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A feasibility study on biweekly administration of docetaxel for patients with recurrent ovarian cancer☆

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Abstract

Objective. A recent study demonstrated that docetaxel (DTX) was an effective agent for second line chemotherapy against ovarian cancer. Weekly administration of taxane compounds had been more effective compared with a 3 week interval administration in ovarian cancer. The role of biweekly administration of DTX has been unknown. We conducted a dose determination and feasibility study of biweekly DTX administration in patients with ovarian cancer.

Methods. Patients with histologically confirmed epithelial ovarian cancer who received one or more regimens of prior chemotherapy with more than 4 weeks of treatment-free interval were eligible. DTX was administered as 1-h intravenous infusion every two weeks for at least four courses. The starting dose was 40 mg/m^2 (level 1) and the dose was escalated to 50 mg/m^2 (level 2) and 60 mg/m^2 (level 3) in consequent patient cohorts.

Results. Nine patients were examined in this study. The treatments were completely performed in all cohorts. Mean treatment delay ranged from 0 to 2.0 days. Dose level did not affect treatment delay. At the first dose level, no patients experienced grade 3/4 neutropenia. Two patients in level 2 experienced grade 3/4 neutropenia. In level 3, all patients had grade 4 neutropenia. Nonhematologic toxicities were tolerable. Of eight patients with measurable disease, all patients in level 1 showed progressive disease, and all patients in level 2 were no-change. There were two responders showing complete response and partial response and one case was no-change in level 3.

Conclusion. The present study showed that biweekly administration of 60 mg/m^2 DTX was feasible for recurrent ovarian cancer.

Introduction

The combination treatment with paclitaxel (PTX) and platinum agents has been the current standard regimen for patients with ovarian cancer [1–3]. Although patients with ovarian cancer show good response to primary chemotherapy, the majority of them ultimately develop chemoresistance [4]. As a result, most patients with relapsed ovarian cancer require second-line chemotherapy of which the standard regimen has not yet been established. Therefore, the search for an effective second-line chemotherapy is essential to improve the prognosis of patients with ovarian cancer.

Docetaxel (DTX), which is another taxane, has greater cytotoxic potency than PTX [5–7]. Recent studies suggested that DTX was effective for PTX and/or platinum resistant cancer [8–10]. DTX may be a beneficial agent for second line chemotherapy for ovarian cancer. In the literature, weekly administration of taxane compounds was well tolerated compared with 3 week interval administration

☆ Sankai Gynecology Study Group study

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11,12]. Although the administration of a weekly DTX markedly reduced the severity of myelosuppression and allowed the higher dose intensity 12], weekly administration is sometimes inconvenient because of frequent hospital visits. Biweekly administration of DTX may be a more convenient and tolerable schedule than a 3 week interval or weekly administration. There were, however, few reports concerning biweekly DTX administration 13]. We conducted this dose determination and feasibility study for further evaluation on the efficacy of biweekly DTX administration in patients with recurrent ovarian cancer.

In our previous study, we demonstrated that 70 mg/m^2 DTX administered every 3 weeks showed the satisfactory responses (overall response rate, 24.0%), but frequently unacceptable toxicities. Incidence of grade 3/4 neutrocytopenia, leukocytopenia were 92% and 80%, respectively. G-CSF was given in 60% of patients. Febrile neutropenia requiring antibiotics occurred in 16% of the patients 14]. In contrast, there was no severe adverse event in the Japanese phase II trial for ovarian and cervical carcinomas using triweekly DTX of 60 mg/m^2 15]. In the present study we evaluated the feasibility of biweekly administration of DTX up to 60 mg/m^2 with or without G-CSF support by escalating the dose from 40 mg/m^2.

Patients and methods

The present study was conducted between December 2000 and March 2002 in Tottori University Hospital, Kawasaki Medical School Hospital, Kawasaki Hospital, and Yamaguchi University Hospital.

The eligibility for entry into this study included the following: histologically confirmed diagnosis of epithelial ovarian cancer; one or more regimens of prior chemotherapy and more than 4 weeks of treatment-free interval; if patients previously had received radiation therapy, biological response modifiers, or hormone therapy, the treatment-free interval was more than 2 weeks; a life expectancy of over 3 months. Additionally, all patients were required to have Eastern Cooperative Oncology Group performance status <3, age ranging from 20 to 75 years, adequate bone marrow function, liver function, and renal function defined by neutrophil >2000/mm^3, platelets >100,000/mm^3, serum AST and ALT <100IU/l (if she has liver dysfunction due to liver metastasis, those levels <150 IU/l), serum ALP <750IU/l, serum total bilirubin <1.5 mg/dl, and serum creatinine <1.5 mg/dl. Measurable disease was not required.

The following patients were excluded from this study: septicemia or severe infection; pulmonary fibrosis or interstitial pneumonia; symptomatic neuropathy over grade 2; grade 2 edema; severe cardiac disease or arrhythmia; concomitant malignancy; symptomatic pleural effusion; allergic reactions to Polysorbate 80, and suspicious of pregnancy.

All patients were required to provide written informed consent. The protocol was approved by the local institution review board.

DTX was administered intravenously for 1 h every 2 weeks and at least four courses were continued. The starting dose was 40 mg/m^2 (level 1) and the dose was escalated to 50 mg/m^2 (level 2) and 60 mg/m^2 (level 3) in consequent patient cohorts. No further dose escalation was contemplated because the standard schedule dose not allow doses of 75 mg/m^2 and beyond in a large percentage of patients.

