浸潤と分化度がリンパ節転移と密接に関連していることを示した[3]．非類内膜型の組織型，子宮下部・頸部浸潤，脈管侵襲などは筋層浸潤や分化度とともに重要なリンパ節転移リスク因子であり，重要な予後因子である．子宮体部にとどまっていると考えられた体癌が手術摘出物の病理組織学的所見から実際は体部外へ進展していることがしばしば認められること，また術後病理組織学的所見から再発・死亡リスクについての重要な情報が得られるためFIGO進行期は手術進行期に改訂された．このFIGO(1988)進行期にはリンパ節転移が進行期決定因子として組み込まれており，リンパ節郭清/生検を含むsurgical stagingを行うことが必要となった．

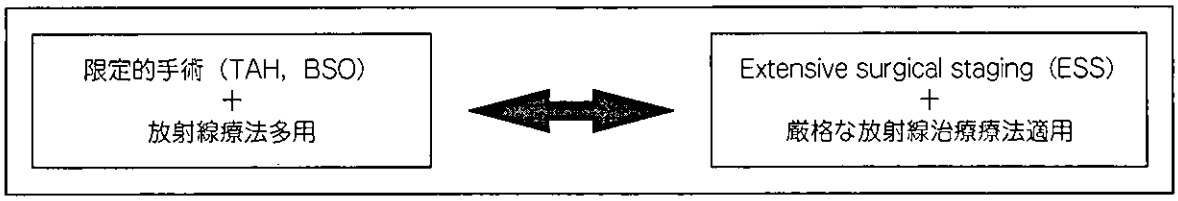
FIGO(1988)進行期分類では骨盤あるいは傍大動脈節転移はⅢc期とされる．しかし全例に手術的にリンパ節転移の有無を検索すべきか，リンパ節の検索を行う場合に郭清すべきかあるいはいわゆるサンプリングで行うべきか，対象となるリンパ節部位はどこかなどの点についてはいまだに大いに議論のあるところである．当然ながらリンパ節転移リスクが十分に低ければ郭清/サンプリングの必要はない．前述したGOGの報告ではG1で内膜限局であれば骨盤リンパ節，傍大動脈節ともに転移リスクは極めて低く(0/44)，逆にG3で筋層浸潤が＞2/3の場合のリスクは骨盤リンパ節34％(22/64)，傍大動脈節23％(15/64)と高い．われわれの教室の成績もリンパ節転移は内膜限局で0/37，筋層浸潤＜1/2で8.1％，筋層浸潤＞＝1/2で35.1％と筋層浸潤と密接に関連しており，内膜限局では郭清/サンプリングは不要であることを示している．

2．リンパ節転移の有無を検索するには傍大動脈節を含めた系統的郭清，骨盤リンパ節のみの選択的郭清，サンプリング(骨盤リンパ節の一部，傍大動脈節の一部)のいずれが適切か：体癌のリンパ節転移は骨盤から傍大動脈領域にわたる広い範囲に起こるため一部の検索では不十分である—How and Why

体癌のリンパ節転移部位について詳細に検討すると，リンパ節転移が多く認められるのは閉鎖節，総腸骨節，傍大動脈節(326b1)である．頸癌とは大きく異なり，個々のリンパ節転移部位としてみた場合これらは同等であり，いずれかを郭清し，あるものは手を付けずに残すというのでは合理性がないといえよう．頻度はそれらよりも低いが下腸間膜動脈と腎静脈の間に位置する傍大動脈節(326b2)，腸骨間節，外腸骨節，深鼠径節，仙骨節など広い範囲のリンパ節に転移が起こりうる．孤立性転移部位は閉鎖節が最も多い．しかし傍大動脈節のみに転移を認める場合もある．部位別の転移頻度と孤立性転移のパターンからすると傍大動脈節転移には骨盤リンパ節転移と同時におこるルートと骨盤リンパ節転移の拡大の結果おこるルートとが存在すると推測される[4]．

3．体癌治療における婦人科腫瘍医の役割：リンパ節郭清を含むstagingは手技と周術期管理に熟練した婦人科腫瘍医を擁する施設で行われることが患者のQOLの面から重要である—Who，When，Where and Why

最近の報告は習熟した腫瘍専門医が行えば後腹膜リンパ節郭清を含むsurgical stagingは比較的安全に行えることを示している[5]．FIGO手術進行期の導入は体癌治療の考え方に大きなインパクトを与えた[6]．現在体癌治療に用いられる治療戦略は，リンパ節郭清/サンプリングを含まない限定的な手術(単摘＋両附属器摘出)を行い，術後放射線治療を多用するという治療方針と，完全なsurgical stagingを行いリンパ節転移がなければ骨盤照射は行わないという治療方針に大別され，これにそれぞれの施設でいくらかの修正が加えられていると考えられる(図1)．体癌術後にルーチンに放射線治療を行うことは治療成績改善に寄与せず患者にbenefitはない[7][8]．正確なstagingに基づき術後補助療法の要否を層別化し不要な補助療法を避けることはQOL(morbidity)の面から患者にと



（図1） 体癌のFIGO手術進行期分類の導入による治療パラダイムの変化

って大きなbenefitであり，surgical stagingが導入されたことは体癌治療に大きなparadigm転換をもたらしたと考えられる[6]．体癌治療を婦人科腫瘍専門医が行った場合と一般婦人科医が行った場合のstagingの内容，術後治療およびQOL(morbidity)を比較した最近の報告をみると，専門医が治療した場合の郭清/サンプリング施行率が83%であったのに対して，一般医ではわずか26%にとどまっており，平均リンパ節個数も19.5対7.7と大きな開きがある．それにも関わらず術中出血量や輸血施行率は同じか専門医の方が少ないという結果であった．強調されるべき点は完全なsurgical stagingを行うことにより術後放射線治療施行率を一般医の21.7%から専門医の8.7%と1/3に減らすことができたということである[9]．不要な放射線治療併用を避け得たことは患者のQOLと医療資源の適正使用という面からリンパ節郭清を含むsurgical stagingの意義を示したものといえよう．また傍大動脈節郭清を加えた場合にはリンパ節再発を有意に減らし，傍大動脈節郭清を行うことが多変量解析でもより長期の生存期間と関連することが示されている[10]．骨盤リンパ節転移陽性例の半数以上が傍大動脈節転移を伴っており，骨盤リンパ節郭清のみを行った場合には多くの転移陽性傍大動脈節を残すことになることからも納得しうることである．当然ながら悪性腫瘍，骨盤内手術，肥満は術後血栓症発症のリスク因子であり血栓症予防措置と迅速・適切な血栓症診断と治療には十二分に配慮すべきである[11]．

4．今後の展望：郭清を含めた治療の個別化が必要である—Future direction

腫瘍マーカー，MRI，組織型・分化度などからリンパ節転移リスクを評価する試みもされているが[12][13]，現時点では後腹膜リンパ節転移など病理組織学的因子を術前に把握することに有用であることが確定された手段はなく，高分化型で内膜限局の腫瘍など極めてリンパ節転移のリスクが低い場合を除き，可能であれば完全なstaging手術を行うべきである．一方リンパ節転移陽性例については予後の面からさらに群分けすることができる．多変量解析を用いて予後因子の解析を行うと，転移個数や筋層浸潤が漿膜まで及ぶなどといった腫瘍の拡大(Tumor extension)と脈管侵襲，核異型度，組織型，p53変異などといった腫瘍の悪性度(Tumor biology)の因子が独立した因子として現れてくる[14]~[17]．傍大動脈郭清を含めた手術治療を行い長い生存期間を得る傍大動脈節転移患者も多い[18][19]が，現在用いうる補助療法(放射線あるいは化学療法)の組み合わせによる治療戦略が奏功しない体癌があることも事実であり，今後molecular markerの網羅的検索などの新しい技術の導入がリンパ節郭清を含めた治療そのものの個別化を可能とするかが今後の課題である．

まとめ

体癌治療において，リンパ節郭清を含む完全なsurgical stagingは(What)，熟練し

た婦人科腫瘍医がスタッフなどの充実した施設で(When, Who, Where)行うことにより，合併症を大きく増加させることなく遂行でき，不要な術後療法を減らし術後療法による合併症を回避でき，また予後改善効果も期待できることから(Why)，内科的合併症などのために不適当と判断される場合を除いて標準治療とみなされるべきである．その際，傍大動脈節は骨盤リンパ節と同程度の転移頻度を示す部位であり，骨盤リンパ節とともに傍大動脈節も郭清すべきである(How)．

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Lymph-vascular space invasion and number of positive para-aortic node groups predict survival in node-positive patients with endometrial cancer☆

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Abstract

Objective. The aim of this study was to determine pathologic variables associated with disease-specific survival of node-positive patients with endometrial carcinoma treated with combination of surgery including pelvic and para-aortic lymphadenectomy and adjuvant chemotherapy.

