manner in both cell lines (Fig. 1C and S1B). In contrast, the number of annexin V-positive cells did not change, even at the higher doses of cisplatin. The cleavage of caspase-3 and poly-ADP-ribose polymerase (PARP) was observed in SKOV-3 cells at 24 h but not at 3 h (Fig. 1D). Thus, cisplatin impaired the viability of SKOV-3 and OVCAR-3 cells in a dose-dependent manner, although it was impossible to detect the change within a short period of time after cisplatin treatment by conventional methods.

Early decreased glucose uptake after cisplatin treatment correlated with later cell death. To predict later cell death at an early time point after cisplatin treatment, we examined glucose uptake by the cells. Flow cytometry was performed to monitor glucose uptake by detecting the uptake of 2-NBDG fluorescence within the cells. Simultaneous PI staining was performed to exclude dead cells from the assay. At 3 h after treatment with 20 μg/mL cisplatin, the uptake of 2-NBDG in SKOV-3 and OVCAR-3 cells was significantly decreased (Fig. 2A–C). The SKOV-3 cells required a higher dose of cisplatin than the OVCAR-3 cells to achieve a decrease in glucose uptake (Fig. 2C) parallel to the relative resistance of SKOV-3 cells against cisplatin demonstrated by the cell count and MTS assay (Fig. 1A). The drastic decrease in glucose uptake by OVCAR-3 cells at 24 h (Fig. 2C) might reflect the severe impairment of cell function before cell death (Fig. S1B). Thus, the early decrease in glucose uptake at a high dose of cisplatin correlated with the sensitivity to the drug in ovarian cancer cell lines. In contrast, the change in the glucose concentration of the culture medium was undetectable 3 h after cisplatin treatment for both cell lines (Fig. 2D). The results indicate that the 2-NBDG method is useful for detecting the subtle change in cellular glucose uptake.

Membrane localization of GLUT1 was attenuated at an early time point after cisplatin treatment. To investigate the mechanism of decreased glucose uptake at an early time point after cisplatin treatment, we examined the expression of mRNA encoded by glucose transporter and glycolysis emzymes. The level of GLUT1 expression was decreased 3 h after 20 μg/mL cisplatin treatment of OVCAR-3 cells but not of SKOV-3 (Fig. 3A,B). The level of GLUT1 expression was decreased 3 h after 20 μg/mL cisplatin treatment of OVCAR-3 cells but not of SKOV-3 (Fig. 3A,B). The GLUT3 gene was not expressed in these cell lines (data not shown). The expression levels of the glycolytic enzymes, phosphofluctokinase-1 (PFK), and phosphoglycerate kinase (PGK), were also reduced in OVCAR-3 cells but not in SKOV-3 cells (Fig. 3A,B). Thus, the expression of genes related to glucose uptake and glycolysis decreased at an early time point after cisplatin treatment only in OVCAR-3 cells but not in SKOV-3 cells. Next, we examined the protein levels of GLUT1 by western blotting. The protein levels of GLUT 1 of neither SKOV-3 nor OVCAR-3 cells changed at 3 h after 20 μg/mL cisplatin treatment (Fig. 3C). Immunocytochemistry for GLUT1 revealed a strong signal localized at the cytoplasmic membrane in both cell lines. After 3 h cisplatin treatment, the membrane localization of GLUT1 was attenuated (Fig. 3D).

OVCAR-3 cell oxygen consumption decreased 3 h after cisplatin treatment but mitochondrial membrane potential was not impaired. To investigate the contribution of mitochondrial respiration to the reduction in glucose uptake, we measured the oxygen consumption of OVCAR-3 cells 3 h after treatment with cisplatin. Oxygen consumption was decreased, even after treatment with 5 μg/mL cisplatin (Fig. 4A). On the other hand, the MTS assay, which reflects the mitochondrial metabolic activity, did not show significant changes at 3 h (Fig. 1B), and the mitochondrial membrane potential at that time point was not decreased with 20 μg/mL cisplatin (Fig. 4B). Taken together, the results indicate that the reduction in oxygen consumption after cisplatin treatment is not likely due to direct mitochondrial impairment, but is a consequence of reduced uptake of glucose, which eventually becomes a substrate of mitochondrial respiration.

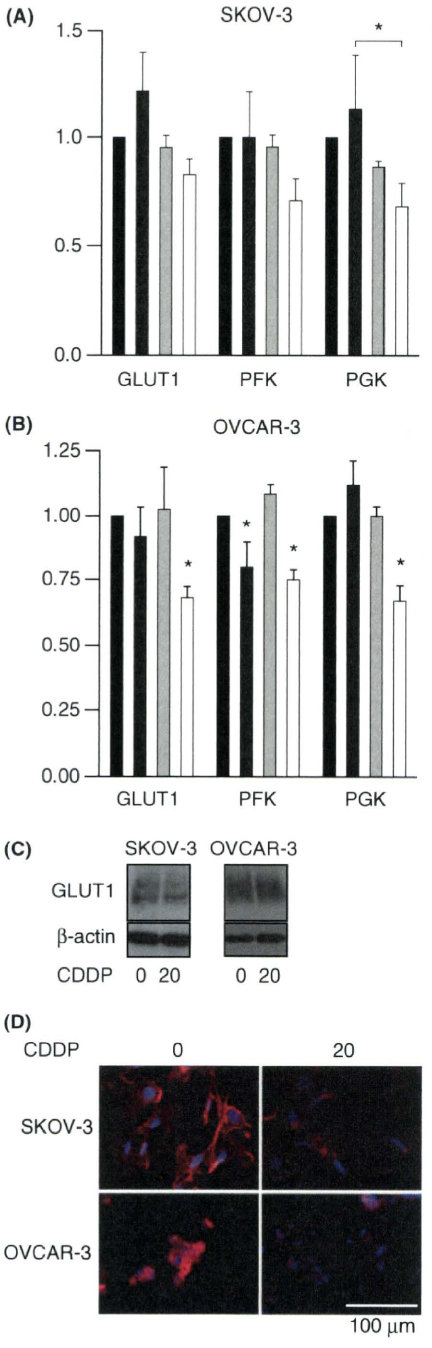

Fig. 3. (A,B) Real-time RT-PCR of genes related to energy metabolism. (A) SKOV-3 cells or (B) OVCAR-3 cells were treated with cisplatin for 3 h. The doses of cisplatin were 0, 5, 10 and 20 μg/mL from left to right. The results are the normalized mean values for three replicates from three independent experiments. $*P < 0.05$ compared with the control unless otherwise indicated. (C) Western blotting for GLUT1 of SKOV-3 and OVCAR-3 cells treated with 20 μg/mL cisplatin for 3 h. (D) Cells were treated with 20 μg/mL cisplatin or water for 3 h and subjected to immunocytochemistry for GLUT-1 (red). Hoechst 33342 staining (blue) indicates the nucleus. CDDP, *cis*-diamminedichloroplatinum(II).

doi: 10.1111/j.1349-7006.2010.01670.x

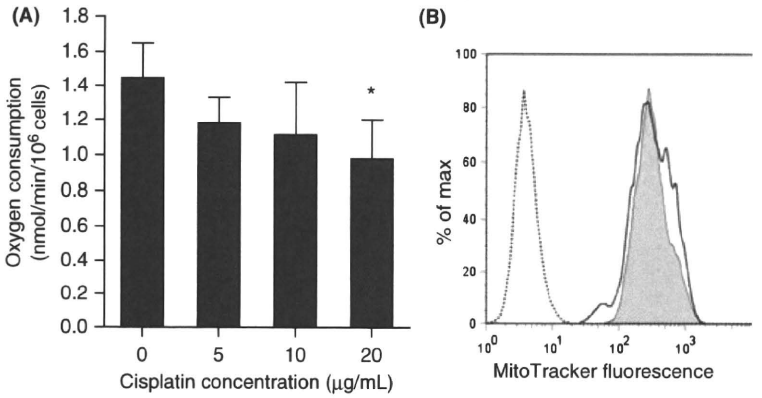

Fig. 4. (A) Oxygen consumption of OVCAR-3 cells after treatment with cisplatin at the indicated doses for 3 h. The results are from three independent replicates. The experiments were repeated three times, and representative results are shown. *$P < 0.05$ compared with the control. (B) Histogram of mitochondrial membrane potential assessed by MitoTracker 3 h after treatment with 20 μg/mL cisplatin. The *y*-axis represents the percentage of maximum cell number counts (Max); the *x*-axis represents the mean MitoTracker fluorescence intensity. The dotted line indicates untreated cells. Gray shading indicates untreated cells with the MitoTracker, and the solid line indicates cisplatin (20 μg/mL)-treated cells with the MitoTracker.