Subsequent cycle was repeated in each cohort when patients met the following criteria: neutrophil >1000/mm^3, platelets >75,000/mm^3, serum AST and ALT <100 IU/l (<150 IU/l with liver dysfunction due to liver metastasis), serum ALP <750 IU/l, body temperature <37.5 °C, and performance status of 0 to 2. An incomplete recovery from nonhematologic toxicities of over grade 2, except for emesis, poor appetite, and fatigue, also required treatment delay. We used 50 μg/body/day of nartograstim (Kyowa Pharmaceutics Inc, Tokyo, Japan) as granulocyte colony stimulating factor (G-CSF) if patients had grade 3 neutropenia with fever or grade 4 neutropenia. Administration of G-CSF was continued until neutrophil counts increased to over 5000/mm^3. DTX was given at least 48 h after the last administration of G-CSF. As a prophylactic treatment, G-CSF was given five times every other day from 48 h after DTX administration in the subsequent course.

If three patients could completely receive four-cycle treatment without having administration delay of more than 1 week in each course, the next cohort underwent an escalated level. If all patients experienced over 1-week delays of treatment, the next level of treatment should be interrupted. When one or two patients experienced over 1 week delay of treatment, another three patients were enrolled into the same level. If fewer than three of six patients showed over 1 week delay, the next cohort underwent escalated level. If the delay occurred in over three of six patients, the treatment was interrupted. Drug effects to each dosage were completely analyzed before proceeding to next dosage. A dose of more than 60 mg/m^2 was not scheduled to be tested in this study. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

The effect of chemotherapy was evaluated 3 weeks after each chemotherapy treatment by magnetic resonance imaging and computerized tomography according to the following criteria: a complete response (CR) was defined as the absence of disease over 4 weeks and a partial response (PR) as a greater than 50% reduction in all measurable lesions without the appearance of new lesions over 4 weeks; no change (NC) was defined as a decrease of less than 50% or an increase of less than 25% in all measurable lesions without the appearance of new lesions. Progressive disease was defined as an increase greater than 25% in measurable disease at a known site, or the appearance of new lesions. In this study, the responders consisted of patients showing CR or PR.

Table 1
Patient characteristics

Age (Mean)	47–71 (55.5)
ECOG performance status	
0	5
1	4
Histology	
Serous adenocarcinoma	8
Clear cell carcinoma	1
Previous chemotherapy	
Paclitaxel + CBDCA	7
CBDCA + THP + CPA	1
CDDP + EPI + CPA	1
CDDP + MMC	1
Ascites	
Presence	2
Absence	7

Note. ECOG, Eastern Cooperative Oncology Group; CBDCA, carboplatin; CDDP, cisplatin; THP, pirarubicin; EPI, epirubicin; CPA, cyclophosphamide; MMC, mitomycin-C.

Results

Ten patients were enrolled in the present study. One patient in level 1 was excluded from this study due to rapid growth of tumor, and then another patient was added into the cohort. Characteristics of nine patients are summarized in Table 1. Except for one patient who previously underwent two regimens of chemotherapy, eight patients had received one regimen of chemotherapy. Two patients previously had undergone radiation therapy. Seven patients had received PTX. The interval from last PTX treatment ranged from 1 to 7 months. In our series, all seven patients did not respond to DTX.

The treatment was completely performed in all cohorts. Mean treatment delay ranged from 0 to 2.0 days. Dose level did not affect treatment delay (Table 2). At the first dose level, no patients experienced grade 3/4 neutropenia. Two patients in level 2 experienced grade 3/4 neutropenia (grade 3, 1 and grade 4, 1). In level 3, all patients had grade 4 neutropenia. G-CSF was given in levels 2 and 3. Table 3 shows therapeutic cycle and frequency of grade 3/4 neutropenia in each cohort. Grade 2 anemia occurred in patients of level 3. Thrombocytopenia was not observed in our series.

All patients experienced grade 2 emesis in level 3. There were two grade 2 alopecia, three diarrhea (grade 1, 2 and grade 2, 1) and one grade 1 myalgia.

Table 2
Treatment delay and treatment course in each cohort

Dose level	Treatment delay (days)		
	2nd course	3rd course	4th course
1	1.3	2.0	1.3
2	0	2.0	1.7
3	0	0.7	0.3

Table 3
Grade 3/4 neutropenia and treatment course in each cohort

Dose level	Number of patients			
	1st course	2nd course	3rd course	4th course
1	0	0	0	0
2	2	2	0	0
3	2	2	2	1

Eight patients (three level 1, two level 2, and three level 3) had measurable lesions. Of eight patients, all patients in level 1 showed PD, and all in level 2 were NC. There were two responders (CR and PR) and one NC in level 3. Two patients with ascites did not respond to DTX and their ascites did not change, although fluid retention is a drawback of DTX.

Discussion

The current management of patients with recurrent ovarian cancer is based on a consideration of the results of the initial chemotherapy. DTX exhibits activity against platinum or PTX resistant ovarian cancer 8,9]. Therefore, DTX may be an effective agent for second line chemotherapy in patients with ovarian cancer.

Dose limiting factor of DTX is neutropenia. The recommended dose is 60–100 mg/m^2 every 3 weeks. At this dose, approximately 74% of patients experience grade 4 neutropenia and 14% have febrile neutropenia 12,16]. We anticipated that severe neutropenia is unavoidable in DTX treatment, therefore prophylactic use of G-CSF was allowed in the protocol. In our series, if three patients could completely receive four-cycle treatment without having administration delay more than 1 week in each course, the next cohort underwent the escalated level. All patients undergoing biweekly administration of 60 mg/m^2 DTX had grade 4 neutropenia, but the treatment was completely performed using G-CSF. The level was judged as tolerable. Thrombocytopenia was not observed in our series. Additionally, we did not find the severe nonhematologic toxicities. We did not conduct the present study to clarify the maximum tolerable dose (MTD) of biweekly DTX administration. Because the aim of this study was to determine whether the dose of DTX up to 60 mg/m^2 biweekly was feasible, the higher dose was not tested.