Methods. Survival of 55 node-positive endometrial carcinoma patients prospectively treated with surgery and adjuvant chemotherapy between 1982 and 2002 at Hokkaido University Hospital was compared to various histopathologic variables. All patients underwent primary surgical treatment including pelvic and para-aortic lymphadenectomy followed by adjuvant chemotherapy consisting of intravenous cisplatin, doxorubicin, and cyclophosphamide. Survival analyses were performed by the Kaplan–Meier curves and the log-rank test. Independent prognostic factors were determined by multivariate Cox regression analysis using a forward stepwise selection.

Results. Among 303 consecutive endometrial cancer patients treated during the period of this study, 55 patients (18.2%), including 44 without peritoneal metastasis (FIGO stage IIIc) and 11 with peritoneal metastasis (FIGO stage IV), were found to have retroperitoneal lymph node metastasis. Multivariate Cox regression analysis revealed that peritoneal metastasis and lymph-vascular space invasion (LVSI) were independently related to poor survival in node-positive endometrial carcinoma. The estimated 5-year survival rate of stage IIIc patients with or without moderate/prominent LVSI was 50.9% and 93.3%, respectively with statistically significant difference ($P = 0.0024$). The estimated 5-year survival rate of stage IV patients was 20.0%. Prognosis of stage IIIc patients could be stratified into three groups by the number of positive para-aortic node (PAN) with an estimated 5-year survival rate of 86.4% for no positive PAN ($n = 23$), 60.4% for one positive PAN ($n = 13$), and 20.0% for ≥ 2 positive PAN ($n = 8$). The difference of survival rate between no or one positive PAN and ≥ 2 positive PAN was statistically significant ($P = 0.0007$ for no positive PAN vs. ≥ 2 positive PAN, $P = 0.0319$ for one positive PAN vs. ≥ 2 positive PAN). Multivariate analysis including number of positive PAN groups showed that LVSI, number of positive PAN groups were independent prognostic factors for survival. Survival of patients with stage IIIc disease could be stratified into three groups by combination of LVSI and number of positive PAN groups with an estimated 5-year survival rate of 93.3% for no or one positive PAN group with nil or minimal LVSI, 62.6% for no or one positive PAN group with intermediate or prominent LVSI, and 20.0% for ≥ 2 positive PAN groups irrespective of LVSI ($P = 0.0002$ for no or one positive PAN group with nil or minimal LVSI vs. ≥ 2 positive PAN groups, $P = 0.0223$ for no or one positive PAN group with nil or minimal LVSI vs. no or one positive PAN group with intermediate or prominent LVSI, $P = 0.0388$ for no or one positive PAN group with intermediate or prominent LVSI vs. ≥ 2 positive PAN groups).

Conclusions. LVSI and number of positive PAN groups were independent prognostic factors for stage IIIc endometrial cancer patients. Postoperative therapy and follow-up modality need to be individualized according to LVSI and the number of positive PAN for stage IIIc patients. New molecular markers to predict the prognosis of endometrial cancer patients preoperatively should be found for individualization

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of treatment. New chemotherapy regimen including taxane needs to be considered as an adjuvant therapy for patients with node-positive endometrial cancer.

Keywords: Endometrial carcinoma; Prognostic factor; Stage IIIc; Para-aortic lymph node metastasis; Lymph-vascular space invasion

Introduction

The incidence of endometrial carcinoma is rapidly increasing in Japan, with an estimated 4800 new cases in the year 2000; this number is still much lower than that in the United States, which was 40,100 in the year 2003 [1].

A majority of patients are diagnosed as being without clinical evidence of extrauterine spread, the International Federation of Gynecology and Obstetrics (FIGO) stages I and II, and have a 5-year survival of approximately 90%. Involvement of pelvic and para-aortic lymph nodes has been recognized as a poor prognostic factor in endometrial carcinoma. The new FIGO surgical staging system classifies endometrial carcinoma with metastasis to the pelvic and/or para-aortic lymph nodes as stage IIIc. The overall 5-year survival for patients with stage IIIc disease varies considerably, depending on the presence of various risk factors, extent of lymphadenectomy, and postoperative adjuvant therapy. Data on surgically staged endometrial cancer patients with nodal involvement have been limited [2–5]. The distribution of nodal disease and its importance in predicting survival are largely unknown, since various procedures have been used to assess para-aortic and pelvic nodes in endometrial cancer, that is, biopsies from enlarged nodes only, selective nodal sampling from multiple sites, pelvic lymphadenectomy, and para-aortic and pelvic lymphadenectomy. It is obvious that para-aortic and pelvic lymphadenectomy is most accurate of these methods. However, para-aortic and pelvic lymphadenectomy is not regarded as the standard surgical procedure for endometrial cancer because the therapeutic significance of the procedure has not yet been sufficiently demonstrated [6,7]. We have routinely performed complete lymphadenectomy in all patients with endometrial cancer because (i) nodal status is the most important prognosticator [2], (ii) results of lymphadenectomy allow tailoring of postoperative adjuvant treatment, (iii) there is an apparent small survival advantage after lymphadenectomy [8], and (iv) there is no increased morbidity with lymphadenectomy [9].

We have previously reported that para-aortic lymph node metastasis is an independent prognostic factor for endometrial cancer patients as well as cell type, grade and LVSI [10,11]. However, the fact that patients with para-aortic lymph node metastasis sometimes have shown long survival suggest that specific prognostic factors may exist for node-positive endometrial carcinoma.

In this study, we tried to identify the independent histopathologic prognostic factors for endometrial carcinoma patients with lymph node metastasis, who were uniformly treated with extensive surgical staging that includes modified radical hysterectomy and systematic pelvic and para-aortic lymphadenectomy followed by adjuvant chemotherapy. We also described the distribution of nodal disease in FIGO stage IIIc endometrial cancer and evaluated whether nodal distribution is related to survival.

Materials and methods

Patients

A total of 303 patients with endometrial carcinoma underwent primary radical surgical treatment from 1982 to 2002 at the Department of Obstetrics and Gynecology, Hokkaido University Hospital. Among 303 patients, 55 patients showed positive retroperitoneal lymph nodes. All subjects underwent modified radical hysterectomy, bilateral salpingo-oophorectomy, and systematic retroperitoneal lymphadenectomy which consisted of complete dissection of pelvic and para-aortic lymph nodes from the femoral ring to the level of the renal vein. All lymphatic tissues that surrounded the arteries and veins were completely removed. The FIGO (1988) stage of the patients was as follows: 44 (80%) stage IIIc and 11 (20%) stage IV. Stage IV disease with distant metastasis (liver or lung metastasis) was excluded from this analysis. We, therefore, defined stage IV patients as node-positive patients with peritoneal metastasis. Median follow-up period was 43 months (1–200 months). The patients' characteristics are shown in Table 1. All patients were treated with an adjuvant chemotherapy of CAP (cyclophosphamide: 350 mg/m^2, adriamycin: 40 mg/m^2 and cisplatin: 50–70 mg/m^2) every 3 weeks.