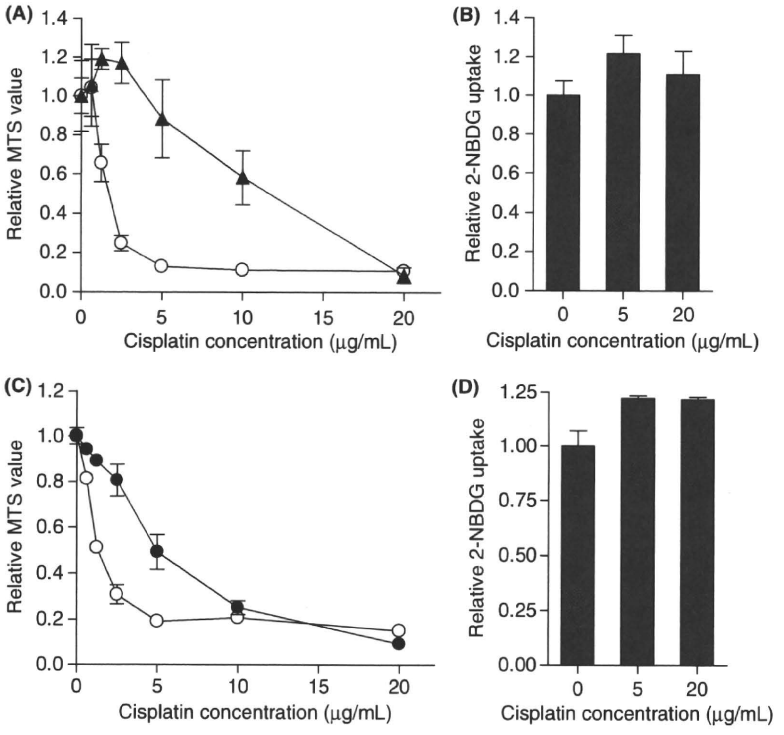

Fig. 5. (A) The MTS values relative to untreated control 48 h after treatment with cisplatin at the indicated doses. Sawano cells are indicated by black triangles. The results from the OVCAR-3 cells are the control indicated by open circles. (B) Sawano cells were treated with cisplatin at the indicated doses and the values of 2-NBDG uptake relative to the untreated group 3 h after treatment are shown. (C) The MTS values relative to untreated control 72 h after treatment of cisplatin at the indicated doses. CisR/SKOV-3 cells are indicated by black circles. The results from control SKOV-3 cells are indicated by open circles. (D) CisR/SKOV-3 cells were treated with cisplatin at the indicated doses, and the values of 2-NBDG uptake relative to the untreated group 3 h after treatment are shown. The results are the normalized mean values for three independent replicates. The experiments were repeated three times, and representative results are shown.

Glucose uptake did not decrease in cisplatin-resistant cells after cisplatin treatment. To generalize the finding that glucose uptake is useful for predicting cisplatin sensitivity, we examined two resistant cell lines. A uterine corpus cancer cell line, Sawano,[30] is known to be resistant to cisplatin. The sensitivity of Sawano cells to cisplatin was confirmed and compared with OVCAR-3 cells (Fig. 5A). Glucose uptake did not decrease in Sawano cells 3 h after treatment with 20 μg/mL cisplatin (Fig. 5B). We also generated cisplatin-resistant SKOV-3 cells, CisR/SKOV-3 (Fig. 5C). Glucose uptake did not decrease in CisR/SKOV-3 cells 3 h after treatment with 20 μg/mL cisplatin (Fig. 5D).

Early glucose uptake decrease after cisplatin treatment was only observed in the *in vitro* cisplatin-sensitive ovarian cancer cells from patient samples. The ovarian cancer cells from patient samples were subjected to primary culture and treated with cisplatin for 3 h. Glucose uptake was measured by the 2-NBDG assay. The 2-NBDG uptake of samples #1 and #2 significantly decreased (Fig. 6A). In contrast, samples #3 and #4 did not exhibit decreased 2-NBDG uptake after cisplatin treatment. These samples were cultured for several days after cisplatin treatment. Cells from #1 to #2 were eradicated 5 days after treatment (Fig. 5B). In contrast, cancer cells were still viable in cells from #3 to #4 10 days after cisplatin treatment

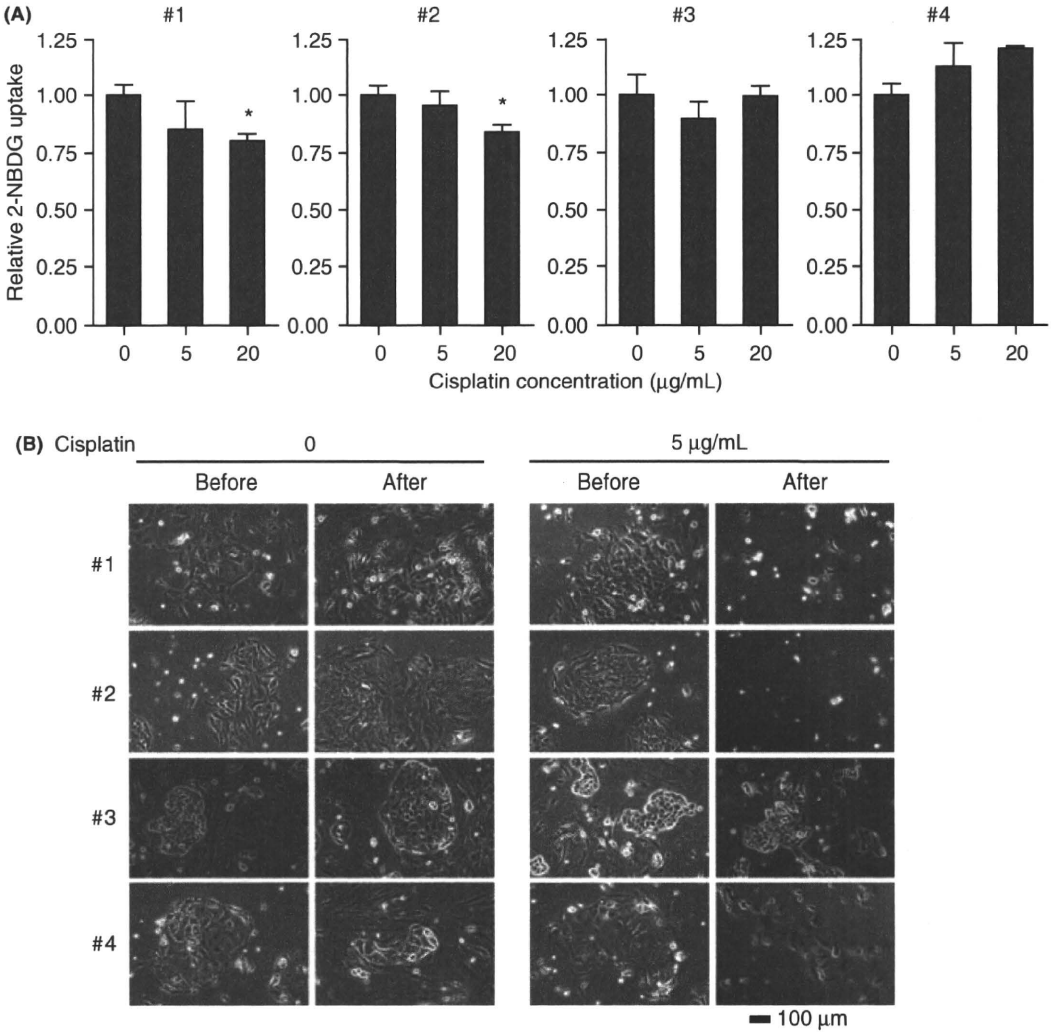

Fig. 6. (A) Ovarian cancer cells from four patients were cultured *in vitro,* treated with cisplatin at the indicated doses, and the values of 2-NBDG uptake relative to the untreated group determined 3 h after treatment. The results are the normalized mean values for three independent replicates. $*P < 0.05$ compared with the control. (B) Phase-contrast pictures of cells before and after culture with or without 5 μg/mL cisplatin, for 5 days in the case of #1 and #2 and 10 days in the case of #3 and #4.

(Fig. 6B). Thus, the early decrease in glucose uptake after cisplatin treatment might be a marker of cisplatin sensitivity in clinical ovarian cancers.

Discussion

The decrease of glucose uptake and oxygen consumption at an early point after cisplatin treatment was observed. It was not likely to be the consequence of non-specific events following exposure to the cytotoxic agent, as other cellular function was maintained, including membrane integrity (Fig. 1A), mitochondrial reductase activity (Fig. 1B), mitochondrial membrane potential (Fig. 4B), mRNA levels of house-keeping genes (TBP in Fig. 3A,B) and GLUT1 protein levels (Fig. 3C). This early decrease in glucose uptake was not observed in cisplatin-resistant cancer cells. These findings were also applicable to primary cultures of clinical samples.

The molecular mechanism underlying the decrease in glucose uptake after cisplatin treatment is not completely understood. Although cisplatin is known to be a DNA-damaging agent, <1% of cisplatin binding is to nuclear DNA, most binding is to mitochondrial DNA, phospholipids and other molecules.[31] Indeed, cisplatin is reported to affect multiple cellular components, not just DNA, but also RNA, plasma membrane, microfilaments, mitochondria and endoplasmic reticulum.[31] Consequently, there are many possible mechanisms for an acute decrease in glucose uptake.

We observed a rapid change in the distribution of GLUT1 after cisplatin treatment. The interaction between cisplatin and the plasma membrane might affect GLUT1 distribution. Cisplatin also possibly binds to tublin and inhibits the transport of GLUT1 to the plasma membrane.

