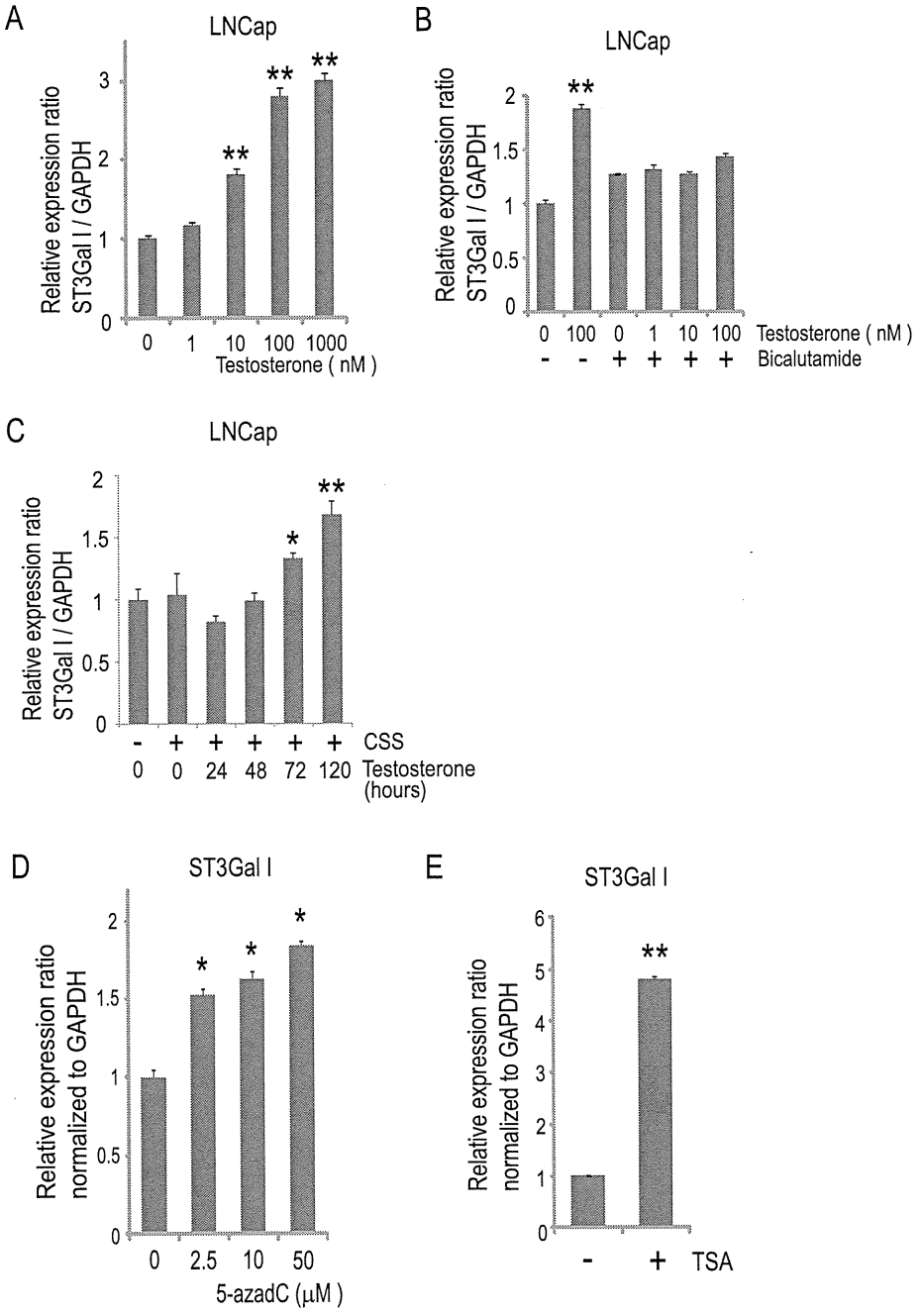

Figure 5. Androgen-dependent and epigenetic regulation of ST3Gal I in LNCap cells. (A) LNCap cells were treated with or without testosterone (0–1000 nM) for 120 h, by refeeding with fresh medium with or without testosterone at 72 h. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. **P<0.001. (B) LNCap cells were treated with or without testosterone (0–100 nM) and simultaneously with or without 10 μM bicalutamide for 120 h, by refeeding with fresh medium with or without testosterone and/or bicalutamide at 72 h. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. **P<0.001. (C) LNCap cells were incubated in charcoal-stripped serum (CSS) for 48 h and then treated with 100 nM testosterone for the indicated times. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. *P<0.05, **P<0.001. (D) LNCap cells were treated with 5-aza-2′-deoxycytidine (5-azadC) (0–50 μM) for 120 h, by refeeding with fresh medium with or without 5-azadC at 72 h. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. *P<0.05. (E) LNCap cells were treated with 5 μM trichostatin A (TSA) for 48 h. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells. **P<0.001.
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were transfected with either scrambled RNA or RelB siRNA and incubated for 120 h. The efficacy of RNAi was assessed by the quantitative real-time PCR analyses (Figure S7). Next, LNCap cells were transfected with either scrambled RNA or RelB siRNA and incubated for 120 h with or without testosterone (Fig. 6). The efficacy of the RelB RNAi was confirmed at the protein level by a Western blot analysis. The levels of RelB protein were not greatly modulated by testosterone treatment (Fig. 6A). The quantitative real-time PCR analyses showed that, without testosterone, RelB siRNA suppressed the expression of ST3Gal I, while no effect was seen on the silenced ST3Gal II. However, the induction of both ST3Gal I and II after testosterone treatment was inhibited by RelB siRNA in the LNCap cells (Fig. 6B). Thus, RelB was required for the androgen-dependent regulation of ST3Gal I and II in LNCap cells.

Discussion

Gangliosides have been widely investigated because of their relationship with cancer progression [3]. GD1a also appears to be related to cancer cell proliferation and metastasis [11–15]. As demonstrated in this study, GD1a was produced in abundance in cancerous tissue samples from human patients with hormone-sensitive prostate cancers as well as those with castration-resistant prostate cancers. However, little is known about the regulation of GD1a production. To address this lack of information, we have been focusing on the transcription of the ST3Gal II gene, which is required for the synthesis of GD1a. We previously reported that ST3Gal II was up-regulated in human castration-resistant prostate cancer cells and that the expression of ST3Gal II is regulated by NF-κB, mainly by RelB [20]. Furthermore, a recent report