The following histopathologic prognostic factors were included in the survival analyses: FIGO (1988) stage, histologic subtype, depth of myometrial invasion, architectural grade (AG), nuclear grade (NG), LVSI, ovarian metastasis, PAN metastasis, and number of positive PAN group. All risk factors except number of positive PAN were determined as previously described [10,11]. We defined a node group by its laterality and location. Pelvic node groups include common iliac nodes, external iliac nodes, internal iliac nodes, obturator nodes, median deep inguinal nodesal deep inguinal nodes, parametrial nodes, and sacral nodes. PAN metastasis was investigated by dividing the metastatic group according to the sites above and below the inferior mesenteric artery and left and right sections bordering on the midline of the aorta as previously described [3]. In this analysis, caval (pre-, peri-, retro-) and aortocaval nodes were

Table 1
Clinicopathologic characteristics of 55 patients with lymph node metastasis

	No.	%
FIGO stage (1988)		
IIIc	44	80.0
IV	11	20.0
Histologic subtype		
Endometrioid	43	78.2
Serous/Clear	12	21.8
Architectural grade		
1	16	29.1
2	27	49.1
3	12	21.8
Nuclear grade		
1	14	25.4
2	25	45.5
3	16	29.1
Depth of myometrial invasion		
< = 1/2	15	27.3
> 1/2	40	72.7
Lymph-vascular space invasion		
Nil/minimal	20	36.4
Moderate/prominent	35	63.6
Cervical invasion		
Negative	28	50.9
Positive	27	49.1
Ovarian metastasis		
Negative	37	67.3
Positive	18	32.7
Para-aortic lymph node metastasis		
Negative	26	47.3
Positive	29	52.7

included in right para-aortic nodes. PAN groups, therefore, consists of four groups.

Statistics

Correlation between the variables was analyzed using chi^2 test. Patients survival was calculated using Kaplan–Meier method. The significance of the survival difference was examined by the log-rank test. Univariate and multivariate survival analyses were performed using the Cox regression model with disease-specific overall survival as the outcome measure. Forward stepwise procedure was used to select the independent variable in multivariate analysis. $P < 0.05$ was considered statistically significant. Statistical analyses were performed with the Statview software package (SAS Institute, Inc, Cary, NC).

Results

Univariate and multivariate survival analysis for all node-positive patients

Age of patients ranged from 40 to 75 (median 58) years. The univariate analysis revealed that the FIGO (1988) stage (IIIc vs. IV, $P < 0.0001$), the histologic subtype (endometrioid vs. serous/clear, $P = 0.0216$), architectural grade (G1/2 vs. G3, $P = 0.0024$), nuclear grade (G1 vs. G2/3, $P = 0.0201$), depth of myometrial invasion (absence or presence of serosal invasion, $P < 0.0001$), LVSI (none/minimal vs. moderate/prominent, $P = 0.0026$), cervical invasion ($P = 0.0159$), and PAN metastasis ($P = 0.0078$) were shown to be related to poor survival. Ovarian metastasis was not related to survival ($P = 0.3306$) (Table 2).

Multivariate analysis, which included the prognostic factors determined by univariate analysis to have statistical significance, was performed using a forward stepwise procedure (Table 3). It was shown that FIGO (1988) stage ($P < 0.0001$) and LVSI ($P = 0.0002$) were independent prognostic factors. We could stratify the patients into three prognostic risk-groups by integrating those two histopathologic risk factors, that is, low risk group (group A: stage IIIc with nil/minimal LVSI, $n = 19$), intermediate risk group (group B: stage IIIc with moderate/prominent LVSI, $n = 25$) and high risk group (group C: stage IV with any LVSI, $n = 11$) with an estimated 5-year survival rate of 93.3%, 50.9%, and 20.0%, respectively (Fig. 1). There was statistically significant difference of survival rate between each group (A vs. B: $P = 0.0024$, B vs. C: $P < 0.0001$, A vs. C: $P < 0.0001$). Because prognostic impact of FIGO (1988) stage was extremely strong for the survival of node-positive patients, we performed further analysis on stage IIIc patients alone ($n = 44$).

Lymph node metastasis in stage IIIc patients

Incidences of pelvic lymph node (PLN) metastasis alone, PAN metastasis alone, and both PLN and PAN metastasis were 52.3% (23/44), 4.5% (2/44), and 43.2% (19/44),

Table 2
Univariate and multivariate Cox regression analysis of prognostic factors of node positive endometrial carcinoma

Univariate		Multivariate		
Prognostic factor	P value	Risk ratio	95% CI	P value
FIGO (1988) stage	< 0.0001	11.2	4.0–31.3	< 0.0001
Histologic subtype	0.0216	–	–	NS
Architectural grade	0.0024	–	–	NS
Nuclear grade	0.0201	–	–	NS
Lymph-vascular space invasion	0.0026	9.3	2.1–41.7	0.0033
Myometrial invasion	< 0.0001	–	–	NS
Cervical invasion	0.0159	–	–	NS
Ovarian metastasis	0.3306	–	–	S
Para-aortic node metastasis	0.0078	–	–	NS

NS: not significant.
FIGO (1988) stage: stage IIIc vs stage IV determined by the presence of peritoneal metastasis.
Tumor cell type: endometrioid vs serous/clear cell.
Architectural grade: G1/2 vs G3.
Nuclear grade: G1 vs G2/3.
Lymph-vascular space invasion: (−)/(+) vs (++)/(+++).
Myometrial invasion: serosal invasion (−) vs (+).
Cervical in vs. ion: (−) vs (+).
Ovarian metastasis: (−) vs (+).
Para-aortic lymph node metastasis: (−) vs (+).

Table 3
Univariate and multivariate Cox regression analysis of prognostic factors of stage IIIc endometrial carcinoma

Univariate		Multivariate		
Prognostic factor	*P* value	Risk ratio	95% CI	*P* value
Histologic subtype	0.7226	–	–	NS
Architectural grade	0.3911	–	–	NS
Nuclear grade	0.0605	–	–	NS
Lymph-vascular space invasion	0.0173	8.8	1.1–71.4	0.0413
Myometrial invasion	0.6036	–	–	NS
Cervical invasion	0.4577	–	–	NS
Ovarian metastasis	0.1815	–	–	NS
Number of positive PAN	0.0016	3.9	1.2–13.0	0.0260

NS: not significant.
Tumor cell type: endometrioid vs serous/clear cell.
Architectural grade: G1/2 vs G3.
Nuclear grade: G1 vs G2/3.
Lymph-vascular space invasion: (−)/(+) vs (++)/(+++).
Myometrial invasion: serosal invasion (−) vs (+).
Cervical invasion: (−) vs (+).
Ovarian metastasis: (−) vs (+).
Number of positive PAN: 0, 1 vs ≥ 2.

respectively. The estimated 5-year survival rate of patients without or with PAN metastasis was 86.4%, 48.1%, respectively (Fig. 2). The difference was statistically significant ($P = 0.0108$).

Prognostic impact of the number of positive PLN groups in stage IIIc patients

The estimated 5-year survival rate for patients with one positive PLN group was 79.3% and that for patients with ≥ 2 positive PLN groups was 60.8%. The difference of survival was not statistically significant. However, 21 of 22 patients (95.5%) with no or one positive PLN group had no or one positive PAN group and only one of 22 patients (4.5%) had two positive PAN groups, while 15 of 22 patients (68.2%) with ≥ 2 positive PLN groups had no or one positive PAN group and 7 of 22 patients (31.8%) had ≥

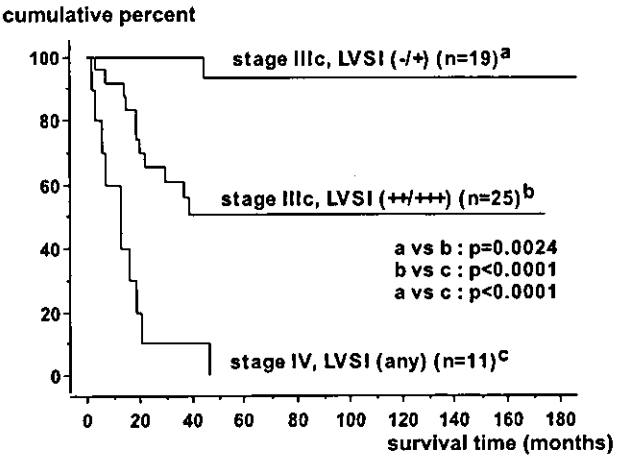

Fig. 1. Survival of node-positive patients with endometrial carcinoma by combination of FIGO (1988) stage and LVSI.