We used G-CSF as a prophylactic treatment in our series. Neutropenia was decreased by the prophylactic treatment of G-CSF, particularly in subsequent course of level 2. In addition, biweekly administration of 60 mg/m^2 DTX was completely performed while using G-CSF. These finding suggest that the prophylactic treatment with G-CSF may be useful for biweekly administration of DTX. In the literature, the overall response rate was 23% including one CR, when patients with PTX-resistant mullerian carcinoma received either 100 or 75 mg/m^2 of DTX every 3 weeks 8]. Addi-

tionally, the subsequent dose-escalation phase II trials while using every 3 weeks administration of DTX did not show a satisfactory response rate (over 20%) until 70 mg/m^2 14,17]. Those findings suggest that the efficacy of DTX may depend on dose intensity. While the number of patients in the present study was too small to draw a conclusion concerning efficacy of DTX, it is an interesting observation that two of three patients who received a biweekly administration of 60 mg/m^2 DTX were responders, whereas no responses were observed when lower doses (50 and 40 mg/m^2) were administered.

Based on this study, a phase II study of biweekly administration of 60 mg/m^2 DTX in recurrent ovarian cancer will be undertaken by our group. Future studies should explore the usefulness of biweekly administration of DTX for second line chemotherapy of ovarian cancer.

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ORIGINAL ARTICLE

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Unknown primary carcinoma: a feasibility assessment of combination chemotherapy with cisplatin and docetaxel

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Abstract
Background. Docetaxel (Taxotere) and cisplatin are two of the most active single agents used in the treatment of solid tumors. We examined the feasibility of using a combination of docetaxel and cisplatin for the treatment of unknown primary carcinoma in order to prepare a larger scale prospective study.
Methods. Eligible patients were aged 18 to 75 years, with an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less and a life expectancy of 8 weeks or more, and had been diagnosed as having unknown primary carcinoma by the required examinations. Patients were not permitted to have received prior chemotherapy and had to have measurable lesions. Docetaxel (60 mg/m^2) was given intravenously over a 1-h period immediately before cisplatin (80 mg/m^2), which was given intravenously over a 2-h period, every 3 weeks. Premedication included dexamethasone, granisetron, and standard hyperhydration.
Results. Twenty-six treatment courses in five patients were tested according to the protocol and feasibility was assessed. Adverse events observed incldued grade 4 neutropenia, leukopenia, grade 3 nausea/vomiting, grade 2 diarrhea, and mucositis. These adverse events were well tolerated, reversible, and manageable. Doses were not reduced and all injections were given or their due date without any delay in all patients. Four patients achieved a partial response and one had stable disease.
Conclusion. Treatment of patients with unknown primary carcinoma with a combination of docetaxel and cisplatin is feasible. Conduct of a phase II trial of this regimen is warranted.

Key words Unknown primary carcinoma · Docetaxel · Pilot study

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Introduction

Unknown primary carcinoma (UPC) is defined as the presence of metastatic cancer documented in the absence of an identifiable primary tumor site.[1] According to this definition, between 0.5% and 7% of cancer patients are diagnosed as having this clinical entity.[2] UPCs are a heterogenous group of neoplasms with widely varying natural histories and biological characteristics. In this broad category, there are four major light-microscopic diagnoses;[3] (i) poorly differentiated neoplasm, (ii) well-differentiated and moderately differentiated adenocarcinoma, (iii) squamous cell carcinoma, and (iv) poorly differentiated carcinoma (with or without features of adenocarcinoma). It has been reported that the prognosis of patients with UPC is poor. The median survival duration is 3 to 4 months, and fewer than 25% of UPC patients are alive 1 year after diagnosis[1]. Previous trials with a variety of chemotherapeutic regimens have produced response rates of less than 30%, with negligible benefit in terms of median survival. Cisplatin-containing regimens have included higher response rates than those without cisplatin in patients with UPC, but complete responses are rare.[4]

Docetaxel (Taxotere; Aventis Pharma, Antony, France) is an antimicrotubule agent that has a unique cellular mechanism of action; the promotion of tubulin polymerization into stable microtubules and the inhibition of tubulin depolymerization.[5] Docetaxel has definite antitumor activity in various solid tumors, such as breast, ovary, and non-small-cell lung cancer.[6–9] We therefore planned a prospective safety and efficacy trial of a combination of cisplatin and docetaxel in patients with UPCs and tested its feasibility in a small number of patients.