We tested the hypothesis that the acute decrease in glucose uptake by cancer cells after cisplatin treatment is a secondary effect of impaired mitochondrial function. Cisplatin accumulates in mitochondria as mentioned above, and cisplatin preferentially binds to voltage-dependent anion channels (VDAC) that exist on the mitochondrial outer membrane.[32] Therefore, cisplatin could affect mitochondrial function. However, we revealed that the down-regulation of glucose uptake

doi: 10.1111/j.1349-7006.2010.01670.x

proceeded changes in the MTS assay, and the mitochondrial membrane potential was not impaired when glucose uptake was already reduced. Mitochondria are not likely to be the primary site of cisplatin effects.

Perturbation of the glycolysis–mitochondria interaction is another possible mechanism. Cancer cells exhibit a high rate of glycolysis,[7] and increased expression of HK2 is thought to be one of the reasons for this high rate. HK2 phosphorylates glucose to produce glucose 6-phosphate (G-6-P) in an ATP-dependent manner. The majority of HK2 binds to VDAC in order to easily use mitochondrial ATP.[33] Cisplatin reportedly releases HK2 from VDAC,[34] after which glucose phosphorylation might become less efficient, followed by the down-regulation of glycolysis-related genes and glucose uptake. In addition, AKT is reportedly activated in cisplatin-resistant ovarian cancer cells due to the anti-apoptosis signals. AKT also enhances the binding of VDAC and HK2,[35] which might explain the minimal change in glucose uptake by cisplatin-resistant cells in our study.

Here, we showed that an early decrease in glucose uptake after cisplatin treatment correlates with later sensitivity to the drug. Although it is possible that the decreased glucose uptake is the direct cause of the cytotoxic effect, the substantial delay in cell death after the reduction suggests that the events are independent. Cellular cisplatin uptake is known to be a critical parameter for sensitivity,[36–39] and the reduction in glucose uptake might reflect the cisplatin levels in the cell.

An early decrease in glucose uptake after treatment with chemotherapeutic drugs was previously reported in other cancers. For example, decreased glucose uptake was observed in a GIST cell line 2 h after imatinib treatment,[18] a breast cancer cell line 1 h after 5-FU,[19] and in a breast cancer cell line 4 h after 5-FU and doxorubicin.[20] To the best of our knowledge, this report is the first to detect early reduced glucose uptake after cisplatin treatment in ovarian cancer.

We used 2-NBDG, a fluorescent D-glucose derivative, to monitor glucose uptake. 2-NBDG was initially developed to distinguish viable *Escherichia coli* in 1996.[40] The uptake of 2-NBDG is inhibited by D-glucose, and it is incorporated into a cell through GLUT.[41] Once inside the cells, 2-NBDG, like other 2-DG derivatives, is rapidly phosphorylated at the C-6 position by hexokinase.[42] Recently, 2-NBDG was shown to be useful for monitoring glucose uptake in a variety of mammalian and cancer cells.[41,43–45] 2-NBDG is much more sensitive than measuring the change in the glucose concentration of the medium. A radioactive isotope has been used to measure glucose uptake, but 2-NBDG can be used more safely in the laboratory. In addition, we showed that flow cytometric analysis with 2-NBDG is useful for detecting subtle and early changes in glucose uptake.

FDG-PET is widely used in clinical practice to diagnose various cancers. A decrease of the standard uptake value (SUV), which represents glucose uptake, can be detected before the size of the tumor changes in lung cancer and esophageal cancer.[11,46] Avril *et al.*[13] characterized a group of ovarian cancer patients with decreased SUV after one or three courses of chemotherapy as metabolic responders and non-responders. The prognosis was better in metabolic responders than non-responders. These reports suggest that glucose metabolism changes in cancer cells *in vivo* over a short period of time after chemotherapy, which is compatible with our *in vitro* results.

Adjuvant therapy improves the prognosis after surgical tumor removal, even in advanced cases,[1,47–49] but PET cannot be used to evaluate chemosensitivity after surgery because it is only useful when the tumor size is more than ~1 cm. An *in vitro* 2-NBDG chemosensitivity assay of the surgically removed specimen can provide information for designing adjuvant therapy. Also, the assay can be applied to recurrent diseases or patients with a tumor that cannot be removed surgically; biopsy samples would be enough for the assay. In addition, the assay can be performed within a short period of time, and does not require a long time culture, although culture conditions require further improvement for application to every patient sample.

Acknowledgments

The authors thank Toshiko Yasuda for secretary assistance. The research was supported (in part) by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

Disclosure Statement

The authors have no conflict of interest.

References

1 Aebi S, Castiglione M. Epithelial ovarian carcinoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2008; **19**(Suppl 2): ii14–6.
2 Bookman MA, McGuire WP III, Kilpatrick D *et al.* Carboplatin and paclitaxel in ovarian carcinoma: a phase I study of the Gynecologic Oncology Group. *J Clin Oncol* 1996; **14**: 1895–902.
3 McGuire WP, Hoskins WJ, Brady MF *et al.* Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996; **334**: 1–6.
4 Ozols RF, Bundy BN, Greer BE *et al.* Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 2003; **21**: 3194–200.
5 Markman M, Rothman R, Hakes T *et al.* Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin. *J Clin Oncol* 1991; **9**: 389–93.
6 Therasse P, Eisenhauer EA, Verweij J. RECIST revisited: a review of validation studies on tumour assessment. *Eur J Cancer* 2006; **42**: 1031–9.
7 Warburg O. On respiratory impairment in cancer cells. *Science* 1956; **124**: 269–70.
8 Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res* 1998; **58**: 1408–16.
9 Macheda ML, Rogers S, Best JD. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol* 2005; **202**: 654–62.
10 Bourguet P, Blanc-Vincent MP, Boneu A *et al.* Summary of the standards, options and recommendations for the use of positron emission tomography with 2-[18F]fluoro-2-deoxy-D-glucose (FDP-PET scanning) in oncology (2002). *Br J Cancer* 2003; **89**(Suppl 1): S84–91.
11 Weber WA, Petersen V, Schmidt B *et al.* Positron emission tomography in non-small-cell lung cancer: prediction of response to chemotherapy by quantitative assessment of glucose use. *J Clin Oncol* 2003; **21**: 2651–7.
12 Wieder HA, Brucher B, Zimmermann F *et al.* Time course of tumor metabolic activity during chemoradiotherapy of esophageal squamous cell carcinoma and response to treatment. *J Clin Oncol* 2004; **22**: 900–8.
13 Avril N, Sassen S, Schmalfeldt B *et al.* Prediction of response to neoadjuvant chemotherapy by sequential F-18-fluorodeoxyglucose positron emission tomography in patients with advanced-stage ovarian cancer. *J Clin Oncol* 2005; **23**: 7445–53.
14 Nishiyama Y, Yamamoto Y, Kanenishi K *et al.* Monitoring the neoadjuvant therapy response in gynecological cancer patients using FDG PET. *Eur J Nucl Med Mol Imaging* 2008; **35**: 287–95.
15 Schwarz JK, Grigsby PW, Dehdashti F, Delbeke D. The role of 18F-FDG PET in assessing therapy response in cancer of the cervix and ovaries. *J Nucl Med* 2009; **50**(Suppl 1): 64S–73S.
16 Minn H, Kangas L, Knuutila V, Paul R, Sipila H. Determination of 2-fluoro-2-deoxy-D-glucose uptake and ATP level for evaluating drug effects in neoplastic cells. *Res Exp Med (Berl)* 1991; **191**: 27–35.
17 Zhou R, Vander Heiden MG, Rudin CM. Genotoxic exposure is associated with alterations in glucose uptake and metabolism. *Cancer Res* 2002; **62**: 3515–20.