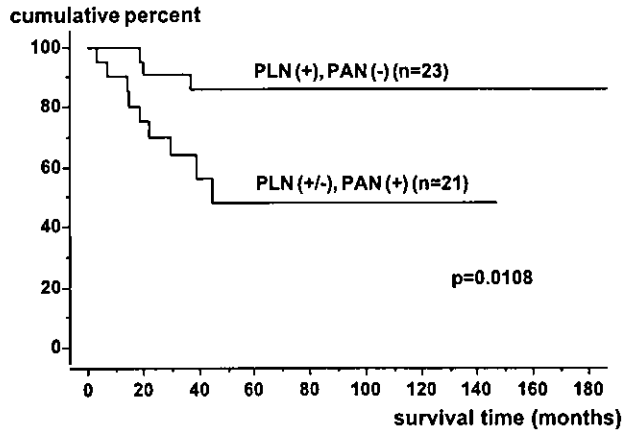

Fig. 2. Survival of patients with stage IIIc endometrial carcinoma by PAN metastasis.

2 positive PAN groups. There was a statistically significant difference in incidence of ≥ 2 positive PAN groups between patients who had no or one positive PLN group and those who had ≥ 2 positive PLN groups ($P = 0.023$). (Table 4).

Prognostic impact of the number of positive PAN groups in stage IIIc patients

Fig. 3 shows the survival of stage IIIc patients according to the number of positive PAN groups. The estimated 5-year survival rate was 86.4% for patients without positive PAN group ($n = 23$), 60.4% for those with one positive PAN group ($n = 13$), and 20.0% for those with ≥ 2 positive PAN groups ($n = 8$). There was statistically significant difference between no positive PAN group and ≥ 2 positive PAN groups ($P < 0.0007$), between one positive PAN group and ≥ 2 positive PAN groups ($P = 0.0319$). There was no statistically significant difference between no positive PAN group and one positive PAN group ($P = 0.1354$).

Univariate and multivariate survival analysis for stage IIIc patients

Since the number of positive PAN group was shown to have significant impact on the survival of stage IIIc patients,

Table 4
Incidence of para-aortic lymph node metastasis according to number of positive pelvic lymph node groups in stage IIIc endometrial cancer patients

	Positive PAN group		Total
	0, 1	≥ 2	
positive PLN group			
0, 1	21	1	22
> 2	15	7	22
Total	36	8	44
			$P = 0.0023$

PLN: pelvic lymph node, PAN: para-aortic lymph node.
There was a statistically significant difference in incidence of ≥ 2 positive PAN groups between patients who had no or one positive PLN group and those who had ≥ 2 positive PLN groups ($P = 0.023$).

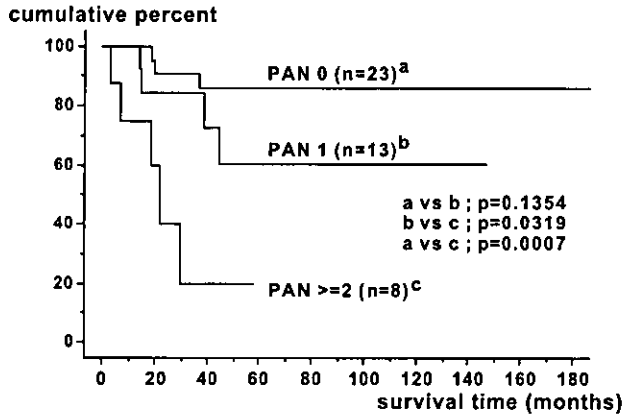

Fig. 3. Survival of patients with stage IIIc endometrial carcinoma by the number of positive PAN group.

we included number of positive PAN groups in the univariate analysis instead of presence or absence of PAN metastasis. The univariate analysis revealed that the LVSI ($P = 0.0173$), number of positive PAN groups ($P = 0.0016$) were shown to be related to poor survival. Histologic subtype ($P = 0.7226$), architectural grade ($P = 0.3911$), nuclear grade ($P = 0.0605$), depth of myometrial invasion ($P = 0.6036$), cervical invasion ($P = 0.4577$), ovarian metastasis ($P = 0.1815$) were not related to poor survival (Table 3, Fig. 4).

Multivariate analysis revealed that both LVSI ($P = 0.0413$) and number of positive PAN groups ($P = 0.026$) were independent prognostic factors. Survival of patients with stage IIIc disease could be stratified into three groups by combination of LVSI and number of positive PAN group with an estimated 5-year survival rate of 93.3% for no or one positive PAN group with nil or minimal LVSI (group D), 62.6% for no or one positive PAN group with intermediate or prominent LVSI (group E), and 20.0% for ≥ 2 positive PAN groups irrespective of LVSI (group F). The difference of survival rate between each group was statistically significant ($P = 0.0002$ for group D vs. group F, $P = 0.023$ for group D vs. group E, $P = 0.0388$ for group E vs. group F.

Discussion

Stages I and II endometrial carcinomas have shown a favorable prognosis by combination of surgery, radiotherapy, and/or chemotherapy. Some histopathologic factors have been found to be related to prognosis of endometrial carcinoma. Lymph node metastasis is one of the most important prognostic factors of endometrial carcinoma and advanced endometrial carcinoma with lymph node metastasis (IIIc/IV) has been shown to have poorer prognosis. In our series of patients treated in the same manner, the prognosis for patients with stage IIIa endometrial carcinoma was excellent with an estimated 5-year survival of over 90% (patients with stage IIIb disease were not found in our series). However, the prognosis for patients with stage IIIc endometrial carcinoma with an estimated 5-year survival rate of 79.6% was poorer than that of stage IIIa in spite of intensive treatment consisting of extended surgery including pelvic and para-aortic lymphadenectomy and systemic adjuvant chemotherapy. We, therefore, performed retrospective analysis on the prognostic factors for node-positive patients to determine appropriate therapeutic and follow-up modality to achieve their favorable prognosis.

Concerning the distribution of lymph node metastases, 95.5% (42/44) of patients with nodal disease had pelvic node metastases and 45.2% (19/42) of patients with PLN metastases had concomitant PAN metastases. McMeekin et al. [4] analyzed nodal distribution in 47 cases of stage IIIc endometrial cancer and found that an increasing number of positive PLN was associated with PAN metastasis. Our result on nodal distribution in stage IIIc patients is similar to McMeekin et al. [4], i.e., patients with single positive PLN group rarely have multiple positive PAN groups. We also found that positive aortic nodes were associated with poorer prognosis than were positive pelvic nodes alone (estimated 5-year survival 48.1% for positive PAN vs. 86.4% for negative PAN, $P = 0.0108$), suggesting that involvement of pelvic lymph nodes alone does not necessarily carry a poor prognosis as previously reported by Onda et al [12] who performed the same operative procedure as ours. This can be explained in part due to the therapeutic significance of our operative procedure including systematic pelvic and para-aortic lymphadenectomy. Para-aortic lymphadenectomy until just below the renal vein may contribute to the favorable survival of patients with multiple positive PLN groups.