Patients and methods

Eligibility

Eligible patients, aged between 18 and 75 years, with an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less and a life expectancy of 8 weeks or more, were required to be diagnosed to have UPC with measurable lesions. No prior chemotherapy was permitted. Pretreatment evaluation consisted of a history and full physical examination, laboratory tests (complete blood counts, and a chemistry profile including liver function tests, electrolytes, and renal function tests, as well as serum calcium, glucose, and uric acid measurements), chest radiograph, computed tomography (CT) of the abdomen and pelvis, and tumor marker screening, including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 15-3, National Cancer Center Stomach (NCCST) 439, CA 19-9, squamous cell carcinoma related antigen (SCC), neuron-specific enolase (NSE), prostate-specific antigen (PSA), β-human chorionic gonadotropin (hCG), Pro-gastrin-releasing peptide (ProGRP), and CA-125. Further investigations, such as upper and lower gastrointestinal (GI) studies, bronchoscopy, mammography, and radionuclide bone scan, were performed only if symptoms or signs suggested the possibility of a primary lesion at a particular site. Biopsy specimens were obtained by excision or core needle biopsy. Baseline tumor imaging was required, and similar techniques must have been used to reevaluate disease progress. Laboratory investigations were performed immediately before each course (except for full blood counts, which were required twice a week). Patients in subsets with specific, well-defined treatments were excluded. These subsets included women with adenocarcinoma that involved only axillary lymph nodes, patients with squamous cell carcinoma that involved only cervical lymph nodes or inguinal lymph nodes, and patients with carcinoma that involved a single potentially resectable tumor site.[10] All patients provided written, informed consent.

Drug administration schedule

Patients were administered docetaxel, 60 mg/m^2 in 250 ml 5% dextrose, as a 1-h intravenous (IV) infusion, followed by cisplatin, 80 mg/m^2 in 500 ml normal saline, as a 2-h IV infusion on day 1. All patients received dexamethasone (8 mg) and granisetron hydrochloride (3 mg) before docetaxel administration. Adequate antiemetics, hydration, and diuretics were administered, as necessary. Treatments were repeated every 3 weeks. Patients were given at least two courses of treatment and then assessed for response according to standard WHO criteria.[11] Those who achieved a complete response (CR) or a partial response (PR) received an additional four courses of the regimen unless disease progression was noted. In patients who showed no response, chemotherapy was stopped after two cycles. Toxicities were assessed according to the National Cancer Institute common toxicity criteria (NCI CTC).

Table 1. Patient characteristics

Variable	*n*
No. of eligible patients	5
Male/female	1/4
ECOG performance status	
0	3
1	1
2	1
Age (years)	
Median (n = 5)	54
Range	67–52
Histology	
Adenocarcinoma	4
Squamous cell carcinoma	1

ECOG, Eastern Cooperative Oncology Group

Table 2. Patients with grade 3 or 4 hematologic toxicities

Toxicity	Grade 3 (*n*)	Grade 4 (*n*)
Leukopenia	1	1
Neutropenia	0	2
Thrombocytopenia	0	0
Anemia	0	0

Results

Treatment

Five consecutive patients (median age, 54 years; range, 67–52 years) were enrolled in this study. The characteristics of these patients are listed in Table 1. A total of 26 treatment cycles were performed in these patients. Six courses of treatment were given to four patients who showed partial response and 2 courses were given to one patient with stable disease.

Toxicity and schedule compliance

Grade 4 neutropenia was observed in two patients (Table 2). This episode was managed with intravenous administration of ceftazidime. Neither patient received granulocyte colony-stimulating factor (G-CSF). Nausea and vomiting were mild (grade 1 or 2). Other symptoms occasionally reported by some patients included diarrhea and mucositis (Table 3), but specific treatments were not required. Severe nephrotoxicity (grade 3 or 4) was not observed. Doses were not reduced in subsequent courses due to the adverse effects. All 26 courses of treatment were conducted on the due dates without any delay.

Discussion

In spite of the employment of highly sensitive and specific diagnostic tools, oncologists fail to identify the primary anatomic site in a certain fraction of patients with metastatic

Table 3. Patients with nonhematologic adverse events possibly or probably related to treatment

Adverse events*	Worst	NCI-CTC Grade (*n*)	
	1–4	3	4
Allergy	0	0	0
Cardiac dysrhythmia	0	0	0
Cardiac ischemia	0	0	0
Diarrhea	1	0	0
Hypotension	0	0	0
Infection	1	0	0
Nausea	1	0	0
Vomiting	1	0	0
Neurosensory	0	0	0
Mucositis	1	0	0

NCI CTC, National Cancer Institute common toxicity criteria
*For each event, the number of patients having one or more adverse events is indicated. These assignments of toxicity represent the worst grade noted in any treatment cycle

cancer. These patients with unidentified primary sites are pragmatically diagnosed as having UPC to facilitate the employment of effective anticancer treatments. Identification of the primary site in patients with metastatic cancer provides the basis for predicting the behavior of the disease and for assigning appropriate therapy focusing on biological characteristics. Therefore, it is a great challenge for medical oncologists to employ systemic therapy without knowing the anatomical site of origin of the disease.

Historically, the empirical approach has been taken to pursue a more effective chemotherapeutic regimen in patients with UPC. Several 5-fluorouracil (5FU)- and/or anthracycline – containing regimens have been studied.[3] The response rates with these regimens have usually been between 10% and 30%, and median survival times (MSTs) were shorter than 4 months.

A number of cisplatin-based regimens have been evaluated. The response rate ranged between 30% and 40%, and the MSTs were about 8 months.[3]

Among the new agents which became available in the 1990s, docetaxel showed a significant level of single-agent activity against various solid tumors, such as breast, ovary, and non-small-cell lung cancer.[6-9] Docetaxel therefore seems to be a good candidate to include in an empirical chemotherapy regimen for patients with UPC. It is to be expected that a combination of docetaxel and cisplatin would have a high potential to control UPC, because both of these drugs are highly effective in a multitude of solid tumors.[12] Although these two agents have not been shown to be synergistic in vitro in various solid tumor cell lines, they appear to be non-cross-resistant clinically and have a predominantly non-overlapping toxicity profile.[13] Phase I trials have been conducted in patients with ovary and lung cancers, and the recommended doses for phase II trials were docetaxel at 60 mg/m^2 and cisplatin at 80 mg/m^2.[14] This pilot trial was conducted in Japanese patients.