18 Cullinane C, Dorow DS, Kansara M *et al.* An in vivo tumor model exploiting metabolic response as a biomarker for targeted drug development. *Cancer Res* 2005; **65**: 9633–6.
19 Engles JM, Quarless SA, Mambo E, Ishimori T, Cho SY, Wahl RL. Stunning and its effect on 3H-FDG uptake and key gene expression in breast cancer cells undergoing chemotherapy. *J Nucl Med* 2006; **47**: 603–8.
20 Direcks WG, Berndsen SC, Proost N *et al.* [18F]FDG and [18F]FLT uptake in human breast cancer cells in relation to the effects of chemotherapy: an in vitro study. *Br J Cancer* 2008; **99**: 481–7.
21 Suttie SA, Park KG, Smith TA. [18F]2-fluoro-2-deoxy-D-glucose incorporation by AGS gastric adenocarcinoma cells in vitro during response to epirubicin, cisplatin and 5-fluorouracil. *Br J Cancer* 2007; **97**: 902–9.
22 Saito Y, Furukawa T, Arano Y, Fujibayashi Y, Saga T. Comparison of semiquantitative fluorescence imaging and PET tracer uptake in mesothelioma models as a monitoring system for growth and therapeutic effects. *Nucl Med Biol* 2008; **35**: 851–60.
23 Behrens BC, Hamilton TC, Masuda H *et al.* Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987; **47**: 414–8.
24 Mabuchi S, Kawase C, Altomare DA *et al.* mTOR is a promising therapeutic target both in cisplatin-sensitive and cisplatin-resistant clear cell carcinoma of the ovary. *Clin Cancer Res* 2009; **15**: 5404–13.
25 Zou C, Wang Y, Shen Z. 2-NBDG as a fluorescent indicator for direct glucose uptake measurement. *J Biochem Biophys Methods* 2005; **64**: 207–15.
26 Okuyama H, Krishnamachary B, Zhou YF, Nagasawa H, Bosch-Marce M, Semenza GL. Expression of vascular endothelial growth factor receptor 1 in bone marrow-derived mesenchymal cells is dependent on hypoxia-inducible factor 1. *J Biol Chem* 2006; **281**: 15554–63.
27 Endo H, Murata K, Mukai M, Ishikawa O, Inoue M. Activation of insulin-like growth factor signaling induces apoptotic cell death under prolonged hypoxia by enhancing endoplasmic reticulum stress response. *Cancer Res* 2007; **67**: 8095–103.
28 Slater TF, Sawyer B, Straeuli U. Studies on succinate-tetrazolium reductase systems. iii. points of coupling of four different tetrazolium salts. *Biochim Biophys Acta* 1963; **77**: 383–93.
29 Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood* 1994; **84**: 1415–20.
30 Satoh T, Nishida M, Miyazaki Y *et al.* [Establishment of a cisplatin-resistant new human endometrial adenocarcinoma cell line, Sawano cells]. *Hum Cell* 1995; **8**: 67–72.
31 Rebillard A, Lagadic-Gossmann D, Dimanche-Boitrel MT. Cisplatin cytotoxicity: DNA and plasma membrane targets. *Curr Med Chem* 2008; **15**: 2656–63.
32 Yang Z, Schumaker LM, Egorin MJ, Zuhowski EG, Guo Z, Cullen KJ. Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. *Clin Cancer Res* 2006; **12**: 5817–25.
33 Pedersen PL. Voltage dependent anion channels (VDACs): a brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the ''Warburg effect'' in cancer. *J Bioenerg Biomembr* 2008; **40**: 123–6.
34 Shulga N, Wilson-Smith R, Pastorino JG. Hexokinase II detachment from the mitochondria potentiates cisplatin induced cytotoxicity through a caspase-2 dependent mechanism. *Cell Cycle* 2009; **8**: 3355–64.
35 Stiles BL. PI-3-K and AKT: onto the mitochondria. *Adv Drug Deliv Rev* 2009; **61**: 1276–82.
36 Gately DP, Howell SB. Cellular accumulation of the anticancer agent cisplatin: a review. *Br J Cancer* 1993; **67**: 1171–6.
37 Kool M, de Haas M, Scheffer GL *et al.* Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res* 1997; **57**: 3537–47.
38 Kim A, Enomoto T, Serada S *et al.* Enhanced expression of Annexin A4 in clear cell carcinoma of the ovary and its association with chemoresistance to carboplatin. *Int J Cancer* 2009; **125**: 2316–22.
39 Blair BG, Larson CA, Safaei R, Howell SB. Copper transporter 2 regulates the cellular accumulation and cytotoxicity of Cisplatin and Carboplatin. *Clin Cancer Res* 2009; **15**: 4312–21.
40 Yoshioka K, Takahashi H, Homma T *et al.* A novel fluorescent derivative of glucose applicable to the assessment of glucose uptake activity of Escherichia coli. *Biochim Biophys Acta* 1996; **1289**: 5–9.
41 Yamada K, Nakata M, Horimoto N, Saito M, Matsuoka H, Inagaki N. Measurement of glucose uptake and intracellular calcium concentration in single, living pancreatic beta-cells. *J Biol Chem* 2000; **275**: 22278–83.
42 Yoshioka K, Saito M, Oh KB *et al.* Intracellular fate of 2-NBDG, a fluorescent probe for glucose uptake activity, in Escherichia coli cells. *Biosci Biotechnol Biochem* 1996; **60**: 1899–901.
43 O'Neil RG, Wu L, Mullani N. Uptake of a fluorescent deoxyglucose analog (2-NBDG) in tumor cells. *Mol Imaging Biol* 2005; **7**: 388–92.
44 Nitin N, Carlson AL, Muldoon T, El-Naggar AK, Gillenwater A, Richards-Kortum R. Molecular imaging of glucose uptake in oral neoplasia following topical application of fluorescently labeled deoxy-glucose. *Int J Cancer* 2009; **124**: 2634–42.
45 Yamada K, Saito M, Matsuoka H, Inagaki N. A real-time method of imaging glucose uptake in single, living mammalian cells. *Nat Protoc* 2007; **2**: 753–62.
46 Wieder HA, Beer AJ, Lordick F *et al.* Comparison of changes in tumor metabolic activity and tumor size during chemotherapy of adenocarcinomas of the esophagogastric junction. *J Nucl Med* 2005; **46**: 2029–34.
47 Early Breast Cancer Trialists' Collagorative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; **365**: 1687–717.
48 Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004; **350**: 351–60.
49 Young RC. Early-stage ovarian cancer: to treat or not to treat. *J Natl Cancer Inst* 2003; **95**: 94–5.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. SKOV-3 cells and OVCAR-3 cells were treated with cisplatin at the indicated doses. (A) Cell death was assessed by the number of dead cells per total cell number. The values relative to the untreated control group are shown. The white bars indicate 3 h and black bars 24 h after cisplatin treatment. The results are the normalized mean values for three independent replicates. The experiments were repeated three times, and representative results are shown. (B) The apoptotic events in OVCAR-3 cells were assessed by flow cytometry with annexin V/PI staining.

Appendix S1. Materials and methods.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

doi: 10.1111/j.1349-7006.2010.01670.x

ANTICANCER RESEARCH *30*: 4347-4352 (2010)

Disease-free Interval after Primary Treatment Predicts Prognosis of Recurrent Endometrial Carcinoma

YUTAKA UEDA[1,*], YUKI MATSUMURA[1,*], TOMOMI EGAWA-TAKATA[1], TAKAHITO MIYAKE[2], TAKASHI MIYATAKE[1], KIYOSHI YOSHINO[1], MASAMI FUJITA[1], SHINYA MATSUZAKI[1], TAKUHEI YOKOYAMA[1], YUKARI MIYOSHI[1], MASATO YAMASAKI[2], TAKAYUKI ENOMOTO[1] and TADASHI KIMURA[1]

[1]*Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Suita, Osaka, 565-0871, Japan;*
[2]*Department of Obstetrics and Gynecology, Osaka Rosai Hospital, Kita-ku, Sakai, Osaka, 591-8025, Japan*

Abstract. *Aim: The aim of this study was to determine if the disease-free interval after initial surgical resection has any useful prognostic value for recurrent endometrial carcinoma patients. Patients and Methods: Between 1998 and 2007, complete resection of endometrial carcinoma was achieved in 536 cases at the Departments of Obstetrics and Gynecology of the Osaka University and Osaka Rosai Hospitals of Osaka, Japan. Clinical characteristics of these cases were retrospectively reviewed. Results: Recurrence was subsequently detected in 54 cases. Overall survival after recurrence in 27 patients with recurrences earlier than 12 months who received no postoperative therapy, radiation, and chemotherapy as an adjuvant therapy were significantly shorter than that of those with recurrences later than 12 months with similar treatments. Multivariate analysis demonstrated that the disease-free interval was an independent factor for prognosis. Conclusion: We demonstrate a significantly worse prognosis in cases with early versus late recurrence of resected endometrial carcinomas, irrespective of the type of adjuvant therapy.*

The incidence of uterine endometrial carcinoma, already the most common malignancy of the female pelvis and the fourth most common cancer of women in the United States, has increased steadily during the last three decades (1, 2).

*Both Authors contributed equally to this study.

Correspondence to: Dr. Takayuki Enomoto, Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan. Tel: +81 668793351, Fax: +81 668793359, e-mail: enomoto@gyne.med.osaka-u.ac.jp

Key Words: Endometrial cancer, recurrence, disease-free interval, prognosis, overall survival.

The prognostic factors for the disease include the tumor histological type and differentiation, disease stage, peritoneal cytology, myometrial invasion and extrauterine metastasis (1, 2). In the past, radiation therapy alone was performed for endometrial cancer; however, current treatment, even for early stage endometrial cancer without any risk factors, usually consists of hysterectomy and salpingo-oophorectomy, with or without retroperitoneal lymph node dissection. Patients with poor prognostic factors in the past often underwent postoperative irradiation therapy; however, a randomized study revealed that combined chemotherapy was superior to whole abdominal irradiation as the adjuvant therapy in advanced diseases (EORTC #55872) (3). Cytoreductive surgery for extrauterine disease followed by systemic chemotherapy is now performed as the suggested standard of care (4).

A previous systematic review showed that 13% of treated endometrial cancer cases went on to develop a recurrence (5). Unfortunately, 3 to 19 years after treatment for such recurrence, only 7.7% of the patients survived without evidence of the disease (2). Recurrences restricted to the vaginal vault have been shown to be relatively better treated with radiotherapy. However, in most cases of relapse, the disease has spread to other sites, including pelvic and para-aortic lymph nodes, the peritoneum of the pelvis and abdominal cavity, and the lungs. For these cases, systemic chemotherapy is usually superior.