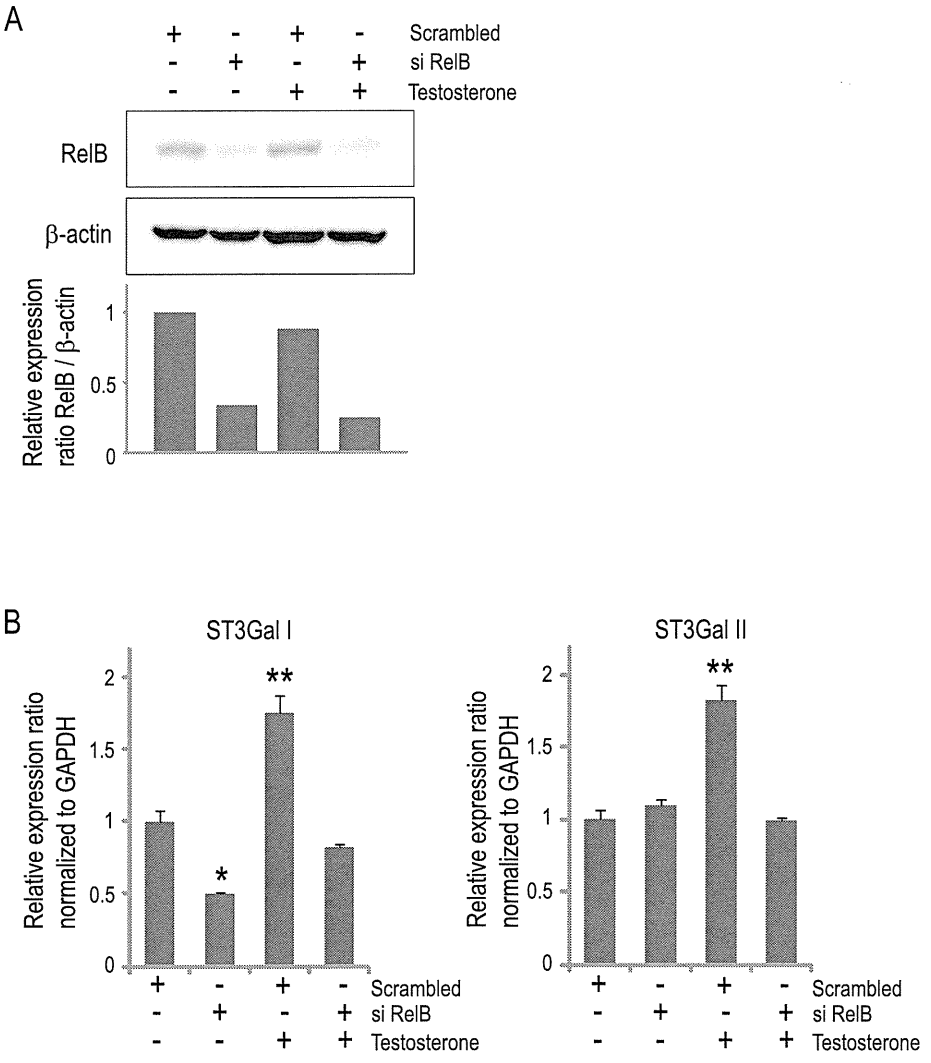

Figure 6. RelB is required for the androgen-dependent regulation of ST3Gal I and II in LNCap cells. (A) LNCap cells were transfected with either scrambled RNA or RelB siRNA and incubated for 120 h with or without 100 nM testosterone. Protein extracts were prepared using RIPA lysis buffer, and the RelB expression level of each sample was analyzed by a Western blot analysis. The expression relative to β-actin is shown in each lane after normalizing the values to the expression level of the scrambled RNA–transfected and testosterone-untreated cells. (B) LNCap cells were transfected with the either scrambled RNA or RelB siRNA and incubated for 120 h with or without 100 nM testosterone. The quantitative real-time PCR analyses for ST3Gal I and II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of the scrambled RNA–transfected and testosterone-untreated cells. *P<0.05, **P<0.001.
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showed that RelB is activated in human prostate cancers in patients with high Gleason scores [32]. As demonstrated in this study, the expression of ST3Gal II was constitutively activated and androgen-independent in castration-resistant prostate cancer cells (Fig. 2, Figure S2) because the CpG island in the ST3Gal II promoter was hypomethylated (Fig. 4). However, in androgen-depleted LNCap cells, a hormone-sensitive prostate cancer cell line, ST3Gal II was not up-regulated in spite of the activation of RelB.

We herein demonstrated that the expression of the ST3Gal II required for the production of GD1a was epigenetically silenced under androgen-depleted conditions and was up-regulated by androgen-treatment in hormone-sensitive prostate cancer cells. The CpG island of the ST3Gal II promoter was hypermethylated under androgen-depleted conditions and was demethylated by androgen treatment in hormone-sensitive prostate cancer cells (Fig. 4). The presence of the androgen may promote a chromatin environment where RelB can activate the transcription of ST3Gal II in hormone-sensitive prostate cancer cells. Thus, in hormone-sensitive prostate cancers, the production of GD1a may be regulated by androgen, which can modulate the methylation state of the CpG sites in the promoter region of a sialyltransferase gene.

Although effects of androgen on NF-κB activity have been reported, the topic remains controversial. One report showed that androgen treatment repressed the NF-κB activity through maintenance of the IκBα protein levels [33], but another report showed that the NF-κB DNA binding activity increased after androgen treatment [34]. In the present manuscript, the NF-κB activity was not significantly different between the testosterone-treated LNCap cells compared to the cells cultured without testosterone (Figure S1). The two routes in NF-κB signaling are the canonical pathway, which involves the complex formed between RelA and p50, and the non-canonical pathway, in which the complex formed between RelB and p52 is involved [35]. The different subunits of NF-κB may be differently regulated by androgens. Although the levels of nuclear RelA and p52 were not elevated after androgen treatment in a previous report [34], it is still unknown whether androgens can modulate the level of RelB. In the present study, we showed that the levels of RelB protein were not greatly modulated by androgens in the cell lines examined (Fig. 6).

Although GD1a is synthesized from GM1 mainly by ST3Gal II, ST3Gal I may also contribute to the synthesis of GD1a [6,21–24]. We found that the expression of ST3Gal I was up-regulated by 5-azadC treatment in LNCap cells, indicating that the expression of ST3Gal I may be regulated by DNA methylation, as is the case for ST3Gal II. However, methylation at the CpG island in the ST3Gal I promoter was not detected in LNCap cells by a MSP analysis. This suggests that the methylation of a genome region other than the CpG island may affect the expression of ST3Gal I. Thus, the methylation status of CpG islands which affect the gene expression are different between ST3Gal I and II. As previously reported [20], in LNCap cells, ST3Gal I was expressed, while ST3Gal II expression was silenced. The differences in the methylation of the CpG island between ST3Gal I and II may result in the differences in the expression of these two genes in LNCap cells. Based on publically available data and previous studies [36,37], we identified that ST3Gal II has one highly probable androgen receptor recruitment site −39.5 kb of the transcription start site of the ST3Gal II p1 promoter, and that the ST3Gal I has two highly probable androgen receptor recruitment sites −13.9 kb and −42.6 kb of the transcription start site of the ST3Gal I p1 promoter. Thus, as there are differences in the methylation status of CpG islands, the regulation of promoter methylation by the androgen receptor might also be different between ST3Gal I and II.

The progression of many cancers is epigenetically regulated. DNA demethylation changes have been reported to occur later in prostate carcinogenesis [38,39]. We first demonstrated that the androgen-dependent activation of transcription was induced by demethylation of CpG promoter region in ST3Gal II. Recently, several reports have shown that DNA methylation can be controlled by hormone receptors. For example, the estrogen receptor directly modifies the methylation status of the pS2 gene [40,41], and the glucocorticoid receptor could also modify the DNA methylation status [42]. Another report showed that DNA methylation/demethylation was hormonally altered to control the transcription of the cytochrome p450 27B1 gene, and that the 5-methyl-CpG binding domain family (MBD) protein activated by hormonal stimulation seemed to complete the DNA demethylation in the MBD-bound promoter [43]. In the present study, we demonstrated that demethylation of the ST3Gal II promoter was induced by androgen treatment in hormone-sensitive prostate cancer cells. Although the mechanism is currently unclear, the MBD protein may be involved in this type of androgen-induced DNA demethylation. Further research is needed to elucidate the mechanism underlying the hormonal control of the DNA methylation/demethylation of the ST3Gal II promoter.

Androgen plays a pivotal role in the development, growth, and progression of prostate cancers [25]. Although we have herein shown the androgen-dependent activation of ST3Gal II by the demethylation of CpG promoter region, other genes may also be epigenetically regulated by androgen treatment in hormone-sensitive prostate cancer cells. Although we focused on DNA methylation at the CpG island in the ST3Gal promoter in the current manuscript, it is known that DNA methylation is linked to histone deacetylation [44,45]. Therefore, in future studies, we plan to elucidate the mechanism underlying the regulation on histone modification by androgen, in addition to the effects on DNA methylation.

GD1a should be focused also from the view of cancer therapy. Several gene therapy approaches for the treatment of prostate cancer have been clinically tested [46]. Oncolytic viruses have been developed to selectively augment the anti-tumor effects, and some viruses, such as adenovirus and the herpes simplex virus, are used to combat prostate cancers [47,48]. Recently, we reported that inactivated Sendai virus particles (HVJ-E) selectively induced apoptosis in human castration-resistant prostate cancer cells by retinoic acid-inducible gene-I (RIG-I)–mediated gene expression and the induction of anti-tumor immunities [17]. A ganglioside, GD1a, which is enriched in human castration-resistant prostate cancer cells [17–20] is one of the receptors for the Sendai virus [16]; therefore, HVJ-E is expected to be a novel therapeutic tool for prostate cancers. However, HVJ-E did not induce apoptosis in LNCap cells, a human hormone-sensitive prostate cancer cell line, because these cells did not express a viral receptor ganglioside, such as GD1a [17], on their cell surface. The present study showed that GD1a was produced in clinical samples of hormone-sensitive prostate cancers and of castration-resistant prostate cancers. We are currently analyzing the mechanism underlying the cancer-selective apoptosis induced by HVJ-E, and our preliminary data suggest that both castration-resistant and hormone-sensitive prostate cancers may be treated by HVJ-E. Thus, this study will also be important to determine the indications for treating prostate cancer patients with HVJ-E.