No clinical trials of this regimen has been conducted so far in patients with UPC. Therefore, we thought that a small-scale pilot trial, to estimate feasibility by evaluating the toxicity and efficacy of this regimen, would be necessary before going into a full-scale phase II trial. The major toxicity of this combination was neutropenia, and others observed included diarrhea and mucositis. Toxicity was mild and clinically manageable. No modification of the dose or the schedule was needed in this patient set. Four of the five patients had a partial response to this regimen (response rate, 80%; 95% confidence interval [CI], 37.5%–96.3%). The lower limit of the 95% CI (37.5%) was thought to be good enough for the response rate for UPCs. This means that the use of this regimen is ethically acceptable. We therefore conclude from this small-scale study that the safety and efficacy of this regimen is warranted to be evaluated in a full-scale phase II trial.

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Prognostic significance of positive peritoneal cytology in endometrial carcinoma confined to the uterus

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A retrospective analysis was performed to evaluate the prognostic significance of peritoneal cytology in patients with endometrial carcinoma limited to the uterus. A total of 280 patients with surgically staged endometrial carcinoma that was histologically confined to the uterus were examined clinicopathologically. The median length of follow-up was 62 (range, 12–135) months. All patients underwent hysterectomy and salpingo-oophorectomy with selective lymphadenectomy, and only three patients received adjuvant postoperative therapy. No preoperative adjuvant therapy was employed. In all, 48 patients (17%) had positive peritoneal cytology. The 5-year survival rate among patients with positive or negative peritoneal cytology was 91 or 95%, respectively, showing no significant difference (log-rank, $P=0.42$). The disease-free survival rate at 36 months was 90% among patients with positive cytology, compared with that of 94% among patients with negative cytology, and the difference was not significant (log-rank, $P=0.52$). Multivariate proportional hazards model revealed only histologic grade to be an independent prognostic factor of survival ($P=0.0003$, 95% CI 3.02 – 40.27) among the factors analysed (age, peritoneal cytology, and depth of myometrial invasion). Multivariate analysis revealed that histologic grade ($P=0.02$, 95% CI 1.21–9.92) was also the only independent prognostic factor of disease-free survival. We concluded that the presence of positive peritoneal cytology is not an independent prognostic factor in patients with endometrial carcinoma confined to the uterus, and adjuvant therapy does not appear to be beneficial in these patients.
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Malignant peritoneal cytology is recognised as an adverse prognostic factor in some gynaecologic malignancies. In ovarian cancer, there is a general consensus that postoperative adjuvant chemotherapy should be given to patients with positive peritoneal cytology even if the tumour is limited to the ovaries, that is, the International Federation of Gynecology and Obstetrics (FIGO) stage IC.

As for the positive prognostic value of peritoneal cytology in endometrial carcinoma confined to the uterus, there is still controversy, and conflicting results have appeared in the literature. Accordingly, there is no evidence as to the indication for and efficacy of adjuvant treatment in the case of positive peritoneal cytology. Several studies have reported the prognostic value of positive cytology, and proposed various modalities of adjuvant therapy, that is, multiagent chemotherapy, progestins, whole abdominal radiation, and intraperitoneal radioactive chromic phosphate (^{32}P) (McLellan *et al*, 1989; Lurain, 1992). On the other hand, investigators who found that malignant peritoneal cytology has poor prognostic value, found that adjuvant therapy was not beneficial (Yazigi *et al*, 1983; Konski *et al*, 1988; Lurain *et al*, 1989; Kadar *et al*, 1992). The question of the prognostic significance of malignant cytology in endometrial carcinoma confined to the uterus remains unanswered.

This retrospective clinicopathological study was undertaken to identify the prognostic significance of positive peritoneal cytology in endometrial carcinoma confined to the uterus.

PATIENTS AND METHODS

Patients

We reviewed the medical records and the cytologic and pathologic materials that had been obtained from 392 patients with surgically treated endometrial carcinoma at the Gynecology Division of the National Cancer Center Hospital, Tokyo, between 1990 and 1998. This study included patients who met the following criteria: the patient underwent primary surgery consisting of total abdominal hysterectomy and salpingo-oophorectomy with selective pelvic and/or para-aortic lymphadenectomy; the patient had no histologic evidence of extrauterine disease; peritoneal cytology was determined in a peritoneal washing obtained by laparotomy immediately upon entering the peritoneal cavity during primary surgery; and the patient had a histologic subtype of endometrioid adenocarcinoma or adenosquamous carcinoma. Patients with uncommon histologic subtypes (mucinous, serous, clear cell, and/or squamous cell carcinoma), and those who had other simultaneous primary malignancy were excluded. All of the

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patients were surgically staged according to the FIGO staging system (1988), and histologic typing was evaluated according to the criteria of the WHO International Histologic Classification of Tumors.