Most recurrences are detected within the first two years after primary surgery (6-9). The prognostic significance of this disease-free interval (DFI) has been highly controversial (6, 7, 9-17). These studies included some patients who were treated by radiotherapy alone without surgery, and the majority of patients whose tumor was surgically resected either received no adjuvant therapy or underwent postoperative radiotherapy. Our own data (unpublished) provided us with evidence that the majority of refractory or

0250-7005/2010 $2.00+.40

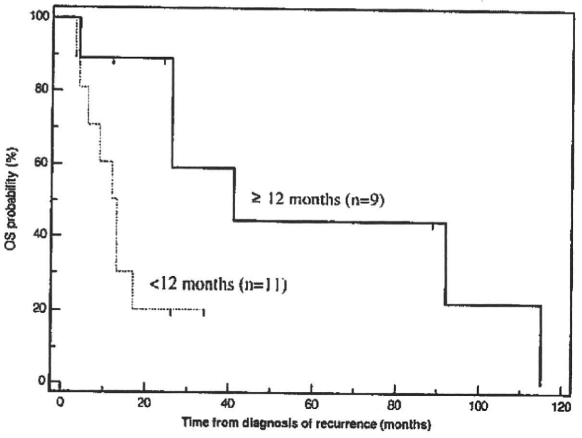

Figure 1. *Overall survival (OS) after recurrence by disease-free interval in those who did not undergo adjuvant therapy. The median OS after recurrence is shown in the cases where, at the initial treatment for endometrial cancer, any adjuvant therapy following surgery was performed. The OS of the early recurrent cases (<12 months from the initial surgery) was significantly shorter than that of the late ones (≥12 months) (p=0.028 by the log-rank test).*

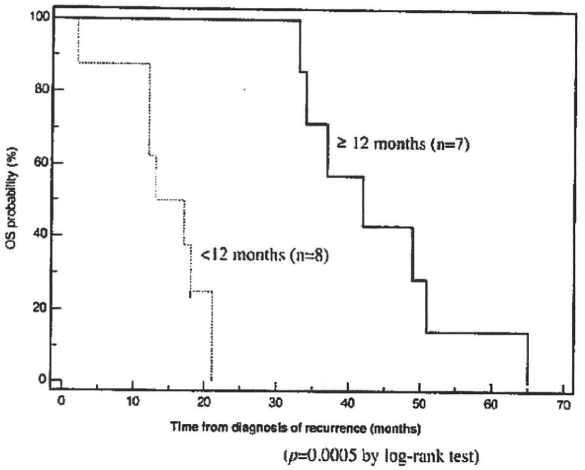

Figure 2. *Overall survival (OS) after recurrence by disease-free interval in those who underwent adjuvant radiotherapy. The median OS after recurrence is shown in the cases where adjuvant radiation was performed at the initial treatment for endometrial cancer. The OS of the early recurrent cases (<12 months from the initial surgery) was significantly shorter than that of the late ones (≥12 months) (p=0.0005 by the log-rank test).*

recurrent diseases occurring within 6 months of first-line chemotherapy are non-responsive to the current regimens of second-line chemotherapy. The recent widespread trend is to use chemotherapy as the preferred adjuvant therapy instead of radiotherapy for resected endometrial cancer.

We have now re-examined the prognostic significance of the time to recurrence in cases in which no adjuvant therapy was performed, those in which radiation was performed, and those which underwent adjuvant chemotherapy. We performed a comparison of the prognosis of the patients whose tumor recurred within 12 months from the primary surgical therapy and those detected after 12 months and we investigated the association of DFI with other factors, such as the type of adjuvant therapy, and its prognostic significance of recurrent endometrial carcinoma.

Patients and Methods

Patients. During the 10-year study period of 1998 to 2007, 555 endometrial carcinomas were diagnosed and treated within the Departments of Obstetrics and Gynecology of the Osaka University Hospital and of the Osaka Rosai Hospital of Osaka, Japan. The patients were all ethnically Asian. The diagnosis was histologically confirmed by pathologists within the respective Departments of Pathology. Complete surgical removal of the disease by total abdominal hysterectomy, bilateral salpingo-oophorectomy, staging, and a maximum cytoreduction, including a retroperitoneal lymph-node dissection, was provisionally achieved in 536 out of the 555 cases. Following surgery, adjuvant radiotherapy or chemotherapy was indicated in 284 cases having risk factors that included serous and clear cell adenocarcinomas, grade 3 endometrioid carcinoma with myometrial invasion, outer-wall myometrial invasion, remarkable lymph-vascular involvement, or development of tumors outside of the uterus. Fifty-one patients did not agree to receive any adjuvant therapy; 138 patients underwent adjuvant chemotherapy (paclitaxel, epirubicin and carboplatin (TEC) in 98 patients; paclitaxel and carboplatin (TC) in 29 patients; cisplatin, adriamycin and cyclophosphamide (CAP) in 8 patients; and some other regimen in 3 patients); 95 patients received adjuvant radiation therapy. Adjuvant therapy was not indicated for the 252 patients with low or no risk factors. After the initial tumor treatment, all patients received periodic intensive follow-up that included combinations of a pelvic-rectal examination, vaginal-vault cytology, and transvaginal ultrasonography (TV-USG), a computed tomography (CT) scan, chest X-ray and tumor marker analyses.

In the 45 cases in which recurrent diseases were detected, intensive rescue treatments, including surgical resection, radiation, and chemotherapy, were performed, except for those patients whose performance status was judged to be too poor for them to receive any therapy, or those who preferred watchful observation over intensive treatment.

Methods. We retrospectively reviewed the patients' characteristics and the clinicopathological features of all cases of endometrial carcinoma recurrence, utilizing their clinical records, which included physical examination notes, radiological reports, operation records and histology reports. Progression-free survival (PFS) after recurrence was measured from the date of diagnosis of the recurrence to the date of subsequent radiologic or pathologic relapse of the disease, or, in subsequently disease-free patients, to the date of their last known follow-up visit. Overall survival (OS) after recurrence was defined as the period from the diagnosis of the recurrence to the patient's disease-specific death, or to the date of their last known follow-up.

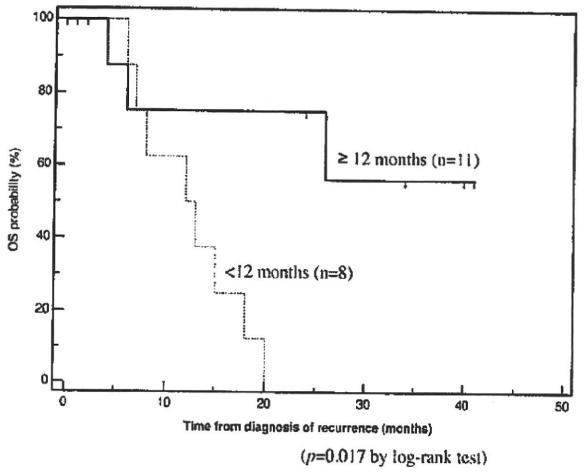

Figure 3. *Overall survival (OS) after recurrence by disease-free interval in those who underwent adjuvant chemotherapy. The median OS after recurrence is shown in the cases where adjuvant chemotherapy was performed at the initial treatment for endometrial cancer. The OS of the early recurrent cases (<12 months from the initial surgery) was significantly shorter than that of the late ones (≥12 months) (p=0.017 by the log-rank test).*

Statistical analysis. The clinicopathological characteristics of patients with recurrences that were diagnosed between early (<12 months from the initial treatment) and late (≥12 months) time periods were analyzed by Fisher's exact test, Pearson's chi-square (χ^2) test and the Mann-Whitney *U*-test. The patients with recurrence were divided into three groups according to the kind of adjuvant therapy they received: no adjuvant therapy group, radiation group, and chemotherapy group. Survival curves for the patients with early and late recurrences in each group were constructed using the Kaplan-Meier method and these results were evaluated for statistical significance by the log-rank test. A multivariate Cox proportional hazards model was used to determine the significantly important factors for survival of patients with recurrent endometrial cancer. The variables considered were: age of the patients, histology, the initial stage, adjuvant therapy, DFI, recurrent site, symptoms present at the time of detection of recurrence, and the treatment given for the recurrent disease. The results were considered to be significant when the *p*-value was less than 0.05.

Statements of ethics or conflicts of interest. Our Institutional Review Board and Ethics Committee approved this study. There were no known conflicts of interest.

Results

Clinical characteristics of the patients with recurrent endometrial cancer. During their post-surgical follow-up period (median 43 months, range 2-108 months), a recurrence was detected in 54 (10%) out of the 536 completely resected cases. The clinical characteristics of these cases are shown in Table I. Adjuvant therapy was performed in 34 of the 54 cases at initial treatment of their endometrial carcinoma. Fifteen patients received radiation and 19 patients underwent chemotherapy. Twenty-seven cases (50%) recurred in less than 12 months from the initial surgery and the other 27 cases (50%) recurred after 12 months.

Survival prognosis of the early and late recurrent cases. The OS of the early recurrent cases was significantly worse than that of the late ones ($p<0.0001$ by the log-rank test). Histology (endometrioid *versus* non-endometrioid), initial stage (I/II *versus* III/IV), adjuvant therapy (none *versus* radiation *versus* chemotherapy), symptom at recurrence (symptomatic *versus* asymptomatic), site of recurrence (localized *versus* metastatic) and treatment for recurrence (none *versus* performed) did not show any significant difference between early and late recurrent cases. However, the median ages of patient with early and late recurrences were 57 (33-83) and 62 (41-77) years, exhibiting a statistically significant difference ($p=0.021$ by the Mann-Whitney *U*-test) (data not shown).