In this study, we firstly reported that the survival of patients with stage IIIc endometrial carcinoma can be stratified by the number of positive PAN groups. The survival of patients without positive PAN (PLN metastasis

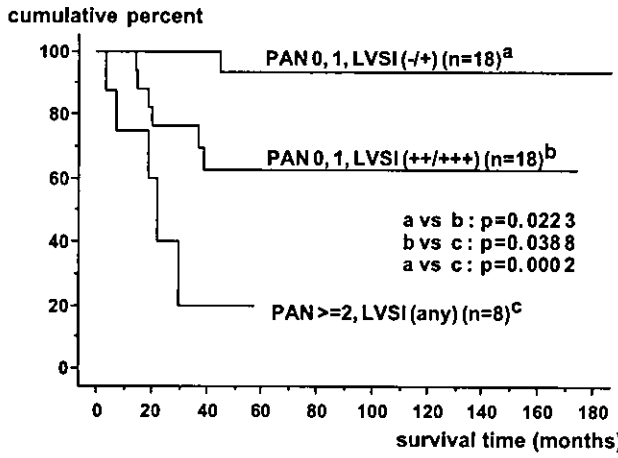

Fig. 4. Survival of patients with stage IIIc endometrial carcinoma by combination of LVSI and number of positive PAN group.

alone), with one positive PAN group, and with ≥ 2 positive PAN groups was 86.4%, 60.4%, 20.0%, respectively. The survival of patients with ≥ 2 positive PAN groups was much worse than others. Notably, there was no statistically significant difference between the patients without positive PAN group and those with one positive PAN group (P = 0.14), suggesting that single PAN metastasis is still a local disease that can be cured by complete lymphadenectomy and subsequent chemotherapy. When patients have multiple PAN metastasis, lymphadenectomy does not prolong survival.

Radiotherapy and chemotherapy have been employed as adjuvant therapies for endometrial cancer. Radiotherapy has been considered as a standard adjuvant therapy in Western countries. The result of GOG 122, however, clearly demonstrated that adjuvant chemotherapy (adriamycin and CDDP) significantly improved progression free survival and overall survival than adjuvant radiotherapy (whole abdominal radiotherapy) for stage III/IV patients [13], indicating that chemotherapy should be considered as a standard adjuvant therapy for endometrial cancer. Systemic chemotherapy has been widely accepted as a standard adjuvant therapy for endometrial cancer in Japan. Adriamycin has been used as a key drug for endometrial cancer. We have used CAP regimen for endometrial carcinoma with risk factors for recurrence. However, the poorer survival of stage IIIc patients with multiple positive PAN groups than single positive PAN group, who received adjuvant chemotherapy (CAP), clearly indicates that we should consider a new chemotherapeutic regimen to improve prognosis of node-positive patients. The most promising drug for endometrial cancer is taxane [14].

In this study, we found that LVSI and number of positive PAN group are independent prognostic factors by multivariate analysis, indicating that para-aortic lymphadenectomy should be routinely included in the surgical procedure for endometrial cancer to predict the survival of node-positive patients. We also conclude that we should investigate LVSI with more careful attention for node-positive patients. Careful investigation of LVSI, however, is time-consuming and it is impossible to evaluate LVSI preoperatively and during operation by frozen section. To individualize the therapeutic modality for each patient, we need to search for new molecular markers which can be easily assessed and reflect disease status in regard to LVSI preoperatively. There have been few reports on the useful molecular markers for survival of endometrial carcinoma. We reported that p53 overexpression by immunohistochemical staining was found to be an independent prognostic factor and the estimated 5-year survival rate of patients with stage III/IV disease without p53 overexpression was significantly better than that with p53 overexpression, indicating that p53 missense mutation, which is closely related to immunohistochemical p53 overexpression, have a significant prognostic impact on the survival of advanced endometrial carcinoma [15]. Kanamori et al. [16] reported that PTEN expression was found to be associated with prognosis for patients with advanced endometrial carcinoma undergoing postoperative chemotherapy. Yokoyama et al. [17] reported that high levels of immunoreactivity for vascular endothelial growth factor (VEGF)-D in stromal cells and its receptor, VEGF-R-3 in carcinoma cells were independent prognostic factors in endometrial carcinoma. We need to further investigate more useful prognostic factors for endometrial carcinoma using molecular biological techniques.

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Functional analysis of *p53* gene and the prognostic impact of dominant-negative *p53* mutation in endometrial cancer†

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KEYWORDS

endometrial cancer • *p53* • mutation • dominant negative • survival • serous adenocarcinoma

ABSTRACT

In addition to the loss of function, mutant p53 can possess a dominant-negative effect on wild-type p53 and may also exert gain-of-function activity. It is not clear whether the functional status of *p53* mutation contributes to differences in outcome in endometrial cancer. We collected a total of 92 RNA samples of high quality from endometrial cancer tissues, and the samples were subjected to yeast functional assay and sequencing for *p53* mutations. The detected mutant *p53* genes were further investigated for their dominant-negative activity using a yeast-based transdominance assay. *p53* mutation was found in 24 out of 92 (26.1%) tumors, of which 10 exhibited no dominant-negative activity (recessive mutation) and 14 showed dominant-negative activity. Dominant-negative *p53* mutation was related to advanced stages ($p = 0.01$), nonendometrioid type tumors ($p = 0.01$) and grade 3 tumors ($p = 0.04$). The patients with dominant-negative mutation had significantly shorter survival than patients with no mutation ($p < 0.0001$) and those with a recessive mutation ($p = 0.01$) in the *p53* gene. No difference in survival was found between the patients with tumors harboring a recessive *p53* mutation and those with tumors harboring a wild-type *p53*. Multivariate analysis revealed that dominant-negative *p53* mutation ($p = 0.019$), FIGO stage ($p = 0.0037$) and histologic subtype ($p = 0.014$) were independently related to patient survival. Dominant-negative *p53* mutation was the most important prognostic factor for stage III/IV endometrial cancer ($p = 0.0023$). In conclusion, dominant-negative *p53* mutation is often found in advanced stages and aggressive histologic subtypes of endometrial

cancer and it is a strong predictor of survival of patients with advanced endometrial cancer. To elucidate further the role of *p53* mutation in endometrial cancer, it is necessary to investigate gain-of-function activity involving dominant-negative p53 mutant proteins. © 2005 Wiley-Liss, Inc.

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ARTICLE TEXT

The *p53* tumor suppressor gene is mutated in about 50% of all tumors,[1][2][3] and more than 19,000 different somatic mutations have been identified.[4] Mutation of the *p53* gene plays a key role in the carcinogenesis and progression of many different malignancies, including endometrial cancer. p53 overexpression has been shown to predict patient survival in endometrial cancer.[5][6][7] p53 overexpression, as determined by immunohistochemistry (IHC), is a surrogate marker of missense mutation of p53 protein. Because MDM2 is a transcriptional target of p53, loss of p53 function reduces the production of MDM2. MDM2 degrades p53 protein through ubiquitination of the protein, and reduced MDM2 production will lead to an accumulation of p53 protein in the nucleus, which is detected as overexpression by IHC.[8] IHC is a convenient method for the investigation of p53 status. However, it can be affected by many factors, such as antibody used, antigen retrieval technique and subjectivity of criteria for p53 overexpression. p53 overexpression does not necessarily correspond to *p53 gene* mutation. One report showed that only 32% of tumors with exclusively nuclear staining were found to contain a *p53* gene mutation.[6] Dominant-negative *p53* mutation will lead to decreased MDM2 production, irrespective of the status of the second allele of *p53*. *p53* mutations abolishing the production of p53 protein will lead to loss of p53 protein when it is associated with loss of heterozygosity (LOH) of the second allele. Therefore, p53 overexpression can be related to both dominant-negative mutation and recessive missense mutation with LOH. It is therefore reasonable to expect that p53 overexpression does not necessarily correspond to a dominant-negative mutation of *p53*.

At least 2 distinct pathways have been proposed that contribute to cisplatin-induced apoptosis *in vitro*. One involves p53 tumor suppressor protein, and the other is mediated by the p53-related protein p73. Inhibition of p73 function by dominant-negative p73 proteins or by mutant p53 abrogates apoptosis and cytotoxicity induced by these agents.[9] Therefore, investigation into the status of p73 in endometrial cancer is expected to provide further information regarding the response to adjuvant chemotherapy in cases involving this type of cancer.