Supporting Information

Figure S1 NF-κB activity after testosterone treatment in LNCap cells. LNCap cells were transfected with a NF-κB luciferase reporter construct and incubated for 120 h with or without 100 nM testosterone. The luciferase activity was measured, and the results are shown as the means ± S.E. (n = 3).
(EPS)

Figure S2 Androgen-independent regulation of ST3Gal II in PC3 cells. PC3 cells were incubated in charcoal-stripped serum (CSS) for 48 h and then treated with 100 nM testosterone for the indicated times. The quantitative real-time PCR analyses for ST3Gal II were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells.
(EPS)

Figure S3 Testosterone does not induce global DNA demethylation in LNCap cells. (A) The results of the differential restriction analysis. The genomic DNA isolated from untreated LNCap cells or LNCap cells treated with 100 nM testosterone for 120 h was digested using *Msp*I or *Hpa*II for 16 h at 37°C. The digested DNA was analyzed in 2% agarose gels stained with ethidium bromide. (B) The results of the MSP analysis of GSTP1. The genomic DNA isolated from untreated LNCap cells or LNCap cells treated with 100 nM testosterone for 120 h was amplified with primers specific for the unmethylated (USP) or the methylated (MSP) GSTP1 promoter after treatment with sodium bisulfite (M, methylated control; UA, unmethylated control A; UB, unmethylated control B). The MSP analyses were repeated 3 times with the same result and a representative image shown in the figure.
(EPS)

Figure S4 Androgen-independent regulation of ST3Gal VI in LNCap cells. LNCap cells were treated with testosterone (0–1000 nM) for 120 h, by refeeding with fresh medium with or without testosterone at 72 h. The quantitative real-time PCR analyses for ST3Gal VI were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells.
(EPS)

Figure S5 Androgen-independent regulation of ST3Gal I in PC3 and PNT2 cells. PC3 and PNT2 cells were treated with testosterone (0–1000 nM) for 120 h, by refeeding with fresh medium with or without testosterone at 72 h. The quantitative real-time PCR analyses for ST3Gal I were performed, and the expression levels are reported as the means ± S.E. (n = 3) of the fold difference in mRNA after normalizing the values to the expression level of untreated cells.
(EPS)

Figure S6 Control of DNA methylation at the CpG island in the ST3Gal I promoter in prostate cancer cells. (A) The CpG island in the ST3Gal I p1 promoter and the location of the MSP primers. The vertical bars represent CpG sites and TSS represents the transcriptional start site. (B) The MSP analysis of ST3Gal I. DNA was isolated from castration-resistant prostate cancer cell lines (PC3 and DU145) or LNCap cells which were treated with or without 100 nM testosterone for 120 h, and then treated with sodium bisulfite, and was finally amplified with primers specific for the unmethylated (USP) or the methylated (MSP) form of the CpG island in the ST3Gal I promoter (M, methylated control; UA, unmethylated control A; UB, unmethylated control B). The MSP analyses were repeated 3 times with the same result and a representative image shown in the figure.
(EPS)

Figure S7 The efficacy of RelB RNAi as assessed by the quantitative real-time PCR analyses. LNCap cells were transfected with either scrambled RNA or RelB siRNA and incubated for 120 h. The total RNA from LNCap cells transfected with either the scrambled RNA or RelB siRNA were subjected to the quantitative real-time PCR analyses, and the results are shown as the means ± S.E. (n = 3). **P<0.001.
(EPS)

Author Contributions

Conceived and designed the experiments: KH YK. Performed the experiments: KH YM. Analyzed the data: KH YM MM KN YN NN YK. Contributed reagents/materials/analysis tools: KH YM MM KN YN NN YK. Wrote the paper: KH YM NN YK.

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Postoperative whole pelvic radiotherapy plus concurrent chemotherapy versus extended-field irradiation for early-stage cervical cancer patients with multiple pelvic lymph node metastases

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ABSTRACT

Objectives. The aim of this study was to compare the efficacy of postoperative pelvic radiotherapy plus concurrent chemotherapy with that of extended-field irradiation (EFRT) in patients with FIGO Stage IA2–IIb cervical cancer with multiple pelvic lymph node metastases.

Methods. We retrospectively reviewed the medical records of patients with FIGO Stage IA2–IIb cervical cancer who had undergone radical surgery between April 1997 and March 2008. Of these, 55 patients who demonstrated multiple pelvic lymph node metastases were treated postoperatively with pelvic radiotherapy plus concurrent chemotherapy (n=29) or EFRT (n=26). Thirty-six patients with single pelvic node metastasis were also treated postoperatively with pelvic radiotherapy plus concurrent chemotherapy. The recurrence rate, progression free survival (PFS), and overall survival (OS) were compared between the treatment groups.

Results. Pelvic radiotherapy plus concurrent chemotherapy was significantly superior to EFRT with regard to recurrence rate (37.9% vs 69.2%, p=0.0306), PFS (log-rank, p=0.0236), and OS (log-rank, p=0.0279). When the patients were treated with pelvic radiotherapy plus concurrent chemotherapy, there was no significant difference in PFS or OS between the patients with multiple lymph node metastases and those with single node metastases. With regards to grade 3–4 acute or late toxicities, no statistically significant difference was observed between the two treatment groups.

Conclusions. Postoperative pelvic radiotherapy plus concurrent chemotherapy is superior to EFRT for treating patients with FIGO Stage IA2–IIb cervical cancer displaying multiple pelvic lymph node metastases.

Introduction

Early stage cervical cancer has traditionally been treated with either radical hysterectomy or primary radiotherapy with similar survival outcomes. According to previous reports, the 5-year survival rate of patients with FIGO Stage Ib–IIa cervical cancer treated with radical surgery ranges from 83 to 91%, which is comparable to the 74–91% reported for those treated with radiotherapy alone [1–3]. In patients with FIGO stage IIb disease, a recent retrospective analysis conducted by a Japanese group suggested similar treatment outcomes for patients treated with radical hysterectomy and those treated by definitive radiotherapy, both of which showed estimated 5-year survival rates of 69% [4].

Several risk factors have been identified that adversely impact on the outcome of patients with early stage cervical cancer who undergo radical surgery as their primary treatment [5–7]. Generally, patients with risk factors such as positive pelvic nodes, parametrial invasion, or a positive surgical margin are regarded as at "high-risk" of recurrence. Moreover, patients with a tumor that is confined to the cervix who display risk factors such as a large tumor, lymph vascular space invasion, or deep stromal invasion are considered to be at "intermediate-risk" of recurrence [5–7]. Postoperative radiotherapy with or without concurrent chemotherapy is usually recommended for patients that display these risk factors.

Among these reported prognostic factors, nodal metastasis remains the single most important prognostic factor in cervical

Abbreviations: MRI, magnetic resonance imaging; OS, overall survival; PFS, progression free interval; ICRT, intracavitary radiotherapy; EBRT, external beam radiotherapy; RT, radiotherapy; CCRT, concurrent chemoradiotherapy; PALN, para-aortic lymph node.

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cancer. It has been reported that pelvic lymph node metastasis is associated with a 30–50% reduction in 5-year survival rates [8].

The number of pelvic node metastases is reported to be a predictor of para-aortic node (PALN) metastasis. According to a previous report, PALN metastasis was observed in 0.5% of patients with one or no metastatic pelvic nodes compared with 27.6% of patients with two or more positive nodes [9]. Moreover, Kim et al. reported that the risk of PALN failure after postoperative pelvic radiotherapy in patients with two or more metastatic pelvic lymph nodes was 10 times higher than that in those with one or no positive nodes [10]. These findings indicate that a significant number of patients with early stage cervical cancer, especially those suffering from multiple pelvic node metastases, harbor occult para-aortic nodal metastases.

With the aim of controlling occult para-aortic nodal metastases and prolonging survival, RTOG initiated a phase III study examining the role of postoperative EFRT for patients with bulky Ib–IIb cervical cancer. The study demonstrated that postoperative EFRT significantly reduced extrapelvic recurrence and improved 10-year overall survival [11]. However, a subsequent RTOG study in the setting of definitive radiotherapy that compared EFRT with pelvic radiotherapy combined with concurrent chemotherapy using cisplatin and fluorouracil demonstrated that pelvic radiotherapy with concurrent chemotherapy is significantly superior to EFRT in terms of survival in patients with FIGO stage Ib–IIb cervical cancer [12]. In addition, a prospective randomized clinical trial (GOG 109/SWOG 87–97) addressing the role of postoperative adjuvant CCRT in early-stage cervical cancer patients with high-risk prognostic factors demonstrated that the addition of concurrent cisplatin-based chemotherapy to pelvic radiotherapy improved survival [13].

On the basis of the results of the above-mentioned trials [12,13], concurrent chemotherapy combined with pelvic radiotherapy has become a standard treatment for cervical cancer [14,15]. However, there has been no direct comparison between postoperative concurrent chemotherapy and pelvic radiotherapy with EFRT in patients with high-risk early stage cervical cancer.

In the current study, we retrospectively evaluated whether postoperative pelvic radiotherapy plus concurrent chemotherapy is superior to EFRT in patients with FIGO Stage IA2–IIB cervical cancer with multiple pelvic node metastases.

Materials and methods

Patients

Permission to proceed with the data acquisition and analysis was obtained from the institutional review board of Osaka University Hospital and Osaka Medical Center for Cancer and Cardiovascular Diseases. A list of patients who had undergone radical hysterectomy (type III) and pelvic lymphadenectomy for FIGO Stage IA2–IIB cervical cancer from April 1998 to March 2008 was generated from our institutional tumor registries. Then, through a chart review, 55 patients who had multiple pelvic nodes metastases and were treated postoperatively with either pelvic radiotherapy plus concurrent chemotherapy (CCRT-group) or extended-field radiotherapy (EFRT-group) were identified, and their clinical data were retrospectively reviewed. A group of patients with early-stage cervical cancer with single pelvic node metastasis that had received postoperative pelvic radiotherapy plus concurrent chemotherapy were also identified through the chart review and used as the control (N1-CCRT-group). Of a total of 36 patients in the N1-CCRT-group, 21 were treated inside the context of the previous clinical study [16].