The OS of the early and late recurrent cases were evaluated in each adjuvant therapy group. In those who did not undergo any adjuvant therapy, recurrence was detected early (<12 months) in 11 patients and late (≥12 months) in 9 patients. The median OS of the early and the late recurrent cases were 13 months and 41 months, respectively, demonstrating a statistically significant difference ($p=0.028$ by the log-rank test) (Figure 1). In those who received radiation as an adjuvant therapy, recurrence was detected early in 8 patients and late in 7 patients. The median OS of the early and the late recurrent cases were 15 months and 42 months, respectively, also demonstrating a statistically significant difference ($p=0.0005$ by the log-rank test) (Figure 2). In those who underwent adjuvant chemotherapy, recurrence was detected early in 8 patients and late in 11 patients. The median OS of the early recurrent cases was 12.5 months. On the other hand, it was not possible to calculate the OS of the late ones because more than the half (8 out of 11) of the patients were still alive at the time of our last analysis. However, OS curves for 8 early and 11 late recurrences were constructed using the Kaplan-Meier method, demonstrating a statistically significant difference between them ($p=0.017$ by the log-rank test) (Figure 3).

Multivariate Cox proportional hazards analysis for recurrent endometrial cancer. The prognostic significance of factors including the age of the patients, histology, the initial stage, adjuvant therapy, DFI, recurrent site, presence of symptoms at the time of the detection of recurrence and the treatment for recurrent diseases in the recurrent endometrial carcinoma cases were evaluated by a multivariate Cox proportional hazards model. The age of the patients (<60 *versus* ≥60 years), initial stage (stages I/II *versus* stages III/IV), symptoms at detection of recurrence (symptomatic *versus*

Table I. *Clinical characteristics of patients with recurrent endometrial cancer.*

	Characteristic
Number (cases)	54
Median age (year)	59 (33-83)
Histology (cases)	
Endometrioid	40
Non-endometrioid*	14
Initial stage (cases)	
I/II	17
III/IV	37
Adjuvant therapy (cases)	
None	20
Radiation	15
Chemotherapy	19
Symptoms at recurrence (cases)	
Asypmtomatic	29
Symptomatic	25
Site of recurrence (cases)	
Local alone	29
Distant**	25
DFI (cases)	
≥12 months	27
<12 months	27
Treatment for recurrence (cases)	
None	9
Performed	45

*Non-endometrioid: serous adenocarcinoma, clear cell adenocarcinoma, mucinous adenocarcinoma and other histological types. **Distant: recurrence out of the pelvis, with or without local vaginal vault recurrence.

Table II. *Multivariate Cox proportional hazards analysis for prognostic factors of recurrent endometrial carcinoma.*

Variable	Adjusted HR	95% CI	*p*-Value
Age (years)			
<60	1		
≥60	0.796	0.347-1.828	0.59
Histology			
Endometrioid	1		
Non-endometrioid*	2.773	1.221-6.300	0.015
Initial stage			
I/II	1		
III/IV	1.089	0.483-2.457	0.84
Adjuvant therapy			
None	1		
Radiation	1.572	0.678-3.646	0.29
Chemotherapy	1.151	0.395-3.348	0.55
Symptoms at recurrence			
Asyptomatic	1		
Symptomatic	1.214	0.575-2.564	0.61
Site of recurrence			
Local alone	1		
Distant**	0.917	0.449-1.869	0.81
DFI			
≥12 months	1		
<12 months	9.724	3.166-29.870	<0.0001
Treatment for recurrence			
Performed		1	
None	6.428	2.341-17.650	0.0003

*Non-endometrioid: serous adenocarcinoma, clear cell adenocarcinoma, mucinous adenocarcinoma and other histological types. ** Distant: recurrence out of the pelvis, with or without local vaginal vault recurrence.

asymptomatic) and site of recurrence (local alone *versus* distant) were not demonstrated to be significant predictors for prognosis of recurrent endometrial carcinoma cases (Table II). The type of adjuvant therapy (none *versus* radiation *versus* chemotherapy) also did not exhibit statistical significance for prognosis.

However, having a non-endometrioid type of histology showed an adjusted hazard ratio (HR) of 2.773 (95% confidence interval (CI)=1.221-6.300, p=0.015), and receiving no treatment for the recurrent disease also showed an adjusted HR of 6.428 (95% CI=2.341-17.650, p=0.0003). An adjusted HR for DFI <12 months was 9.724 (95% CI=3.166-29.870, p<0.0001), demonstrating that DFI was an independent predictor for prognosis of recurrent endometrial cancer cases.

Discussion

Endometrial adenocarcinoma, already the most common female pelvic malignancy in the United States, is increasing (1, 2). Although early endometrial cancer can sometimes be treated successfully by surgical resection alone, the current trend for initial treatment consists of surgical resection and postoperative chemotherapy. We have recently shown that even for advanced cases with distant metastasis, cytoreductive surgery for the metastases followed by postoperative chemotherapy can be effective (4).

Despite good initial intensive treatments, recurrences still develop in around 13% of patients (5). For these recurrences, combinations of surgery, radiation and chemotherapy are performed. However, in most cases of relapse the disease has spread to distant sites, and for these cases aggressive systemic chemotherapy is usually required (1,2).

The prognostic significance of the DFI following surgery has been highly controversial. A recent study demonstrated a significant impact of DFI (<24 *versus* >24 months) on the OS of patients with recurrent endometrial cancer (18). In their series, 7% of the patients were treated by radiation alone without surgery. Adjuvant radiation therapy following primary surgery was performed in 70.1% of the recurrent cases. Another recent study did not, however, demonstrate a significant impact of DFI (<12 months *versus* ≥12 months) on OS (17). In their study, 10% of the patients were treated

by radiation, not by surgery. The number of patients who received adjuvant therapy following primary surgery and the types of therapy were not described. Based on our own unpublished data, which show that the majority of refractory or recurrent diseases, occurring within 6 months of a first-line chemotherapy are non-responsive to the current regimens of second-line chemotherapy, DFI may be a possible predictor for prognosis of recurrent endometrial cancer cases, especially in those cases where adjuvant chemotherapy was performed.

In our present examination of the significance of DFI on the prognosis of patients with recurrent endometrial cancer, only the patients who were treated by surgery during the primary treatment were enrolled in the study. The recurrent cases were divided into three groups by kinds of adjuvant therapy: no adjuvant therapy group, radiation group, and chemotherapy group. Recurrence was detected in 54 (10%) out of the 536 completely resected cases, compatible with a previously described recurrence rate (5). The reason why the median age of 57 (33-83) years for early recurrence is somewhat younger than that of 62 (41-77) years for late recurrence is unclear. The OS of the early recurrent cases was significantly shorter than that of the late recurrences in all the adjuvant therapy groups: p=0.028 for those who did not receive any adjuvant therapy, p=0.0005 for those who underwent adjuvant radiation, and p=0.017 for those who received adjuvant chemotherapy. These results clearly show, for the first time, that DFI is an important predictor for prognosis of recurrent endometrial carcinoma, irrespective of the type of adjuvant therapy.

Moreover, a multivariate Cox proportional hazards model analysis reveals that having a non-endometrioid type of tumor histology, no treatment for recurrent disease, and DFI were each of independent significance for OS after recurrence. Especially significant was an adjusted HR for DFI <12 months, which was 9.724 (95% CI=3.166-29.870, p<0.0001), demonstrating that among those clinical factors studied, DFI was the most important predictor for prognosis of recurrent endometrial cancer cases (Table II). The type of adjuvant therapy (none *versus* radiation *versus* chemotherapy) also did not exhibit statistical significance for prognosis. On the other hand, symptoms at detection of recurrence, which our previous study had suggested to have a prognostic significance by univariate analysis, did not exhibit a significant impact on prognosis when examined by multivariate Cox analysis (19).

Our present study, for the first time, clearly demonstrates that DFI after recurrence is an extremely important predictor for prognosis of recurrent endometrial cancer cases. Recurrent endometrial cancer is usually highly difficult to cure completely, with the exception of when the recurrence is completely restricted, which can usually be successfully treated by surgery or radiation therapy (1, 2, 20). Especially, early recurrent tumors after previous chemotherapy was shown to exhibit extreme resistance to second-line chemotherapy (21).

In our studies, we have found that appropriate treatment for recurrent diseases does significantly improve the prognosis when compared to observation alone or palliative care. However, we have also found that the prognosis of cases which recur the earliest remains quite poor. Further investigations are required to find more useful predictors for recurrent endometrial cancer outcome and to establish better treatment plans for this disease.

Acknowledgements

We would like to thank Dr. G. S. Buzard, CDCP, for his constructive critiques and editing of our manuscript.

References

1 DiSaia PJ and Creasman WT: Clinical Gynecologic Oncology (6th ed.). St. Louis: Mosby, pp. 137-67, 2002.

2 Berek JS: Novak's Gynecology (13th ed.): Baltimore: William and Wilkins, pp. 1143-85, 2002.

3 Aapro MS, van Wijk FH, Bolis G, Chevallier B, van der Burg ME, Poveda A, de Oliveira CF, Tumolo S, Scotto di Palumbo V, Piccart M, Franchi M, Zanaboni F, Lacave AJ, Fontanelli R, Favalli G, Zola P, Guastalla JP, Rosso R, Marth C, Nooij M, Presti M, Scarabelli C, Splinter TA, Ploch E, Beex LV, ten Bokkel Huinink W, Forni M, Melpignano M, Blake P, Kerbrat P, Mendiola C, Cervantes A, Goupil A, Harper PG, Madronal C, Namer M, Scarfone G, Stoot JE, Teodorovic I, Coens C, Vergote I and Vermorken JB. European Organisation for Research and Treatment of Cancer Gynaecological Cancer Group: Doxorubicin *versus* doxorubicin and cisplatin in endometrial carcinoma: definitive results of a randomised study (55872) by the EORTC Gynaecological Cancer Group. Ann Oncol *14*: 441-448, 2003.