Cytopathology

Cytological specimens were obtained by laparotomy upon entering the peritoneal cavity immediately before the primary surgery. Approximately 30 ml of sterile saline was instilled into the pelvis over the uterus, and then aspirated in the cul-de-sac. When a sufficient amount of ascites was present, the fluid was removed with a 20–30-ml syringe. The samples were subjected to cytocentrifugation onto slide glasses at 1700 rpm for 60 s at room temperature. The slides were then fixed in 95% ethanol, followed by Papanicolau stain, and alcian blue stain. Additional slides were stained immunocytochemically for CEA (Mochida, CEA010, Tokyo, Japan), and also for epithelial antigen defined by an antibody BerEP4 (DAKOPATTS, Glostrup, Denmark). Two to three cytotechnologists and cytopathologists independently examined all the slides to make a consensus diagnosis. A patient was considered to have positive peritoneal cytology if adenocarcinoma cells were detected regardless of the number of cancer cells. In this study, in cases where atypical cells were present but could not be definitively identified as cancer cells, the peritoneal cytology was considered to be negative.

Treatment

Our standard primary treatment for early-stage endometrial carcinoma was surgery consisting of extrafascial total abdominal simple hysterectomy, bilateral salpingo-oophorectomy and selective pelvic and/or para-aortic lymphadenectomy. In cases in which preoperative endometrial biopsy revealed histologic grade 1 tumour and no macroscopic myometrial invasion was found during the operation, lymphadenectomy was not performed. Para-aortic lymphadenectomy was performed if para-aortic node metastasis was diagnosed by pathologic sampling during the operation. Preoperative adjuvant therapy was not employed in any patient, and postoperative adjuvant therapy was not indicated for patients with limited disease.

The primary diagnosis of endometrial carcinoma was made by endometrial biopsy, which had been performed as an office procedure. Hysteroscopy was not performed prior to surgery. Before the surgery, the patients were examined by computed tomography and magnetic resonance imaging. Following the surgery, asymptomatic patients underwent pelvic examination, Pap smear, chest radiograph, ultrasonography, and/or determination of serial tumour markers every 4–6 months. Symptomatic patients underwent the appropriate examination where indicated.

Statistical methods

Survival and disease-free survival (DFS) curves were obtained by the Kaplan–Meier method and the survival curves were compared by nonparametric survival analysis (log-rank test). Variables that showed a significant association with survival or DFS, and peritoneal cytology were included in multivariate analysis based on the Cox-proportional hazards model. Patients who died of other causes were included as deaths in the survival analysis. Follow-up continued through 30 November, 2001. These statistical analyses were performed using the Statview statistical software package (version 5.0; SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

In all, 280 patients met the study criteria, with a mean age of 56 years (range, 27–81 years) and a median length of follow-up of 62 months (range, 12–135 months). Of the patients, 112 who underwent surgery for endometrial carcinoma (mean age, 57 years) were excluded. Of these, 46 patients had extrauterine disease including stage III and IV. The remaining patients were excluded because of uncommon histologic subtype, other simultaneous malignancies, and/or inadequate cytologic materials. Of the 280 subjects, 48 patients (17%) had positive peritoneal cytology and 232 (83%) had negative cytology. The characteristics of the patients are summarised in Table 1. The histologic subtypes were the endometrioid type in 270 cases (96%) and the adenosquamous type in 10 cases (4%). The FIGO stage was as follows: 35 patients (12%) had stage IA disease, 123 (44%) had stage IB, 41 (15%) had stage IC, 5 (2%) had stage IIA, 28 (10%) had stage IIB, and 48 (17%) had stage IIIA. In total, 149 patients (53%) underwent simple hysterectomy and salpingo-oophorectomy with lymphadenectomy; 108 (39%) underwent simple hysterectomy and salpingo-oophorectomy without lymphadenectomy; and 23 (8%) underwent radical hysterectomy. Preoperative radiation therapy, chemotherapy, and progestin therapy were not administered to any patient. Only three patients received postoperative adjuvant therapy. These three patients with stage IIB carcinoma had deep cervical involvement, and external beam radiotherapy to the whole pelvis (total dose of 50 Gy) was administered postoperatively.

Survival

The cumulative survival was assessed in subgroups according to peritoneal cytology (positive or negative), age (over 60 years or 60 years and under), histologic grade (grade 1, grade 2, or grade 3),

Table 1 Patient characteristics

	Positive cytology n=48 (%)	Negative cytology n=232 (%)
Age (y)		
Over 60	12 (25)	76 (33)
60 or under	36 (75)	156 (67)
Histologic grade		
Grade 1	34 (81)	147 (63)
Grade 2	10 (17)	56 (24)
Grade 3	4 (2)	29 (13)
Myometrial invasion		
Absent	5 (10)	35 (15)
< 1/3	20 (42)	106 (46)
1/3–2/3	11 (23)	52 (22)
>2/3	12 (25)	39 (17)
Cervical involvement		
Absent	34 (70)	198 (85)
Mucosal	7 (15)	6 (3)
Stromal	7 (15)	28 (12)
Lymph – vascular space invasion		
Absent	34 (71)	172 (74)
Present	14 (29)	60 (26)
Lymph node status		
Negative	32 (67)	140 (60)
Not resected	16 (33)	92 (40)

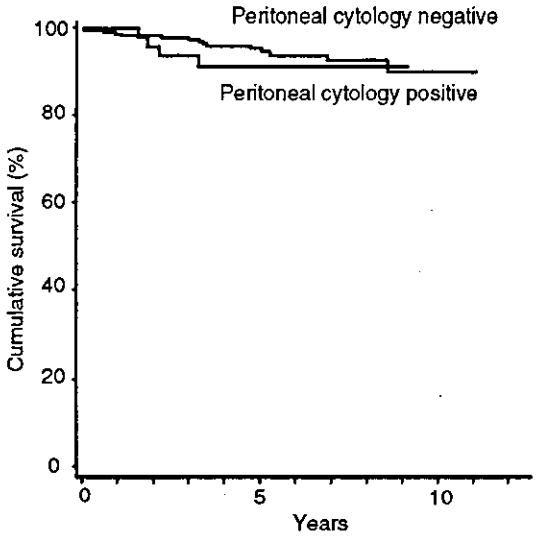

Figure 1 Survival of patients with endometrial carcinoma confined to the uterus according to the presence or absence of malignant peritoneal cytology.