Treatments

All patients included in this study were treated with radical hysterectomy (type III) and pelvic lymphadenectomy. The lymphadenectomy procedure included complete bilateral pelvic lymphadenectomy with the aim of removing all of the external iliac, internal iliac, common iliac, obturator, suprainguinal, and presacral lymph nodes. Preoperative PALN evaluation was performed in all patients via a CT scan of the pelvis and abdomen as part of the initial evaluation. The intra-operative assessment of PALN was routinely performed by palpation. When PALN metastasis was suspected by CT scan or palpation, a biopsy was taken for confirmation whenever possible. Patients who had biopsy-confirmed PALN metastasis were excluded from the study.

Postoperatively, patients were treated in accordance with the institutional treatment guidelines. In Osaka University Hospital, the patients were treated with pelvic radiotherapy plus concurrent chemotherapy when their pathological report displayed any of the following "high-risk" prognostic factors: a single pelvic lymph node metastasis, positive parametrial involvement, or a positive surgical margin or one of the following "intermediate-risk" prognostic factors: deep stromal invasion, lymphovascular space invasion, or a large tumor (over 4 cm), as reported previously [16]. When a patient's pathological report revealed multiple pelvic node metastases, they were treated with either EFRT, as reported previously [17], or with pelvic radiotherapy plus concurrent chemotherapy according to the physician's preference. In Osaka Medical Center for Cancer and Cardiovascular Diseases, the patients with high-risk or intermediate-risk prognostic factors were all treated with pelvic radiotherapy plus concurrent chemotherapy regardless of the number of pelvic node metastases.

Postoperative radiotherapy was performed within 4 weeks of radical surgery. Pelvic radiotherapy was delivered using a 10 megavolt (MV) X-ray from a linear accelerator using the anteroposterior parallel opposing technique. The superior margin of the external radiation field was located at the top of the fifth lumber vertebra, and the inferior border of the obturator foramen was used as the inferior margin. Laterally, the field extended 2 cm beyond the lateral margin of the bony pelvic wall. We used multi-leaf collimators to block the upper and lower corners of the radiation field. The external irradiation was delivered to the whole pelvis at 2 Gy per fraction in 5 fractions per week, for a total of 25 fractions (50 Gy).

Postoperative EFRT was also delivered to the patients via a 10 megavolt (MV) X-ray from a linear accelerator using the anteroposterior parallel opposing technique. The radiation field encompassed the pelvic and PALN drainage area. The superior margin of the PALN area was located at the bottom of the T12 vertebral body, and the inferior margin was located at the inferior border of the obturator foramen. The lateral margin was 1.5 cm to 2 cm lateral to the widest margin of the bony pelvis. The external irradiation was delivered to the EFRT fields for a total of 45 Gy in 25 fractions, and to the whole pelvis at 1.8 Gy per fraction, for a total of 28 fractions (50.4 Gy).

The patients that displayed vaginal invasion close to the surgical margin also received intracavitary radiotherapy (ICRT) involving 30 Gy in 5 fractions delivered to a depth of 5 mm below the vaginal mucosa after external beam radiotherapy (EBRT).

In our institutions, nedaplatin has been employed as a radiosensitizing agent for patients with cervical cancer [16,18]. Nedaplatin was given intravenously weekly or biweekly during the course of pelvic radiotherapy. Weekly nedaplatin was administered in Osaka University Hospital to 10 patients at a median dose of 40 mg/m^2 for 5 weeks, as described previously [16]. Biweekly nedaplatin was administered in Osaka Medical Center for Cancer and Cardiovascular Disease to 19 patients at a median dose of 70 mg/m^2 for 2 cycles per patient, as recommended by a previous report [19]. The first cycle of nedaplatin was initiated on the first day of radiotherapy treatment.

Toxicity

Clinical data regarding treatment-related complications were also collected. Complications that occurred within 90 days of the start of

primary treatment were considered to be acute complications, and those that occurred more than 90 days after the start of treatment were considered to be late complications. The severity of acute complications was classified according to the NCI Common Terminology Criteria for Adverse Events, Version 2.0. Late complications were graded according to the Radiation Therapy Oncology Group (RTOG) Late Radiation Morbidity Scoring Scheme [20].

Follow-up

The patients were followed-up regularly and observed for acute and late toxicities by both gynecological oncologists and radiation oncologists as reported previously [21]. During the treatment, the patients were evaluated weekly by pelvic examination and complete blood counts. For the patients who were treated with chemoradiation, renal and liver function tests were also performed weekly. After treatment completion, the patients were followed in an outpatient clinic every month in the first year, every 2 months in the second year, every 3 months in the third year, every 6 months in the fourth to fifth year, and annually thereafter until 10 years after treatment. When recurrence was suspected, a biopsy was taken for confirmation whenever possible. Loco-regional recurrence was defined as disease recurring in the pelvis in the CCRT-group or in the pelvis or para-aortic area in the EFRT-group. Recurrences were defined as distant when disease occurred outside the pelvis in the CCRT-group or outside the pelvis excluding the PALN area in the EFRT-group. The median duration of follow-up was 48 months (range: 13–120 months).

Statistical analysis

The differences between groups with respect to clinical stage, histology, parametrial involvement, deep stromal invasion, and surgical margin status were assessed using Fisher's exact test. Age, tumor diameter, and the duration of radiotherapy were analyzed using Wilcoxon's exact test. Pretreatment hemoglobin levels were compared using the Student's t test. Treatment related toxicities and the recurrence rate were compared using Fisher's exact test. The survival analysis was based on the Kaplan–Meier method, and the results were compared using the log-rank test. PFS was defined as the time from the primary diagnosis to the detection of recurrence. OS was defined as the time from the primary diagnosis to death or the latest observation. P-values of <0.05 were considered statistically significant.

Results

Patient characteristics

The characteristics of the 91 patients included in the study are shown in Table 1. Of these, 55 patients displayed multiple pelvic lymph node metastases and were treated with either pelvic radiotherapy plus concurrent chemotherapy (CCRT-group) or EFRT (EFRT-group) postoperatively. Thirty-six patients displayed single pelvic lymph node metastases and received pelvic radiotherapy plus concurrent chemotherapy after surgery (N1-CCRT-group).

The characteristics of the patients in the CCRT- and EFRT-groups are shown in Table 2. Among a total of 55 patients, 26 were treated with EFRT, and 29 were treated with pelvic radiotherapy plus concurrent chemotherapy.

In the CCRT-group, the mean age of the patients was 44 years. Twenty-five patients had SCC, and 4 had non-SCC histology (3 adenocarcinomas and 1 small cell carcinoma). Eight patients displayed parametrial involvement (Stage IIb), and 3 patients demonstrated vaginal invasion close to the surgical margin and were treated with ICRT after pelvic radiotherapy.

Table 1
Patients included in this study.

		CCRT-group	EFRT-group	N1-CCRT-group
Number of patients		29	26	36
Number of positive pelvic nodes	(mean)	3.3	5.0	1
Age	(mean)	46.1	49	49.8
Stage[a]	Ia2–IIa	21	15	21
	IIb	8	11	15
Histology	SCC	25	18	30
	Non-SCC	4	8	6

CCRT, concurrent chemoradiotherapy; EFRT, extended-field radiotherapy.
[a] Pathological stage.

In the EFRT-group, the mean age of the patients was 49 years. Eighteen patients had SCC histology, and 8 had non-SCC histology (8 adenocarcinomas). Eleven patients displayed parametrial involvement (Stage IIb). Two patients demonstrated vaginal invasion close to the surgical margin and received ICRT after EFRT.

There were no statistically significant differences in terms of age, clinical stage, histological distribution, tumor diameter, margin status, pretreatment hemoglobin level, or number of positive lymph nodes between the groups. However, as shown in Table 2, the duration of radiotherapy was significantly longer in the EFRT-group (42 days) than in the CCRT-group (34 days).

Survival outcome

At the time of this report, 8 patients in the CCRT-group (27.6%) and 16 patients in the EFRT-group (61.5%) had died of their disease. When the CCRT-group was compared with the EFRT-group, as shown in Fig. 1 and Table 3, CCRT was significantly superior in terms of recurrence rate ($p=0.0306$), PFS (log-rank; $p=0.0236$), and OS (log-rank; $p=0.0279$). When the patients in the CCRT-group were compared with those in the N1-CCRT-group (Fig. 2 and Table 4), there was no significant difference in recurrence rate ($p=1.0000$), PFS (log-rank; $p=0.9967$), or OS (log-rank; $p=0.7990$). In contrast, when the patients in the EFRT-group were compared with those in the N1-CCRT-group, there were significant differences in recurrence rate ($p=0.0197$), PFS (log-rank; $p=0.0194$), and OS (log-rank; $p=0.0351$).