4 Ueda Y, Enomoto T, Miyatake T, Egawa-Takata T, Ugaki H, Yoshino K, Fujita M and Kimura T: Endometrial carcinoma with extra-abdominal metastasis: improved prognosis following cytoreductive surgery. Ann Surg Oncol *17*: 1111-1117, 2010.

5 Fung-Kee-Fung M, Dodge J, Elit L, Lukka H, Chambers A and Oliver T: Cancer Care Ontario Program in Evidence-based Care Gynecology Cancer Disease Site Group. Follow-up after primary therapy for endometrial cancer: a systematic review. Gynecol Oncol *10*: 520-529, 2006.

6 Sears JD, Greven KM, Hoen HM and Randall ME: Prognostic factors and treatment outcome for patients with locally recurrent endometrial cancer. Cancer *74*: 1303-1308, 1994.

7 Kuten A, Grigsby PW, Perez CA, Fineberg B, Garcia DM and Simpson JR: Results of radiotherapy in recurrent endometrial carcinoma: a retrospective analysis of 51 patients. Int J Radiat Oncol Biol Phys *17*: 29-34, 1989.

8 Poulsen MG and Roberts SJ: The salvage of recurrent endometrial carcinoma in the vagina and pelvis. Int J Radiat Oncol Biol Phys *15*: 809-813, 1988.

9 Vavra N, Denison U, Kucera H, Barrada M, Kurz C, Salzer H and Sevelda P: Prognostic factors related to recurrent endometrial carcinoma following initial surgery. Acta Obstet Gynecol Scand *72*: 205-209, 1993.

10 Curran WJ Jr., Whittington R, Peters AJ and Fanning J: Vaginal recurrences of endometrial carcinoma: the prognostic value of staging by a primary vaginal carcinoma system. Int J Radiat Oncol Biol Phys *15*: 803-808, 1988.

11 Rubin P, Gerle RD, Quick RS and Greenlaw RH: Significance of vaginal recurrence in endometrial carcinoma. Am J Roentgenol Radium Ther Nucl Med *89*: 91-100, 1963.

12 Mandell LR, Nori D and Hilaris B: Recurrent stage I endometrial carcinoma: results of treatment and prognostic factors. Int J Radiat Oncol Biol Phys *11*: 1103-1109, 1985.

13 Ackerman I, Malone S, Thomas G, Franssen E, Balogh J and Dembo A: Endometrial carcinoma – relative effectiveness of adjuvant irradiation *vs*. therapy reserved for relapse. Gynecol Oncol *60*: 177-183, 1996.

14 Pirtoli L, Ciatto S, Cionini L, Taddei G and Colafranceschi M: Salvage with radiotherapy of postsurgical relapses of endometrial cancer. Tumori *66*: 475-480, 1980.

15 Phillips GL, Prem KA, Adcock LL and Twiggs LB: Vaginal recurrence of adenocarcinoma of the endometrium. Gynecol Oncol *13*: 323-328, 1982.

16 Jereczek-Fossa B, Badzio A and Jassem J: Recurrent endometrial cancer after surgery alone: results of salvage radiotherapy. Int J Radiat Oncol Biol Phys *48*: 405-413, 2000.

17 Karagol H, Saip P, Uygun K, Kucucuk S, Aydiner A and Topuz E: Evaluation of prognostic factors and comparison of systemic treatment modalities in patients with recurrent or metastatic endometrial carcinoma. Med Oncol *23*: 543-548, 2006.

18 Sartori E, Laface B, Gadducci A, Maggino T, Zola P, Landoni F and Zanagnolo V: Factors influencing survival in endometrial cancer relapsing patients: a Cooperation Task Force (CTF) study. Int J Gynecol Cancer *13*: 458-465, 2003.

19 Ueda Y, Enomoto T, Egawa-Takata T, Miyatake T, Yoshino K, Fujita M, Matsuzaki S, Yokoyama T, Miyoshi Y and Kimura T: Endometrial carcinoma: better prognosis for asymptomatic recurrences than for symptomatic cases found by routine follow-up. Int J Clin Oncol. 2010 Apr 28. [Epub ahead of print].

20 Barlin JN, Puri I and Bristow RE: Cytoreductive surgery for advanced or recurrent endometrial cancer: a meta-analysis. Gynecol Oncol *118*: 14-8, 2010.

21 Ueda Y, Miyake T, Egawa-Takata T, Miyatake T, Matsuzaki S, Yokoyama T, Yoshino K, Fujita M, Enomoto T and Kimura T: Second-line chemotherapy for advanced or recurrent endometrial carcinoma previously treated with paclitaxel and carboplatin, with or without epirubicin. Cancer Chemother Pharmacol 2010 Jun 20. [Epub ahead of print].

Received June 14, 2010
Revised July 29, 2010
Accepted August 16, 2010

Int J Clin Oncol
DOI 10.1007/s10147-010-0146-6

ORIGINAL ARTICLE

Questionnaire survey of the current status of radical trachelectomy in Japan

Shinya Sato · Daisuke Aoki · Hiroaki Kobayashi · Tsuyoshi Saito · Ryuichiro Nishimura · Tadayoshi Nagano · Nobuo Yaegashi · Takayuki Enomoto · Junzo Kigawa

Received: 21 September 2010 / Accepted: 21 October 2010

Abstract
Background The number of young patients with cervical cancer has been increasing recently in Japan. Radical trachelectomy is a potential option for patients who wish to preserve their fertility, but its status is not clear. The present survey was conducted to clarify the status of radical trachelectomy in Japan.
Methods Questionnaires were mailed to 164 selected institutions based on tumor registration with the Japanese Obstetrics Gynecology Society. The subjects were patients undergoing radical trachelectomy between 2000 and 2008.
Results The response rate to the questionnaire was 88.4% (145/164). Radical trachelectomy was performed on 269 patients in 26 institutions (17.9%) . Most cases (74.7%, 201/269) underwent an abdominal approach. Three institutions had performed more than 21 cases (max. 61 cases), whereas 8 institutions had performed only one case. Twenty pregnancies and 13 deliveries were achieved and the frequency of delivery later than the 29th gestational week was 62% (8/13). "Tumor size ≤2 cm (81%)" and "stage ≤Ib1 (96%)" were commonly regarded as indications for radical trachelectomy. On the other hand, 46% of the centers did not consider the histological type as an indication.
Conclusion This survey is the first report on the current status of radical trachelectomy in Japan. It reveals a difference in the criteria for surgery applied in each institution.

Keywords Cervical cancer · Radical trachelectomy · Fertility-sparing

S. Sato (✉)
Department of Obstetrics and Gynecology,
School of Medicine, Tottori University, 36-1 Nishi-cho,
Yonago, Tottori 683 8504, Japan
e-mail: sshinya@med.tottori-u.ac.jp

D. Aoki
Department of Obstetrics and Gynecology,
School of Medicine, Keio University, Tokyo, Japan

H. Kobayashi
Department of Gynecology and Obstetrics,
Graduate School of Medical Sciences, Kyusyu University,
Fukuoka, Japan

T. Saito
Department of Obstetrics and Gynecology, Sapporo Medical University, School of Medicine, Sapporo, Japan

R. Nishimura
Department of Gynecologic Oncology, Hyogo Cancer Center,
Akashi, Japan

T. Nagano
Department of Obstetrics and Gynecology, Kitano Hospital,
Osaka, Japan

N. Yaegashi
Department of Obstetrics and Gynecology,
Tohoku University School of Medicine, Sendai, Japan

T. Enomoto
Department of Obstetrics and Gynecology,
Osaka University Graduate School of Medicine,
Osaka, Japan

J. Kigawa
Cancer Center, Tottori University Hospital, Tottori, Japan

Published online: 19 November 2010

Introduction

Cervical cancer is the second most common female cancer [1]. Radical hysterectomy is generally considered a therapeutic option for patients with stage Ib cervical cancer; in Japan, most patients with stage Ib are treated with radical hysterectomy. Approximately 15% of all cervical cancers and 45% of surgically treated stage Ib cancers occur in women under the age of 40 [2]. Recently, the incidence of young patients with cervical cancer has increased, and many women are diagnosed with this cancer during their reproductive years [3]. Accordingly, fertility preservation is an important issue for gynecological oncologists.

Radical trachelectomy was introduced as an alternative to radical hysterectomy for the treatment of young patients with cervical cancer wishing to preserve their fertility [4]. There are many reports on radical trachelectomy [5–8]; however, its status is not clear in Japan. We therefore conducted a questionnaire survey to clarify the current status of radical trachelectomy in Japan.

Material and methods

One hundred and sixty-four institutions were selected based on tumor registration with the Japanese Obstetrics Gynecology Society. These included all of the main institutions in Japan, and were sent a questionnaire asking about subjects undergoing radical trachelectomy between 2000 and 2008, including the number of radical trachelectomies and pregnancies after surgery, and eligibility. Data were collected from the patients' medical records.