An understanding of the role played by *p53* mutation in endometrial cancer may lead to tailored treatment planning and more rational targeted approaches for treating this disease. *p53* gene mutation and LOH result in the loss of p53 function. In addition to the loss of function, mutant p53 can possess a dominant-negative effect that suppresses wild-type p53, and which may also exert gain-of-function activity.[10] There have been many studies of the prognostic significance of and/or therapeutic outcome related to the type of *p53* mutations. However, the results of such studies have been inconsistent,[11] and the significance of the dominant-negative function in terms of both prognosis and therapeutic success remains unclear at present.[12]

In order to elucidate the prognostic importance of dominant-negative activity in endometrial cancer, we surveyed the functional status of p53 protein in endometrial carcinoma using a yeast p53 functional assay and a transdominance assay. The yeast p53 functional assay tests the ability of p53 to activate transcription *in vivo* in yeast.[13][14] A modification of this method (transdominance assay) can identify the dominant-negative/recessive properties of the mutant p53.[15] This is the first report to compare directly the functional status of p53 (*i.e.*, the presence or absence of dominant-negative activity) to the survival of patients with endometrial cancer.

Material and methods

Tissue specimens

A total of 92 endometrial carcinoma tissue samples, which were obtained from the resected uterus of patients with endometrial carcinoma treated surgically at the Department of Obstetrics and Gynecology, Mainz University in Mainz, Germany, and at the Department of Gynecology, Hokkaido University Graduate School of Medicine and School of Medicine in Sapporo, Japan, were used for this study. Informed consent was obtained from all study participants. From among the 92 samples, 49 were obtained from German patients and 43 from Japanese patients. Surgical treatment was initiated during the period between April 1990 and January 2000

for the German cohort and between February 1990 and December 2002 for the Japanese cohort. The data from 23 patients of the 43 Japanese cohort participants were reported in a previous study.[16] The clinicopathologic variables, namely, FIGO stage, histologic subtype, grade of tumor and follow-up period, did not differ between the 2 cohorts, with the exception of patient age (Table I). The tissue samples included 79 cases of endometrioid-type adenocarcinoma and 13 cases of nonendometrioid adenocarcinoma. The group of nonendometrioid tumors included 11 serous adenocarcinomas, 1 clear cell adenocarcinoma and 1 squamous cell carcinoma. The treatment strategies employed in the 2 institutes were different. At Mainz University, the surgical procedure involved selective lymph node dissection rather than routine systematic lymphadenectomy. At Hokkaido University, routine systematic pelvic and paraaortic lymphadenectomy was carried out. The modality of adjuvant therapy employed at Mainz University was radiotherapy, whereas that used at Hokkaido University was chemotherapy.

Table I. Clinicopathologic Characteristics of Patients with Endometrial Carcinoma Treated in Mainz University and Hokkaido University

	Mainz, Germany (n = 49)	Sapporo, Japan (n = 43)	p
Age			
< 60	13	26	
≥ 60	36	17	0.001
FIGO stage			
I/II	37	28	
III/IV	12	15	0.27
Histologic subtype			
Endometrioid	44	35	
Nonendometrioid	5	8	0.25
Grade			
1	17	14	
2	19	17	
3	13	12	0.98
Follow-up period (months)	1-130 (median, 58.5)	5-125 (median, 41.0)	0.11

RNA extraction and reverse transcription (RT)-PCR

Total RNA was extracted from 100-200 mg of each frozen tissue sample by the guanidinium/phenol/chloroform method (TRIzol reagent; Gibco-BRL, Gaithersburg, MD). RNA integrity was verified by electrophoresis on 1% agarose gel. *p53* cDNA was synthesized at 37°C for 1 hr with 200 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco-BRL) from 1-3 μg of total RNA in 20 μl of RT buffer containing 25 pmol *p53*-specific primer RT-1 (5′-CGGGAGGTAGAC-3′), 7.5 mM dithiothreitol (DTT), 0.5 mM $MgCl_2$ and 0.5 mM of each dNTP. The *p53* cDNA was PCR-amplified in 20 μl of reaction mixture containing 2 μl of RT reaction product, 1.25 units of *Pfu* DNA polymerase (Stratagene, La Jolla, CA), 10% DMSO, 50 μM of each dNTP and 10 pmol of primers P3 [5′-ATTTGATGCTGTCCCCGGACGATATTGAA(s)C-3′, where (s) represents a phosphorothioate linkage] and P4 [5′-ACCCTTTTTGGACTTCAGGTGGCTGGAGT(s)G-3′]. PCR was run on a Thermal Cycler Model 2400 (Perkin-Elmer, Chiba, Japan) at 96°C for 1 min, then for 35 cycles of 95°C for 40 sec, 65°C for 70 sec and 78°C for 90 sec, followed by 78°C for 2 min. Satisfactory amplification was confirmed by examining the PCR product in a 1% agarose gel. Each crude PCR product was used for the transformation of yeast.

Plasmids

The yeast expression vector pSS16[17] was digested with excess amounts of *Hind*III and *Stu*I, dephosphorylated with calf intestinal alkaline phosphatase (Takara, Otsu, Japan) and electrophoresed on 1% low-melting-temperature agarose (Sea Plaque agarose; FMC, Rockland, ME). The linearized plasmids were recovered from the gel and purified with Wizard PCR prep kit (Promega, Madison, WI). A gap was created between codons 67 and 347.

Yeast p53 functional assay

The yeast functional assay was performed according to a method described previously.[14][18] The yeast reporter strain yIG397[15] was used throughout the study. The strain yIG397 contains an integrated plasmid with the ADE2 (phosphoribosylaminoimidazole carboxylase, EC 4.1.1.21) open reading frame under the control of a p53-responsive promoter. The genotype is *MATa ade2-1 leu2-3 112trp1-1 his3-11 15can1-100 ura3-1::[URA3 3*xRGC-p*CYC1-ADE2*]. When a yeast cell is transformed with a plasmid encoding mutant p53, the cell fails to express ADE2 and forms a red colony due to the accumulation of an oxidized polymerized derivative of phosphoribosyl-aminoimidazole.[19] The yeast was cultured in 100 ml of YPD medium supplemented with 200 μg/ml of adenine until the OD600 value reached 0.8. The cells were pelleted, washed with LiOAc solution containing 0.1 M lithium acetate, 10 mM Tris-HCl, pH 8.0, and 1 mM $EDTANa_2$; the cells were then pelleted again and resuspended in 500 μl of LiOAc solution. For each transformation, 50 μl of yeast suspension were mixed with 1-5 μl of unpurified *p53* cDNA PCR product, 50-100 ng of linearized plasmid, 5 μl of sonicated single-stranded salmon sperm DNA (10 mg/ml) and 300 μl of LiOAc containing 40% polyethylene glycol 4000 (Kanto, Tokyo, Japan). The mixture was incubated at 30°C for 30 min and heat-shocked at 42°C for 15 min. The yeast was then plated on a synthetic dropout (SD) medium minus leucine plus adenine (5 μg/ml) and was incubated for 48 hr in a 30°C humidified chamber. More than 200 colonies were examined on each culture plate. In this assay system, 16% was the cutoff value for *p53* mutation.[20]

Recovery of p53 plasmids from yeast and DNA sequencing

The yeast was digested with Zymolase-100T (Seikagaku-Kogyo, Tokyo, Japan), and p53 expression plasmids were extracted by the alkaline lysis method (QIAprep plasmid kit; Qiagen, Hilden, Germany) and transfected into XL-1 blue *E. coli* by electroporation. The plasmids were recovered, purified and sequenced with a DyeDeoxy Terminator Kit (Perkin-Elmer, Urayasu, Japan) on an ABI 377 automated sequencer (Applied Biosystems, Urayasu, Japan) as specified by the manufacturer's protocol and using the following primers: P3seq, 5′-ATTTGATGCTGTCCCCGGACGATATTGAAC-3′; P11seq, 5′-TACTCCCCTGCCCTCAACAAGATG-3′; P12seq, 5′-TTGCGTGTGGAGTATTTGGATGAC-3′; and P13seq, 5′-GCCCATCCTCACCATCATCACACT-3′.