Table 2
Patient characteristics.

		CCRT	EFRT	P-value
Number of patients		29	26	
Age	(mean)	46.1	49	0.3402
Stage[a]	Ia2–IIa	21	15	0.2730
	IIb	8	11	
Histology	SCC	25	18	0.1924
	Non-SCC	4	8	
Maximal tumor diameter[b]	Median (mm)	35	40	0.3334
Number of positive pelvic nodes	(mean)	3.3	5.0	0.0809
Common iliac nodes involvement	Yes	2	6	0.1306
	No	27	20	
Positive margins	Yes	3	2	1.0000
	No	26	24	
Stromal invasion	Less than one-half	4	4	1.0000
	More than one-half	25	22	
Pretreatment hemoglobin level[c]	Mean (mg/dl)	12.25	12.29	0.8988

CCRT, concurrent chemoradiotherapy; EFRT, extended-field radiotherapy; SCC, squamous cell carcinoma.
[a] Pathological stage.
[b] The maximal tumor diameter was measured three-dimensionally based on T2-weighted images. The longest diameter was considered valid as the maximal tumor diameter.
[c] Hemoglobin level just before the start of adjuvant radiotherapy.

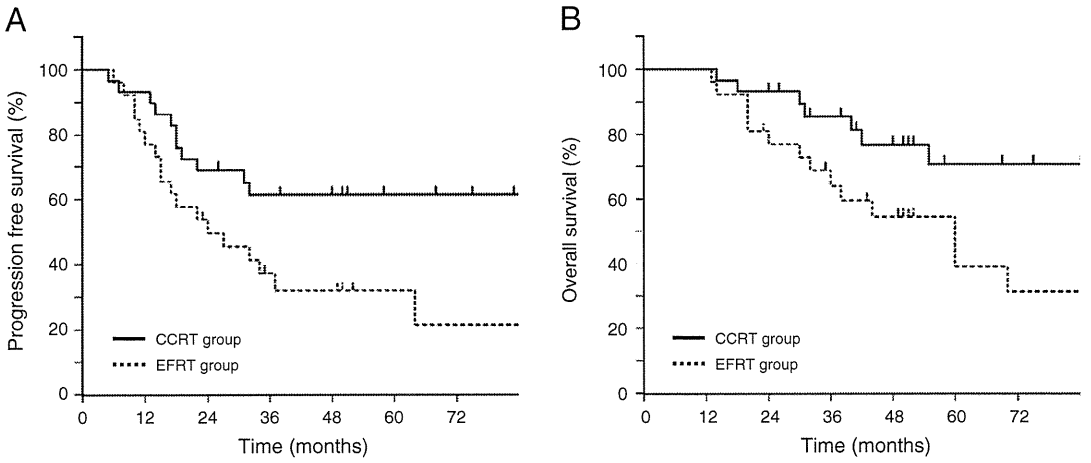

Fig. 1. A: Progression free survival among patients in the CCRT- and EFRT-groups. The progression free survival rate was significantly higher among the patients in the CCRT-group ($p=0.0236$). B: Overall survival in the CCRT- and EFRT-groups. The overall survival rate was significantly higher among the patients in the CCRT-group ($p=0.0279$).

When analyzed according to histology, CCRT was significantly superior to EFRT in terms of PFS (log-rank; $p=0.0462$) and OS (log-rank; $p=0.0349$) in the SCC-group. However, in the patients with non-SCC histology, no significant difference in recurrence rate, PFS, or OS was observed between the two treatment groups.

Pattern of recurrence

Treatment failure was observed in 11 patients (37.9%) in the CCRT-group and 18 patients (69.2%) in the EFRT-group. In the CCRT-group, one patient developed recurrence inside the irradiated field, 6 developed recurrence outside the irradiated field, and 4 developed recurrence both inside and outside of the irradiated field. In the EFRT-group, 6 patients developed recurrence in the irradiated field, 10 developed recurrence outside the irradiated field, and 2 developed recurrence both inside and outside of the irradiated field. The median interval from surgery to recurrence was 17.5 months (range: 5–32 months) in the CCRT-group and 16 months (range: 6–64 months) in the EFRT-group. The use of pelvic radiotherapy plus concurrent chemotherapy resulted in fewer relapses inside the irradiated field than EFRT; however, the pattern of recurrence did not differ statistically between the two treatment groups ($p=0.1680$).

Of a total of 11 patients who developed recurrences in the CCRT-group, 6 (54.5%) developed recurrences in PALN area. In contrast, of a total of 18 patients with recurrences in the EFRT-group, recurrences in PALN area were observed in 5 patients (27.7%).

When examined according to histology, a distinctive pattern of recurrence was observed in the non-SCC group. Two out of 8 recurrences in the EFRT-group and 2 out of 4 recurrences in the CCRT-group involved peritoneal dissemination in the non-SCC-group. No peritoneal dissemination was observed in the SCC-group.

Table 3
Treatment outcome.

		CCRT	EFRT	P-value
Number of patients		29	26	
Duration of radiotherapy	Median (days)	34	42	<0.0001
Number of patients with recurrence	(%)	11 (37.9)	18 (69.2)	0.0306
PFS	Median (months)	38.0	23.5	0.0236
OS	Median (months)	51.0	40.5	0.0279
Number of patients with grade 3–4 acute toxicity	(%)	2 (6.9)	3 (11.5)	0.6586
Number of patients with grade 3–4 late toxicity	(%)	2 (6.9)	3 (11.5)	0.6586

CCRT, concurrent chemoradiotherapy; EFRT, extended-field radiotherapy; PFS, progression free survival; OS, overall survival.

Adverse effects

Generally, both pelvic radiotherapy plus concurrent nedaplatin-based chemotherapy and EFRT were well tolerated. All patients completed the planned external beam radiotherapy, and there were no treatment-related deaths. Among a total of 29 patients in the CCRT-group, although grade 1–2 acute toxicities were commonly observed, only two (6.9%) patients had grade 3 or 4 acute toxicities (Table 3): one patient had neutropenia, and the other had thrombocytopenia. Among a total of 26 patients who were treated with EFRT, three patients (11.5%) had grade 3 or 4 acute toxicities, all of which were neutropenia. No patient developed grade 3–4 non-hematologic toxicities. All cases were manageable by conservative treatment.

Grade 3–4 severe late toxicities were observed in three patients in the EFRT-group (bowel obstruction in two patients and radiation dermatitis in one patient). In the CCRT-group, two patients developed grade 3–4 severe late toxicities, both of which were bowel obstruction. All cases were manageable by conservative treatment. Although the absolute number of acute or late toxicities was lower in the CCRT-group, the difference did not reach statistical significance.

Discussion

Although nodal status is not included in the International Federation of Gynecology and Obstetrics (FIGO) staging criteria, nodal metastasis remains the single most important prognostic factor in early-stage cervical cancer [8]. It was reported that failure to control disease in early-stage cervical cancer is primarily due to an inability to sterilize tumor-containing lymph nodes, and central failure was only responsible for 6% or less of the cases [22].

In general, lymphatic metastasis in cervical cancer proceeds in a stepwise and predictable fashion from the lower pelvis to the upper pelvis including the common iliac nodes, followed by the para-aortic nodes (PALN). Although cervical cancer can reach the common iliac and para-aortic nodes directly via the posterior cervical trunk, this pattern of spread is uncommon [9,23,24].

It is generally accepted that patients with multiple pelvic lymph node involvement carry a risk of para-aortic node metastasis. In addition, a previous surgical staging study conducted by the Gynecologic Oncology Group (GOG) demonstrated a clear correlation between the incidence of para-aortic nodal metastasis and advancing

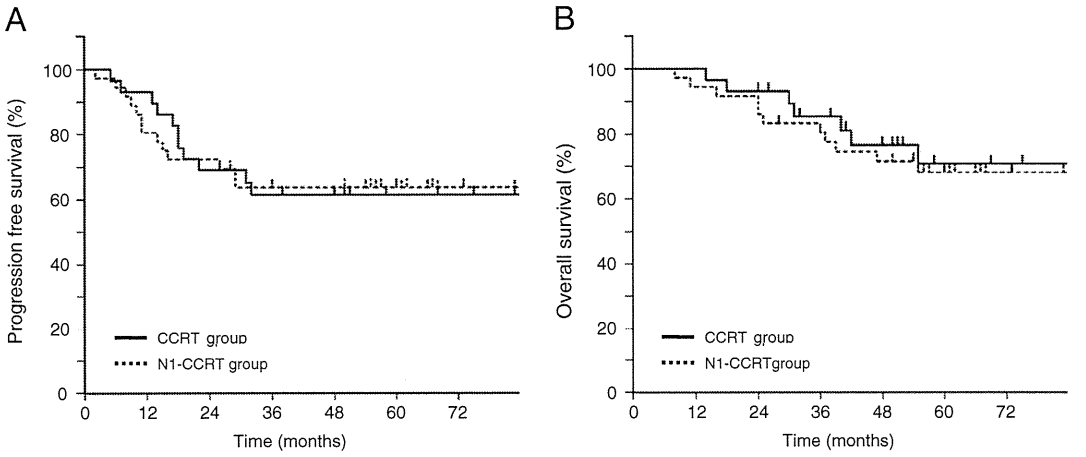

Fig. 2. A: Progression free survival among patients in the N1-CCRT- and CCRT-groups. The progression free survival rate in the CCRT-group was similar to that in the N1-CCRT-group ($p = 0.9967$). B: Overall survival of high-risk patients. The overall survival rate in the CCRT-group was similar to that in the N1-CCRT-group ($p = 0.7990$).