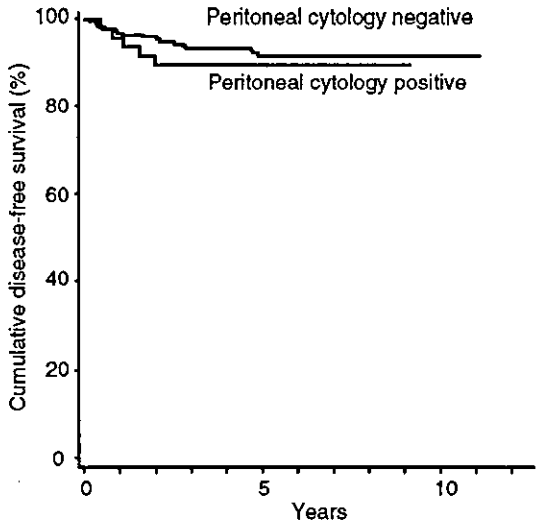

Figure 2 DFS in patients with endometrial carcinoma confined to the uterus according to the presence or absence of malignant peritoneal cytology.

Table 2 Univariate analysis and multivariate proportional hazards model for survival

	Univariate	Multivariate		
	P-value	Hazard ratio	95% CI[a]	*P*-value
Peritoneal cytology	0.42			
Positive		1.82	0.56–5.86	0.31
Age (y)	0.0045			
Over 60		2.50	0.93–6.71	0.06
Myometrial invasion	0.02			
<1/3		0.97	0.10–8.66	0.97
1/3–2/3		0.65	0.061–7.07	0.72
>2/3		1.27	0.13–12.35	0.83
Histologic grade	<0.0001			
Grade 2		3.28	0.81–13.21	0.09
Grade 3		11.02	3.02–40.27	0.0003

[a]95% confidence interval.

depth of myometrial invasion (absent, $<1/3, 1/3-2/3$ or $>2/3$), cervical involvement (absent, mucosal, or stromal), lymph - vascular space invasion (absent or present), and lymph node status (not metastasised or not resected). The 5-year survival rate was 91% among the positive cytology group and 95% among the negative cytology group (Figure 1). There was no significant difference in survival between patients with positive or negative cytology (log-rank, $P=0.42$). There were no significant differences in the survival of patients in subgroups according to cervical involvement (log-rank, $P=0.89$), lymph - vascular space invasion (log-rank, $P=0.40$), and lymph node status (log-rank, $P=0.79$). Significant differences in survival were found among patients in subgroups according to age, myometrial invasion and histologic grade. Multivariate analysis of testing for differences in survival among the subgroups of cytology, age, depth of myometrial invasion, and histologic grade was performed. The proportional hazards model revealed that only histologic grade was an independent prognostic factor and positive cytology was not an independent adverse prognostic factor (Table 2).

Similarly, the DFS was assessed in the same subgroups. The DFS at 36 months was 90% among the patients with positive cytology, compared with 94% among the patients with negative cytology, and this difference was not significant (log-rank, $P=0.52$) (Figure 2). Univariate analysis also revealed no significant differences in the DFS of patients in subgroups according to lymph - vascular space invasion (log-rank, $P=0.29$), and lymph node status (log-rank, $P=0.60$). There were significant differences in the DFS of patients in subgroups according to age, myometrial invasion, histologic grade, and cervical involvement. Among these significant subgroups and the subgroup according to peritoneal cytology, the Cox-proportional hazards model showed that only histologic grade was an independent prognostic factor for DFS, and that positive cytology was not an independent factor (Table 3).

Prognosis and failure sites

Among the 280 patients, 14 patients (5%) suffered tumour recurrence. Table 4 presents the clinical characteristics of the recurrent patients. Peritoneal spread was found in only 20% (one out of five) of the patients with positive cytology who recurred, and the affected site was outside the peritoneal cavity in the remaining 13 patients.

DISCUSSION

In the past 20 years, over 50 reports on the significance of positive peritoneal cytology in endometrial carcinoma have been published, and many conflicting results have appeared in the literature. Based on studies that found that positive cytology is an independent adverse prognostic factor (Harouny *et al*, 1988; Mazurka *et al*, 1988; Brewington *et al*, 1989; Turner *et al*, 1989; Sutton, 1990; Morrow *et al*, 1991; Grigsby *et al*, 1992; Kadar *et al*, 1994; Descamps *et al*, 1997; Kashimura *et al*, 1997; Obermair *et al*, 2001), postoperative adjuvant therapy was recommended for patients with positive peritoneal cytology. Progestins, whole abdominal external radiation, intraperitoneal radioactive chromic phosphate (^{32}P), and multiagent chemotherapy have been proposed. The efficacy of these modalities for treating positive cytology in the absence of other evidence of extrauterine disease is not universally accepted (McLellan *et al*, 1989; Lurain, 1992). On the other hand, investigators who did not find that malignant peritoneal cytology is a significant prognostic factor found no benefit of adjuvant therapy in patients with positive cytology in the absence of other adverse prognostic factors (Yazigi *et al*, 1983;

Table 3 Univariate analysis and multivariate proportional hazards model for DFS

	Univariate	Multivariate		
	P-value	Hazard ratio	95% CI[a]	*P*-value
Peritoneal cytology	0.52			
Positive		0.83	0.24–2.88	0.77
Age (y)	0.005			
Over 60		2.23	0.93–5.32	0.06
Myometrial invasion	0.006			
<1/3		1.94	0.23–16.04	0.53
1/3–2/3		2.16	0.23–19.85	0.49
>2/3		3.63	0.39–33.74	0.25
Histologic grade	<0.0001			
Grade 2		1.32	0.40–4.30	0.63
Grade 3		3.46	1.21–9.92	0.02
Cervical involvement	0.007			
Mucosal		3.47	0.86–14.01	0.07
Stromal		0.55	0.12–2.48	0.44

[a]95% confidence interval.