Transdominance assay

The dominant-negative potential of *p53* mutation was tested using a yeast-based transdominance assay as described previously.[15] Briefly, the yeast functional assay was performed using both a plasmid with wild-type *p53* and a plasmid with mutant *p53* that had been sequence-verified. For each transformation, 50 μl of yeast suspension were mixed with 100 ng of pTSHP53, 100 ng of mutant *p53*-containing pSS16, 50 μg of sonicated single-stranded salmon sperm DNA and 300 μl of LiOAc containing 40% polyethylene glycol 4000. The mixture was incubated at 30°C for 30 min and heat-shocked at 42°C for 15 min. Yeast were then plated on SD medium minus leucine and tryptophan, but which contained a limited amount of adenine (5 μg/ml). The samples were then incubated for 48 hr in a 30°C humidified atmosphere. Double-transformant clones (Leu^+, Trp^+) giving rise to white (Ade^+) or pink/red (Ade^-) colonies were interpreted as expressing recessive and dominant-negative mutations, respectively.

Statistical analysis

The statistical significance of differences between the categorical variables was examined by the chi-square test. Disease-specific survival curves were obtained by the Kaplan-Meier method and the differences between curves were examined by the log-rank test. Independence of prognostic significance was examined using a Cox regression analysis with forward stepwise selection of the variables. $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using the Statview 5.0 software package (SAS Institute, Cary, NC).

Results

p53 status

The wild-type *p53* gene was observed in 68 tumors. A *p53* mutation was found in 24 (26.1%) tumors. An example of a tumor in which the yeast functional assay gave a positive result (*i.e.*, the number of red colonies *vs.* white colonies = 46:54), and in which DNA sequencing analysis revealed a mutation in codon 273, is shown in Figure 1. The *p53* mutations and their respective transdominance property observed here are summarized in Table II. The mutations included 19 missense mutations, 2 nonsense mutations and 3 deletion mutations. Missense mutation accounted for 79% of all the mutations observed in this study. Codon 273 was most frequently mutated (4 273Arg⟶His and 1 273Arg⟶Cys), followed by codon 280 (280Arg⟶Ile, 280Arg ⟶Gly, 280Arg⟶Serand then codon 248 (248Arg⟶Trp and 248Arg⟶Gln). Regarding the transdominance of p53 mutation, 10 mutant p53 proteins (10.9%) exhibited recessive activity and 14 mutants (15.2%) showed dominant-negative activity. Not all of the missense mutations had a dominant-negative effect. Only 13 of 19 (68%) missense mutations of *p53* exhibited dominant-negative activity.

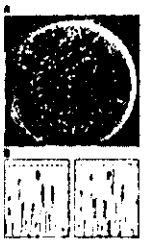

Figure 1. An example of positive yeast p53 functional assay (*a*) and confirmation of p53 mutation by DNA sequencing of the reverse strand: (*b*) wild-type sequence; (*C*) missense mutation of CGT to CAT at codon 273.
[Normal View 59K | Magnified View 243K]

Table II. Discovered *p53* Mutations and its Transdominance in 92 Endometrial Carcinomas

Institute and case number	FIGO stage	Histologic subtype	Grade	Mutation in *p53* gene	Base change	Type of mutation	Transdominance
Hokkaido University							
1	I	Endometrioid adenocarcinoma	2	213 Arg →Stop	CGA→TGA	Nonsense	
2	I	Endometrioid adenocarcinoma	2	321bp deletion (nt 673-993)		Deletion	
3	I	Endometrioid adenocarcinoma	1	133 Met →Arg	ATG→AGG	Missense	
4	I	Endometrioid adenocarcinoma	1	244 Gly→ Asp	GGC→GAC	Missense	DN
5	I	Endometrioid adenocarcinoma	2	363bp deletion (nt 385-747)		Deletion	
6	I	Endometrioid adenocarcinoma	1	108 Gly→ Ser	GGT→AGT	Missense	
7	I	Endometrioid adenocarcinoma	1	241 Ser →Ala	TCC→GCC	Missense	DN
8	I	Serous adenocarcinoma	3	273 Arg →His	CGT→CAT	Missense	DN
9	II	Serous adenocarcinoma	1	240-243 in frame deletion	AGTTCCTGC ATG→GTG	Deletion	DN
10	III	Endometrioid adenocarcinoma	3	264 Leu →Arg	CTA→CGA	Missense	
11	III	Endometrioid adenocarcinoma	2	280 Arg →Ile	AGA→ATA	Missense	DN
12	III	Endometrioid adenocarcinoma	2	173 Val→ Leu	GTG→TTG	Missense	
13	III	Serous adenocarcinoma	2	273 Arg →His	CGT→CAT	Missense	DN
14	IV	Endometrioid adenocarcinoma	3	280 Arg →Gly	AGA→GGA	Missense	DN
15	IV	Serous adenocarcinoma	3	248 Arg →Trp	CGG→TGG	Missense	DN
Mainz University							
1	I	Endometrioid adenocarcinoma	3	306 Arg →Stop	CGA→TGA	Nonsense	
2	I	Endometrioid adenocarcinoma	2	280 Arg →Ser	AGA→AGT	Missense	DN
3	I	Endometrioid adenocarcinoma	3	273 Arg →Cys	CGT→TGT	Missense	DN

4	I	Clear cell adenocarcinoma	3	245 Gly→Val	GGC→GTC	Missense	
5	II	Endometrioid adenocarcinoma	2	257 Leu →Pro	CTG→CCG	Missense	
6	III	Endometrioid adenocarcinoma	3	248 Arg →Gln	CGG→CAG	Missense	DN
7	III	Endometrioid adenocarcinoma	1	273 Arg →His	CGT→CAT	Missense	DN
8	IV	Endometrioid adenocarcinoma	3	175 Arg →His	CGC→CAC	Missense	DN
9	IV	Serous adenocarcinoma	3	273 Arg →His	CGT→CAT	Missense	DN

DN, dominant negative.

p53 mutation was compared to the clinical features (Table III). Total *p53* mutation tended to be related to the following features: nonendometrioid subtype ($p = 0.09$), grade 3 tumors ($p = 0.06$) and Japanese cohort ($p = 0.07$). Dominant-negative mutation was significantly related to advanced FIGO stage ($p = 0.01$), nonendometrioid subtype ($p = 0.01$) and grade 3 tumors ($p = 0.04$).

Table III. Relationship Between *p53* Mutation and Clinical Features of Endometrial Cancer Patients

	All mutations		Dominant-negative mutation	
	Number/total (%)	*p*	Number/total (%)	*p*
Age				
< 60	9/39 (23.1)		5/39 (12.8)	
≥ 60	15/53 (28.3)	0.64	9/53 (17.0)	0.58
FIGO Stage				
I/II	14/65 (21.5)		6/65 (9.2)	
III/IV	10/27 (37.0)	0.19	8/27 (29.6)	0.01
Histologic subtype				
Endometrioid	18/79 (22.8)		9/79 (11.4)	
Nonendometrioid	6/13 (46.2)	0.09	5/13 (38.5)	0.01
Grade				
1, 2	14/67 (20.9)		7/67 (10.4)	
3	10/25 (40.0)	0.06	7/25 (28.0)	0.04
Institute				
Mainz	9/49 (18.4)		6/49 (12.2)	
Sapporo	15/43 (34.9)	0.07	8/43 (18.6)	0.39

Survival analysis

The Kaplan-Meier analysis and log-rank test revealed that the survival of patients in this study was significantly related to conventional prognostic factors: FIGO stage ($p < 0.0001$; Fig. 2), histologic subtype ($p < 0.0001$; Fig. 3) and grade of tumor ($p = 0.037$; Fig. 4). As regards *p53* mutation, dominant-negative *p53* mutation was found to be related to poor patient survival ($p < 0.0001$; Fig. 5). Missense *p53* mutation was also significantly related to patient survival ($p = 0.0001$). The age and institute were not related to survival ($p = 0.17$ and 0.52, respectively). When we further examined the functional status of *p53* mutation in relation to patients' survival, the estimated 5-year survival rate for patients with wild-type p53 ($n = 68$), recessive *p53* mutation ($n = 10$) and dominant-negative *p53* mutation ($n = 14$) was 84.9%, 85.7% and 35.1%, respectively. There was a statistically significant difference in survival between the patients with recessive *p53* mutation and those with dominant-negative *p53* mutation ($p = 0.01$), as well as between the patients with wild-

type *p53* and those with dominant-negative *p53* mutation ($p < 0.0001$). No difference in survival was found between the patients with recessive *p53* mutation and those with wild-type *p53* (Fig. 5). Furthermore, the survival of patients with missense *p53* mutation tended to be related to the transdominance property of mutant *p53*. The 5-year survival rate was 75.0% for patients with recessive missense mutations ($n = 10$) and 34.6% for patients with dominant-negative missense mutations ($n = 14$; $p = 0.09$).