Results

Responses to questionnaire were obtained from 145 institutions (response rate 88.4%). Of 145 institutions, 26 (17.9%) performed radical trachelectomy, and 269 patients had undergone radical trachelectomy (abdominal approach 201, laparoscopic approach 40, vaginal approach 28). The number of surgeries ranged from 1 to 61 (Table 1). Three institutions had treated over 20 cases, whereas 8 institutions had treated only one case. Among the 269 patients who had undergone radical trachelectomy, 20 women became pregnant, and 13 delivered. There were only 3 term deliveries (Table 2).

The indications for radical trachelectomy were widely distributed (Fig. 1). "Tumor size ≤2 cm (81%)" and "stage ≤Ib1 (96%)" were commonly regarded as indications. On the other hand, 46% of the institutions did not consider the histological type as an indication.

Table 1 Distribution of patients undergoing radical trachelectomy

Number	Institutions
≤5	17
6–10	2
11–20	4
≥21	3

The number of surgeries ranged from 1 to 61. Three institutions had performed more than 21 cases, whereas 8 institutions had performed only one case

Table 2 Obstetric outcomes

Gestational week	Number
≤21 weeks	7
21–28 weeks	5
29–32 weeks	2
33–36 weeks	3
≥37 weeks	3
Total	20

Twenty pregnancies and 13 deliveries were achieved and the frequency of delivery later than the 29th gestational week was 62% (8/13)

Discussion

The recommended surgical treatment for women with stage Ib2–Ib1 cervical cancer is a radical hysterectomy and bilateral pelvic lymphadenectomy [9]; however, many women diagnosed with cervical cancer have not completed their childbearing. There is increasing evidence in the literature that radical trachelectomy is a viable option for young women with cervical cancer who wish to preserve their fertility [10, 11]; however, the eligibility for this procedure is controversial. Additionally, the status of radical trachelectomy is not clear [3, 4, 12]. This is the first report concerning its status in Japan.

Because the response rate was extremely high (88.4%) in this survey, our results should be reliable and describe the actual situation in Japan. Although 26 institutions had performed radical trachelectomy, over half of the patients (55.3%) had received the surgery in only 3 institutions. On the other hand, 8 institutions had treated only one case. In our series, 74.7% patients had undergone radical trachelectomy with an abdominal approach. Additionally, total laparoscopic surgery was performed in only one institution. In the literature, the vaginal approach has been slow to be adopted, mainly because most gynecological oncologists are not trained in radical vaginal surgery [3].

As regards the obstetric outcomes, 20 women became pregnant, resulting in 7 abortions before 22 gestational

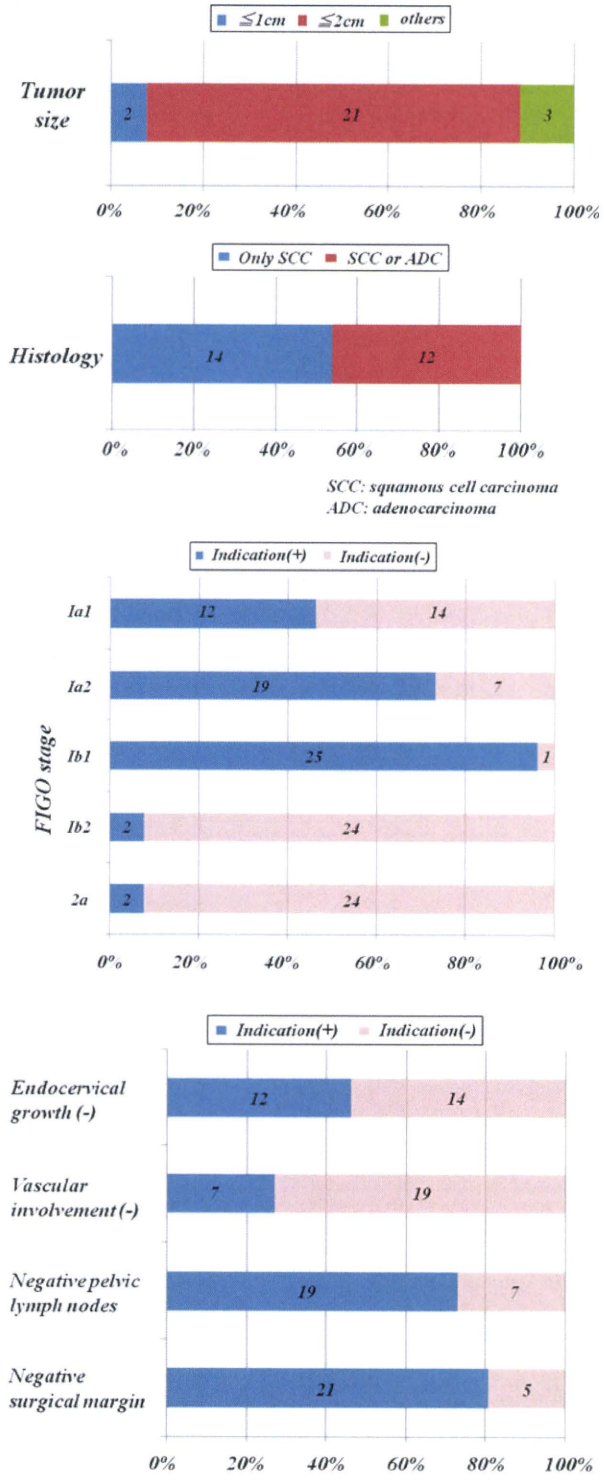

Fig. 1 Surgical indications for radical trachelectomy. Different criteria for radical trachelectomy were applied in each institution. "Tumor size ≤2 cm (81%)" and "stage ≤Ib1 (96%)" were commonly regarded as indications for radical trachelectomy. On the other hand, 46% of the centers did not consider the histological type as an indication

weeks and 13 deliveries. The frequency of delivery later than the 29th gestational week was 62% (8/13). Few obstetric outcomes with radical trachelectomy have been reported in Japan. Those results suggested that the management after radical trachelectomy, including high risk pregnancy, is important.

The criteria for performing radical trachelectomy have been reported by several authors [3–6]. The suggested criteria were, in principle, as follows: "A desire for future fertility", "FIGO stage Ia1 with lymphovascular invasion, stage Ia2, or Ib1", "Tumor size ≤2 cm", "Tumor limited to the cervix", and "No evidence of pelvic lymph node metastasis and/or other distant metastasis". Indications such as "Tumor size ≤2 cm" and "Stage Ia1–Ib1" were commonly acceptable in our series. On the other hand, this survey revealed a difference in the surgical criteria applied in each institution. Radical trachelectomy for patients with tumor size >2 cm or over stage Ib2 was carried out in only a few institutions, and the outcome of patients was not good (data not shown).

The present study suggests that a consensus on radical trachelectomy is necessary in Japan. Further experience of radical trachelectomy in Japan will help to delineate the indications for surgery and the prognosis.

Acknowledgments We thank all the institutions that participated in this study.

Conflict of interest No author has any conflict of interest.

References

1. Scarinci IC, Garcia FA, Kobetz E et al (2010) Cervical cancer prevention: new tools and old barriers. Cancer 116:2531–2542
2. Covens A, Rosen B, Murphy J et al (2001) Changes in the demographics and perioperative care of stage Ia(2)/Ib(1) cervical cancer over the past 16 years. Gynecol Oncol 81:133–137
3. Gien LT, Covens A (2010) Fertility-sparing options for early stage cervical cancer. Gynecol Oncol 117:350–357
4. Ramirez PT, Schmeler KM, Soliman PT et al (2008) Fertility preservation in patients with early cervical cancer: radical trachelectomy. Gynecol Oncol 110:S25–S28
5. Milliken DA, Shepherd JH (2008) Fertility preserving surgery for carcinoma of the cervix. Curr Opin Oncol 20:575–580
6. Nishio H, Fujii T, Kameyama K et al (2009) Abdominal radical trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer in a series of 61 women. Gynecol Oncol 115:51–55
7. Rodriguez M, Guimares O, Rose PG (2001) Radical abdominal trachelectomy with uterine conservation and subsequent pregnancy in the treatment of early invasive cervical cancer. Am J Obstet Gynecol 185:370–374
8. Cibula D, Slama J, Svarosky J et al (2009) Abdominal radical trachelectomy in fertility-sparing treatment of early-stage cervical cancer. Int J Gynecol Cancer 19:1407–1411

9. Nagase S, Inoue Y, Umesaki N et al (2010) Evidence-based guidelines for treatment of cervical cancer in Japan: Japan Society of Gynecologic Oncology (JSGO) 2007 edition. Int J Clin Oncol 15:117–124
10. Beiner ME, Hauspy J, Rosen B et al (2008) Radical vaginal trachelectomy vs. radical hysterectomy for small early stage cervical cancer: a matched case–control study. Gynecol Oncol 110:168–171
11. Petignat P, Stan C, Magevand E et al (2004) Pregnancy after trachelectomy: a high-risk condition of preterm delivery. Report of case and review of the literature. Gynecol Oncol 94:575–577
12. Boss EA, van Golde RJT, Beerendonk CCM et al (2005) Pregnancy after radical trachelectomy: a real option? Gynecol Oncol 99:S152–S156