tumor stage. In this report, 5% of stage I, 17% of stage II, and 25% stage III patients demonstrated biopsy-proven para-aortic metastasis [25]. On the basis of a randomized controlled study [11], the patients at high risk of para-aortic node metastasis have traditionally been treated with prophylactic EFRT after radical surgery in our institution [17]. Although a previous randomized controlled study demonstrated the superiority of pelvic radiotherapy plus concurrent chemotherapy over EFRT in patients with early-stage cervical cancer in the setting of definitive radiotherapy for cervical cancer [12] and pelvic radiotherapy plus concurrent chemotherapy has become a standard treatment for this patient population, no direct comparison of postoperative pelvic radiotherapy plus concurrent chemotherapy and EFRT has been made.

Although our study is retrospective, our results suggested that postoperative pelvic radiotherapy plus concurrent chemotherapy is superior to EFRT with regard to recurrence rate, PFS, and OS for treating early-stage cervical cancer patients with multiple lymph node metastases. Moreover, pelvic radiotherapy plus concurrent chemotherapy resulted in improved outcomes without increasing the incidence of serious side effects. The estimated 5-year overall survival rate of 71% in the CCRT-group in the current study is comparable to the 5-year overall survival rate of 75% observed in the previous randomized clinical trial of cisplatin-based concurrent chemoradiotherapy [13,26].

We employed nedaplatin as a radio-sensitizing agent. Nedaplatin (*cis*-diammine-glycoplatinum), a derivative of cisplatin, was developed in 1983 by Shionogi Pharmaceutical Company with the aim of producing a treatment with a similar effectiveness to cisplatin but decreased renal and gastrointestinal toxicities [27]. In preclinical evaluations of cervical cancer, nedaplatin demonstrated similar antitumor activity to cisplatin [28,29]. Its lower incidence of nephrotoxicity in comparison to cisplatin has been demonstrated to be associated with differences in the kidney distributions of these drugs. When the two agents were administered at the same dose, the amount of nedaplatin that accumulated in the rat kidney was approximately 40% of that of cisplatin, which explains why nedaplatin is associated with less nephrotoxicity than cisplatin [30,31].

The radio-sensitizing properties of nedaplatin in the setting of postoperative adjuvant radiotherapy have been evaluated in one Phase I [32] and two retrospective studies [16,19], in which the authors recommended weekly 35–40 mg/m^2 nedaplatin or biweekly 70 mg/m^2 nedaplatin. In the current study, of a total of 29 patients that were treated with pelvic radiotherapy plus concurrent chemotherapy, 19 received biweekly nedaplatin and 10 received weekly nedaplatin during the course of pelvic radiotherapy. Recurrences were observed in 7 patients (37%) who had received biweekly nedaplatin and in 4 patients (40%) who had received weekly nedaplatin, indicating that treatment failure was not influenced by the concurrent chemotherapy treatment schedule employed in the current study.

As shown in Fig. 2 and Table 4, when the patients were treated with pelvic radiotherapy plus concurrent chemotherapy, there was no significant difference in PFS or OS between the patients with multiple lymph node metastases and those with single node metastases, indicating that the addition of concurrent nedaplatin to pelvic radiotherapy abolished the adverse prognostic impact of multiple lymph node metastases. Our finding is consistent with those of a previous report that demonstrated the effect of the addition of concurrent chemotherapy to pelvic radiotherapy is more profound in patients with multiple lymph node metastases than in patients with single node metastases [26].

Although postoperative EFRT resulted in fewer PALN recurrences than pelvic radiotherapy plus concurrent chemotherapy, it failed to improve the overall recurrence rate and the survival outcome in this patient population. These results may indicate the potential activity of concurrent chemotherapy to control occult systemic metastasis.

The rate of severe late complications in the EFRT-group in our study (11.5%) was similar to those found in previous series [11]. Although not statistically significant, the rate of severe late complications in the EFRT-group (11.5%) was higher than that observed in the CCRT-group (6.9%). As it is well tolerated by patients and displays

Table 4
The activity of postoperative CCRT according to the number of pelvic node metastases.

			CCRT	N1-CCRT	P-value
Patient characteristics	Number of patients		29	36	
	Age	(mean)	46.1	49.8	0.2144
	Stage[a]	Ia2–IIa	21	21	0.3007
		IIb	8	15	
	Histology	SCC	25	30	1.0000
		Non-SCC	4	6	
Treatment outcome	Patients with recurrence	(%)	11 (37.9)	13 (36.1)	1.0000
	PFS	Median (months)	38.0	54.5	0.9967
	OS	Median (months)	51.0	56.5	0.7990

CCRT, concurrent chemoradiotherapy.
[a] Pathological stage.

significant activity, we believe that pelvic radiotherapy plus concurrent nedaplatin-based chemotherapy is a reasonable treatment for this patient population.

We have to recognize the limitations of our study. One is the relatively small sample size. Moreover, due to the retrospective nature of this study, potential biases may have influenced the results, such as the heterogeneity of the patient population and the considerable selection bias exercised by physicians in determining which patients should be considered for pelvic radiotherapy plus concurrent chemotherapy. In addition, the educational level and/or the socio-economical status of the patients might also have affected treatment selection. These factors can only be eliminated in a prospective randomized controlled study.

Although nedaplatin-based CCRT was well tolerated, 7% of patients experienced severe acute or late complications. Thus, further efforts need to be made to reduce severe complications. One strategy is to use intensity-modulated radiation therapy (IMRT) to achieve more conformal dose distributions. According to several retrospective studies, IMRT shows a reduced incidence of acute toxicities compared with conventional techniques in patients with gynecological malignancies including uterine cervical cancer [33]. Thus, to confirm the benefit of IMRT, a clinical trial of pelvic IMRT combined with concurrent weekly nedaplatin in the setting of adjuvant therapy for cervical cancer needs to be conducted in the future.

Although the pelvic radiotherapy plus concurrent nedaplatin-based-chemotherapy resulted in improved survival, a significant number of patients still suffered recurrences and died of their disease. Of the 5 patients in the CCRT-group that developed recurrences inside the irradiated field, only 1 patient developed recurrence at the vaginal apex, indicating that the routine addition of ICBT to the upper vagina only benefits a small proportion of patients. Therefore, to further improve the prognosis of this patient group, novel treatment strategies need to be investigated.

One strategy that might improve the patient outcome is the use of EFRT combined with concurrent chemotherapy. The activity and feasibility of EFRT combined with concurrent chemotherapy has been investigated in several phase I/II clinical studies in cervical cancer patients that were positive or at high risk for PALN metastasis. According to previous clinical trials in which concurrent weekly cisplatin was employed as a radiosensitizer, the reported incidence of acute grade 3–4 gastrointestinal or hematologic toxicities was 10–80% [34–36]. Similarly, in a phase II clinical trial of EFRT plus concurrent nedaplatin, roughly 40% of patients experienced grade 3–4 gastrointestinal or hematologic toxicities [37]. These results indicate the limitation of performing EFRT plus concurrent chemotherapy using conventional radiation techniques. However, recently, preliminary data from small clinical studies suggested a preferable toxicity profile of extended-field IMRT with concurrent cisplatin in patients with cervical cancer [38]. Thus, the activity and feasibility of extended-field IMRT with concurrent cisplatin or nedaplatin should be further investigated in prospective settings. In addition, novel treatments such as the use of new cytotoxic agents in concurrent chemotherapy, the co-administration of nedaplatin with molecularly targeted agents, or the addition of consolidation chemotherapy after postoperative pelvic radiotherapy plus concurrent chemotherapy should also be investigated in future clinical trials.

Conflict of interest statement
The authors declare that they have no conflicts of interest.