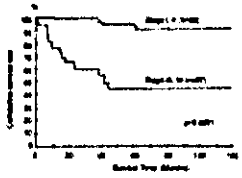

Figure 2. Kaplan-Meier analysis and log-rank test for survival of patients according to FIGO stage.
[Normal View 6K | Magnified View 15K]

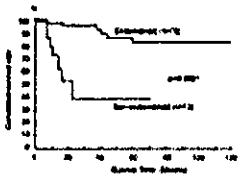

Figure 3. Kaplan-Meier analysis and log-rank test for survival of patients according to histologic subtype of tumors.
[Normal View 6K | Magnified View 15K]

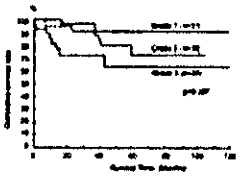

Figure 4. Kaplan-Meier analysis and log-rank test for survival of patients according to grade of tumors.
[Normal View 7K | Magnified View 16K]

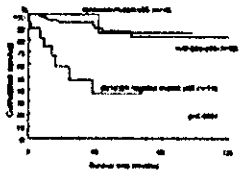

Figure 5. Kaplan-Meier analysis and log-rank test for survival of patients with wild-type, recessive mutant and dominant-negative mutant p53.
[Normal View 7K | Magnified View 17K]

Using multivariate Cox regression analysis, we found that dominant-negative *p53* mutation ($p = 0.019$), FIGO stage ($p = 0.0037$) and histologic subtype ($p = 0.014$) were independent prognostic factor for the patients in this study (Table IV). When only advanced-stage tumors were taken into consideration, dominant-negative *p53* mutation ($p = 0.0023$) was the most significant predictor of patient survival.

Table IV. Cox Regression Analyses for Patients with Endometrial Carcinoma

Variable	Univariate Cox analysis *p*	Multivariate Cox analysis			
		coefficient	Standard error	Hazard ratio	*p*
Dominant negative *p53* mutation	< 0.0001	1.37	0.58	3.9	0.019
FIGO stage	0.0002	1.81	0.62	6.1	0.0037
Histologic subtype	0.0004	1.69	0.58	5.4	0.014
Grade	0.048				NS
Age	0.19				NS
Institute	0.48				NS

Discussion

The functional activities of mutant p53 proteins have been grouped into 5 categories: retained wild-type activity, loss of function, gain of function, dominant-negative effect and temperature sensitivity.[4] The

dominant-negative activity of *p53* mutation corresponds to the capacity of the mutant protein to complex with the product of the remaining wild-type allele to inactivate its function. Thus, dominant-negative *p53* mutation results in the total abrogation of p53 protein function, even if there is still wild-type protein expressed in the cell. Although the importance of analysis of the functional types of *p53* mutation, *i.e.*, the dominant-negative effect and gain of function, in terms of researching carcinogenesis and searching for novel human cancer therapies has been repeatedly emphasized,[10][12][21] it remains unclear whether or not the dominant-negative activity of mutant p53 proteins has a detrimental effect on the survival of cancer patients. Recessive *p53* mutation accompanied by loss of the second allele may be equal to dominant-negative *p53* mutation in terms of loss of function of the gene. Although we did not investigate LOH in this study, the present results did suggest that dominant-negative *p53* mutation is closely related to poor survival of patients with endometrial cancer, even after adjusting for established prognostic factors, that is, tumor stage, grade and histologic type. Future studies including the LOH status of the second allele will be of interest in this context. It is important to investigate whether or not the dominant-negative *p53* mutation exerts an influence on patient survival, not only through a loss of function, but also by other mechanisms attributable to mutant p53 protein such as certain gain-of-function activities.

Also of interest in this context is that dominant-negative *p53* mutation was found to be closely related to the survival of patients with advanced-stage endometrial cancer. Because the prognosis of patients with early-stage endometrial cancer is generally excellent, gynecologic oncologists need to focus increasingly on the survival of patients with advanced endometrial cancer. The histopathologic prognostic factors for endometrial cancer include depth of myometrial invasion, cervical involvement, serosal invasion, adnexal metastasis, positive peritoneal cytology, vaginal metastasis, lymph node metastasis, peritoneal metastasis and bladder/rectal involvement, which are incorporated in the FIGO surgical staging system.[22] The grade of tumor, histologic subtype and lymph-vascular space invasion, which represent the aggressiveness of a tumor, are also important histopathologic prognostic factors that should be taken into consideration in planning treatment for patients with endometrial cancer.[23] The histologic subtypes of serous adenocarcinoma and clear cell adenocarcinoma exhibit more aggressive biologic behavior than common endometrioid adenocarcinoma and has been shown to lead to disproportionate mortality.[24][25][26] Serous adenocarcinoma is frequently associated with p53 overexpression or *p53* mutation. Our current study has shown that dominant-negative *p53* mutation is an important prognostic factor, in addition to the established predictors of survival, namely, FIGO stage and histologic subtype, in cases of endometrial cancer. This suggests that dominant-negative *p53* mutation may be a reasonable target for a novel therapy for cases of endometrial cancer with a poor prognosis.

In the present study, only 68% of the missense mutations in the cases of endometrial cancer studied here exhibited dominant-negative activity. This finding suggests that determining the dominant-negative activity of a *p53* mutation is more important than merely determining the presence or absence of a *p53* mutation as part of a tailored treatment or rational targeted treatment for endometrial cancer. Because of the high frequency of *p53* mutations in human cancers, and due to the pivotal role of p53 in regulating growth, apoptosis and DNA repair, the introduction of the wild-type p53 gene has been regarded as a reasonable strategy for a gene therapy designed to restore the lost activity of p53.[27] However, this approach has achieved substantial effectiveness to date.[28] A possible reason for the unsatisfactory results may be the accumulation of dominant-negative mutant p53, which results in a high amount of mutant protein, which would override the effects of the wild-type protein introduced by gene therapy. This explanation may in part account for the recently reported failure of gene therapy in the study of ovarian cancer.[29] In addition to overriding wild-type p53, some dominant-negative mutants are known to cause a gain of function related to tumor progression.[10][12][30] Such mutants include 175 Arg⟶His, 273 Arg⟶His and 248 Arg⟶Trp, which accounted for 6 of the 14 dominant-negative mutants identified in the present study. The gain-of-function property of the p53 mutants is considered to lend further malignant phenotypes to the tumor cells, such as enhancement of tumorigenicity, metastatic potential and therapy resistance. These properties may account for the extremely poor survival of endometrial cancer patients with dominant-negative p53 mutations. The 6 cases with gain-of-function mutations in this study included 1 case of stage I serous adenocarcinoma (case 8 in the Japanese cohort), 1 case of stage III endometrioid adenocarcinoma (cases 7 in the German cohort), 1 case of stage III serous adenocarcinoma (case 13 in the Japanese cohort), 1 case of stage IV endometrioid adenocarcinoma (case 9 in the German cohort) and 2 cases of stage IV serous adenocarcinoma (case 15 in the Japanese cohort and case 9 in the German cohort). The gain-of-function mutation appears to be related to advanced-stage tumors, although the number of cases studied was not large enough to draw any conclusions.

In summary, this study indicates that dominant-negative mutation of *p53* gene is often found in the advanced stages and aggressive histologic subtypes of endometrial cancer. Moreover, such mutation is a strong predictor of the survival of patients with advanced endometrial cancer. Further investigation will be needed in order to clarify whether or not identification of the dominant-negative property of *p53* mutation may be useful for tailoring the treatment of endometrial cancer.

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