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Dissection of Unsuspicious Para-aortic Lymph Nodes Does Not Improve Prognosis of Advanced Endometrial Carcinoma with Intra- or Extra-abdominal Metastasis

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Abstract. *Background: The aim of this study was to analyze the significance of dissection of unsuspicious para-aortic lymph nodes (PAN) in patients with advanced endometrial carcinomas with intra- or extra-abdominal metastasis. Patients and Methods: We conducted a retrospective comparison of the results of PAN dissection versus non-dissection for endometrial carcinomas with macroscopic metastatic lesions beyond the uterus (without significant swelling of the regional lymph nodes, including PAN), whose lesions were completely resected. Results: Disease-free survival and overall survival did not exhibit a significant difference between the two groups. Multivariate Cox proportional hazards analysis demonstrated that PAN dissection was not an independent prognostic factor for survival. The frequency of PAN involvement at the first recurrence did not differ between the two groups. Conclusion: For advanced endometrial carcinomas with macroscopic metastatic lesions beyond the uterus, without significant swelling of regional lymph nodes, PAN dissection may be omitted without a significant adverse effect on prognosis and survival.*

Uterine endometrial carcinoma, already the most common malignancy of the female pelvis, has had an apparent increasing incidence during the last three decades in the United States (1). When this carcinoma is still confined to the uterus (stage I) it has a good prognosis; however, tumors which progress beyond the uterus confer a dramatically poorer prognosis. For endometrial carcinoma cases with intermediate or high-risk factors of recurrence, a retrospective (SEPAL) cohort study has suggested that there is a therapeutic role to be gained for a combined dissection of the pelvic lymph nodes (PLN) and para-aortic nodes (PAN) (2). However, according to a retrospective study recently conducted by our group on endometrial carcinoma cases in which postoperative adjuvant chemotherapy using combined platinum, anthracycline and taxane derivatives was performed, not only was PAN dissection not associated with a better survival, but in fact, the procedure was adversely associated with significantly increased operative time and total blood loss (Okazawa *et al.*, submitted).

For advanced endometrial carcinoma cases with intra- or extra-abdominal metastasis, a significant role for cytoreductive surgery was demonstrated (3, 4). In these studies, resection of the metastatic lesions and swollen PAN was performed with or without regard to dissection of any unswollen PAN. The survival significance of co-dissection of the unswollen PAN has, thus, remained unclear. In our present study, we performed a retrospective analysis of the survival effect of PAN dissection for advanced endometrial carcinoma cases with intra- or extra-abdominal metastasis beyond the uterus (excluding overt PLN or PAN metastasis) which received postoperative adjuvant chemotherapy using platinum and taxane (with or without anthracycline).

Patients and Methods

A retrospective comparison was conducted of the efficacy of PAN dissection for patients with endometrial carcinomas with metastatic lesions beyond the uterus (unaccompanied by significant swelling of regional lymph nodes), including PAN whose carcinoma lesions were thought to be completely resected. This study analyzed cases treated during the period 2000-2010 at the Department of Obstetrics and Gynecology of the Osaka University Hospital and the

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Key Words: Endometrial carcinoma, para-aortic lymph node dissection, adjuvant chemotherapy, prognosis.

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Department of Gynecology of the Osaka Medical Center for Cancer and Cardiovascular Diseases. Written informed consent was obtained from all patients before any treatment commenced. This study was approved by the Institutional Review Board and the Ethics Committee.

PAN dissection was performed under highly similar indications in both hospitals, including a myometrial invasion depth of >1/2 of total thickness and/or an atypical histology (such as grade 3 endometrioid adenocarcinoma, or a clear cell or serous papillary carcinoma), for endometrial carcinoma without intra- or extra-abdominal metastasis, irrespective of swelling of the PLN or PAN. In this study, not all cases with obvious macroscopic intra- or extra-abdominal metastasis lesions beyond the uterus, but without overt swelling of the PLN and PAN, received dissection of the PAN. Whether or not a PAN dissection was performed was left to the informed choice of the patient. The cases in which PLN dissection was not performed were excluded from analysis.

Metastasis to PAN was assessed with either a helical computerized tomography (CT) scan or magnetic resonance imaging (MRI). Adjuvant combination chemotherapy using taxane (paclitaxel) and platinum (carboplatin), with or without the anthracycline epirubicin, (TEC) or (TC), respectively, was performed. A regimen of TEC (150 mg/m^2 paclitaxel, 50 mg/m^2 epirubicin, and area under the curve (AUC) 4 carboplatin), based on our own phase I/II studies (5), was administered at the Osaka University Hospital every 3-4 weeks for 6 courses. For the alternative TC therapy, conducted at the Osaka Medical Center for Cancer and Cardiovascular Diseases, paclitaxel (175 mg/m^2) and carboplatin (AUC=5) were administered intravenously every 3-4 weeks for 6 courses, based on published protocols (6, 7).

The clinicopathological features of these cases, including the age of the patient, the disease histology and stage, and the number of the metastatic regions, were retrospectively reviewed utilizing their clinical records, including physical examination notes, radiological and histopathology reports, and operative records. Disease-free survival (DFS) was measured from the initial surgery to the date of the radiologic or pathologic diagnosis of recurrence, or to the date of the last follow-up, and overall survival (OS) was defined as the period from the initial surgery to the patient's disease-specific death, or to the date of the last follow-up.

Statistical analysis. MedCalc (MedCalc Software, Mariakerke, Belgium) was used for the statistical analyses. The distribution of patients' ages was analyzed by the Mann-Whitney *U*-test. The distribution of tumor histology and stage, number of metastatic regions and the frequency of PAN involvement at the first recurrence were all analyzed by the Fisher's exact test. DFS and OS curves were constructed using the Kaplan-Meier method and were evaluated for statistical significance by the log-rank test. Results were considered to be significant when the *p*-value was less than 0.05.

Results

Clinical characteristics of the cases in which PAN dissection was or was not performed. At the Osaka University Hospital and the Osaka Medical Center for Cancer and Cardiovascular Diseases, 34 endometrial carcinomas with macroscopic metastases beyond the uterus, but having no significant swelling of regional PLN or PAN, were reported to have been completely resected during the study period. Among them, systemic PAN dissection was performed in 19 cases. Distributions of age, histology, stage and number of metastatic regions were not significantly different between the cases in which PAN dissection was performed (PAN+ group) and those in which PAN dissection was not performed (PAN– group) (Table I).

Table I. *Characteristics of patients treated with or without para-aortic lymph nodes (PAN) dissection. Background characteristics of the two groups were not significantly different.*

Characteristic	PAN Dissection +	PAN Dissection –	*p*-Value
Number of cases	19	15	-
Median age (years)	59 (34-76)	56 (50-71)	0.48
Histology			0.17
Endometrioid	11	12	
Non-endometrioid	8	3	
Stage			0.56
III	7	7	
IV	12	8	
Number of metastatic regions			0.37
1	6	7	
≥2	13	8	

PAN dissection +: the patients for whom PAN dissection was performed; PAN dissection –: the patients for whom PAN dissection was not performed.

TEC therapy was performed in 10 and TC treatment in 9 out of the 19 cases whose PAN was dissected, and in 7 and 8, respectively, out of the 15 cases whose PAN was not dissected. These proportions for regimens of adjuvant chemotherapy were not significantly different.

Diagnostic significance of PAN dissection. PAN metastasis was histologically diagnosed in 2 out of the 19 cases whose PAN had been found to be unswollen by helical CT or MRI. PLN metastasis was also detected in these cases. Moreover, these two cases were diagnosed as stage IVb due to upper-abdominal metastasis. There was no case in which the stage of the disease was changed after surgery.

Survival effect of PAN dissection. The DFS and OS curves of the PAN+ and PAN– dissection groups are shown in Figure 1. The median follow-up period for the two groups was 24 months (range 3-63 months) and 43 months (7-61 months), respectively. DFS and OS did not exhibit a statistically significant difference between the PAN+ and – groups (p=0.13 by the log-rank test, hazard ratio (HR)=1.959; 95% confidence interval (CI)=0.774-4.965; and p=0.17 by the log-rank test, HR=2.430; 95% CI=0.201-8.396, respectively).

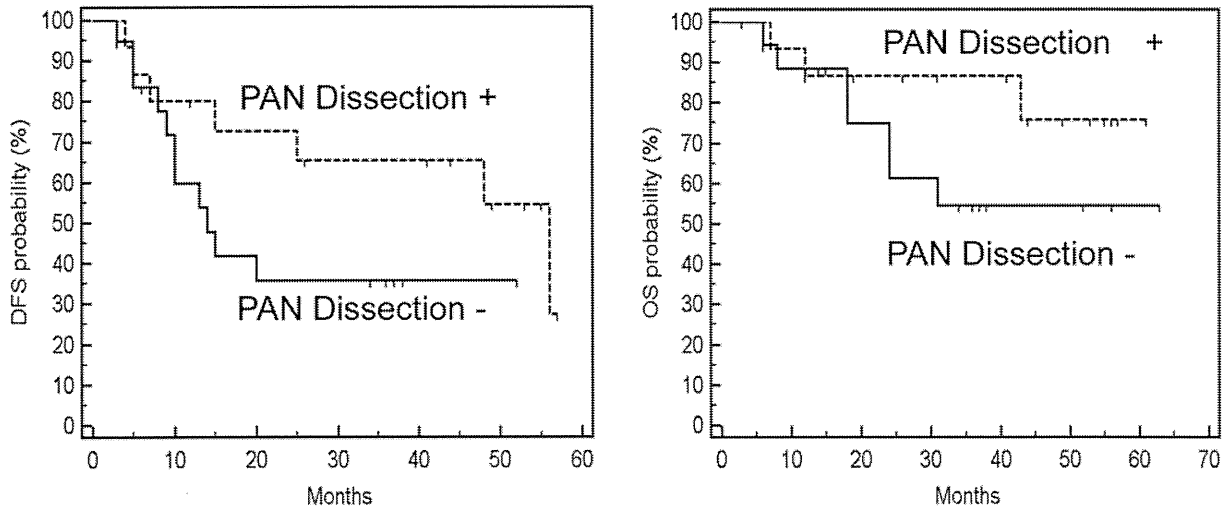

Figure 1. *Disease-free survival (DFS) and overall survival (OS) in the patients treated with or without PAN dissection. Neither DFS nor OS demonstrated significant differences between the patients treated with or without PAN dissection (p=0.13 and p=0.17, respectively, by the log-rank test).*

Multivariate analysis of clinical factors for survival effect. We utilized the multivariate Cox proportional hazards model in order to find evidence to further support our interpretation of these results, namely that the dissection of unswollen PAN does not improve survival of patients with advanced endometrial carcinoma with intra- or extra-abdominal metastasis. We found that the number of metastatic regions was an independent factor for DFS (Table II). The adjusted HR for the number of metastatic regions (1 *versus* ≥2, *i.e.*, single *versus* multiple regions) was 3.633 (95% CI=1.126-11.716; p=0.032) for DFS. The univariate Cox proportional hazards analysis revealed that the DFS probability of cases with a single metastatic region was significantly better than that of cases with multiple metastatic regions (HR=3.506, 95% CI=1.392-8.833; p=0.016) (Figure 2). On the other hand, PAN dissection was shown not to be a significantly independent factor for either DFS or OS (Tables II and Table III).

Frequency of the first recurrence to the PAN. The frequency of PAN involvement at the first recurrence of the tumor was compared between the two treatment groups. The first recurrence at PAN was detected in 2 out of 19 PAN dissection + cases, and in 2 of the 15 PAN dissection – cases. A statistically significant difference was not detected (p=0.80 by Fisher's exact test). Moreover, in one of the two recurrent cases at PAN, pathological diagnosis of PAN metastasis had been made at the initial PAN dissection.

Discussion

For endometrial carcinoma, the therapeutic significance of PAN dissection has been an issue of great debate. In the early stages of endometrial carcinoma, several randomized studies have indicated that systematic dissection of the PLN has little therapeutic value (8, 9). However, for endometrial carcinoma cases with intermediate and high risk factors of recurrence, a retrospective SEPAL cohort study indicated there was a possible therapeutic role for a combined dissection of PLN and PAN (2). However, the adjuvant therapy was not standardized in this study; roughly one-fifth of the cases did not receive an adjuvant therapy, another fifth underwent radiation, and the others had a cisplatin-based chemotherapy.

The combination adjuvant chemotherapy of platinum, taxane and anthracycline has recently come to be regarded as the gold standard for treatment of advanced or recurrent endometrial carcinomas (10-12). Our own previous study showed that PAN dissection was not associated with any better survival in endometrial carcinoma cases in which a postoperative adjuvant chemotherapy using platinum, anthracycline and a taxane derivative was performed. On the contrary, PAN dissection was associated with adverse effects that included increases in both operative times and total blood loss (Okazawa *et al.*, submitted). Still, for more advanced cases of endometrial carcinoma, those with intra- or extra-abdominal metastasis, the significant role of cytoreductive surgery of any visible lesions for increased survivability has been clearly demonstrated (3, 4).

Table II. *Multivariate Cox proportional hazards analysis for disease-free survival (DFS). The adjusted hazard ratio (HR) for the number of metastatic regions being ≥2 was significantly higher than that for the number of metastatic regions being <2 (adjusted HR=3.633, 95% CI=1.126-11.716, p=0.032).*

Variable	Adjusted HR	95% CI	*p*-Value
Age (years)			0.42
<60	1		
≥60	1.551	0.532-4.526	
Histology			0.85
Endometrioid	1		
Non-endometrioid	1.106	0.382-3.203	
Stage			0.71
III	1		
IV	0.713	0.255-1.994	
Number of metastatic resions			0.032
1	1		
≥2	3.633	1.126-11.716	
PAN Dissection			0.29
+	1		
–	1.818	0.604-5.470	

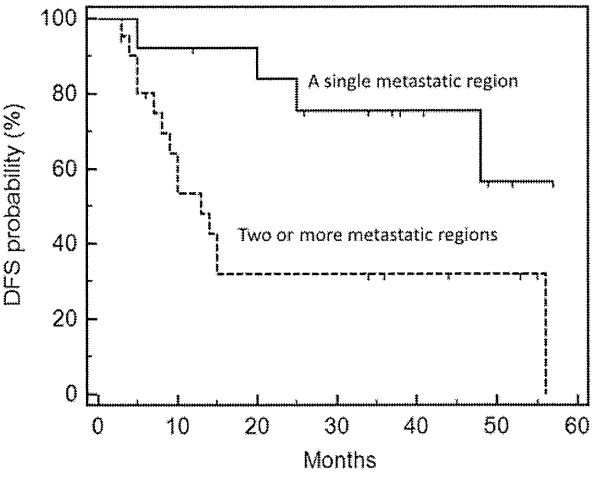

Figure 2. *Disease-free survival (DFS) by the number of metastatic regions. DFS of the patients with two or more metastatic regions was significantly worse than that of the cases with a single metastatic region (p=0.016 by the log-rank test).*

Table III. *Multivariate Cox proportional hazards analysis for overall survival (OS). None of the variables investigated exhibited independent prognostic significance for OS.*

Variable	Adjusted HR	95% CI	*p*-Value
Age (years)			0.63
<60	1		
≥60	1.398	0.366-5.348	
Histology			0.86
Endometrioid	1		
Non-endometrioid	1.132	0.297-4.317	
Stage			0.48
III	1		
IV	0.623	0.166-2.336	
Number of metastatic regions			0.19
1	1		
≥ 2	3.006	0.590-15.306	
PAN Dissection			0.34
+	1		
–	2.049	0.473-8.7311	

HR: Hazard ratio.

Based on these conflicting findings, the prognostic and therapeutic significance of dissection of unswollen PAN for advanced endometrial carcinoma cases with intra- or extra-abdominal metastasis is of great interest to us. First, diagnostic significance was analyzed. In cases in which PAN dissection was performed, there were two cases in which PAN metastasis was pathologically diagnosed. However, in these cases, metastases of the PLN and upper abdomen were also detected and up-staging due to PAN metastasis was not required after PAN dissection. These results imply that diagnostic value of PAN dissection may not be significant. Next, a retrospective comparison of advanced endometrial cases which progressed beyond the uterus (without regional lymph nodes swelling) was conducted. Whether or not a PAN dissection was performed was left to the informed choice of the patients. Those cases whose malignancies were obviously not completely removed by surgery were excluded from the analysis. The clinical characteristics of the patients who received PAN dissection and those who did not receive PAN dissection are shown in Table I. All the patients underwent postoperative adjuvant with either TEC or TC therapy. The proportions of these two regimens were not different significantly in the two groups.

The therapeutic effect of PAN dissection was analyzed. The DFS and OS of the PAN+ group tended to be better than those of the PAN– group, but this improvement did not rise to the level of statistical significance (Figure 1). The multivariate Cox proportional hazards model demonstrated that the number of metastatic regions was an independent factor for DFS; however, PAN dissection was not shown to be a significantly independent factor for either DFS or OS (Tables II and III). These results show that the status of progression of the disease was more important than PAN dissection, implying that dissection of unswollen PAN does not improve survival of patients with advanced disease with macroscopic intra- or extra-abdominal metastasis without regional lymph node swelling.

It might be argued that dissection of even unswollen PAN may be helpful for accurate surgical staging. However, in our study, a PAN metastasis was histologically diagnosed in only

2 out of the 19 cases whose PAN had been determined to be unswollen by helical CT or MRI, and in these cases, due to co-metastasis to other distant sites, the stage of the disease was not further upgraded. Moreover, we found that the frequency of PAN involvement at the first recurrence of the tumor was similar in the PAN+ and PAN– groups. These results may imply that PAN dissection at the initial surgery does not prevent the first recurrence of PAN in these cases.

In this retrospective study, we show that PAN dissection was not an independent prognostic factor for advanced endometrial carcinoma with macroscopic intra- or extra-abdominal metastasis without regional lymph node swelling, in which adjuvant combination TEC or TC chemotherapy was used. These results imply that routine PAN dissection may be objectively omitted for advanced endometrial cases which have progressed beyond the uterus without regional lymph node swelling. Further prospective studies to analyze the significance of PAN dissection are still required.

Conflict of Interest Statement

None of the Authors has any conflict of interest to declare.

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