Hernandez *et al*, 1985; Konski *et al*, 1988; Hirai *et al*, 1989; Lurain *et al*, 1989; Grimshaw *et al*, 1990; Kadar *et al*, 1992; Kennedy *et al*, 1993; Ayhan *et al*, 1994; Ebina *et al*, 1997; Yalman *et al*, 2000). This discrepancy is probably because of the following: (1) the reported incidence of positive cytology was approximately 10% and the number of subjects was small; (2) the difference between the surgical stage and the clinical stage was not always distinguished; (3) various modalities of preoperative and/or postoperative therapies were used; (4) in the statistical analysis, multivariate analysis was not always employed; (5) the objectivity of the cytopathologic diagnosis was not always guaranteed; and (6) a prospective study has not been performed.

The prognosis of endometrial carcinoma appears to be good, and an overall 5-year survival rate of 76% can be achieved (Creasman *et al*, 2001) because the majority of patients with endometrial carcinoma have localised, low-grade disease at the time of primary treatment. Indeed, our data indicated that the 5-year survival rate of patients with endometrial carcinoma confined to the uterus was above 90% regardless of positive peritoneal cytology. Additionally, the Cox-proportional hazards model demonstrated that positive peritoneal cytology was not an independent adverse factor for survival and DFS. Although the number of patients in our study was not as large as that in some other studies, all patients were surgically staged and received no preoperative therapy. Only three patients (1%) were treated with postoperative adjuvant therapy. Considering the above facts, it is doubtful whether patients with no extrauterine disease except for positive peritoneal cytology require more aggressive therapy. As for the statistical power, it was difficult to evaluate the power calculation statistically because the number of statistical events was limited and our study was a retrospective one.

In the study of the Gynecologic Oncology Group (GOG) reported by Morrow *et al* (1991), 895 patients with clinical stage I or II (occult) carcinoma of the endometrium were analysed. In total, 29% of the patients with positive cytology developed recurrence compared with 10.5% of the cytology-negative patients, and a relation between malignant cytology and poor outcome was demonstrated by a multivariate model. This GOG study included patients with extrauterine disease, and 42.9% of the patients with no evidence of extrauterine disease received some form of postoperative radiotherapy. Turner *et al* (1989) demonstrated by multivariate analysis that positive cytology was a poor prognostic factor for both the 5-year survival rate (84 *vs* 96%) and progression-free interval (65% at 5 years *vs* 96%) among 567 patients with surgical stage I disease. In that study, 28 women (4.9%) had positive cytology, and the primary treatment was surgery alone for 90 patients (16%), surgery with preoperative adjuvant radiotherapy in 409 patients (72%), and surgery with postoperative adjuvant radiotherapy in 46 patients (8%). Preoperative radiotherapy may have affected the surgical stage and peritoneal cytology of many patients enrolled in that study.

Similarly, in many previous studies that found that positive peritoneal cytology had no prognostic significance, we found the same problems; for example, many patients received pre- or postoperative adjuvant therapy, or multivariate analysis was not employed. Grimshaw *et al* (1990) showed that there was no significant difference in the 5-year survival rate between patients with positive or negative cytology (80 *vs* 86%) among 305 surgical stage I patients. In that study, statistical significance was analysed with only the Fisher exact test. Kadar *et al* (1992) demonstrated that positive cytology did not influence survival if the disease was confined to the uterus using Cox's proportional hazards model. In that study, treatment variables included the use of adjunctive radiation therapy and the type of radiation therapy used, and 59% (159 out of 269) of the patients received radiation therapy. In the present study, no patient received preoperative therapy and only

Table 4 Clinical characteristics of 14 recurrent patients

Patient no.	Peritoneal cytology	Histologic grade	Depth of invasion	Cervical involvement	Initial failure sites	Time to recurrence (months)	Treatment	Status
1	Positive	1	>2/3	Mucosal	Nodes	24	Not done	DOD[b] (40)
2	Positive	1	<1/3	Mucosal	Peritoneum	9	Chemo	AWD[c] (39)
3	Positive	2	<1/3	Absent	Lung	19	Chemo	DOD (22)
4	Positive	3	>2/3	Mucosal	Lung	6	Chemo	DOD (19)
5	Positive	3	>2/3	Absent	Nodes, bone	24	RT[a]	DOD (26)
6	Negative	1	1/3–2/3	Absent	Vagina	4	RT	NED[d] (116)
7	Negative	1	1/3–2/3	Absent	Vagina	26	RT	NED (64)
8	Negative	1	>2/3	Stromal	Lung, vagina	4	RT, Chemo	NED (72)
9	Negative	1	Absent	Absent	Systemic	26	RT, Chemo	DOD (41)
10	Negative	1	>2/3	Stromal	Lung	13	Surgery	NED (57)
11	Negative	2	>2/3	Absent	Lung	33	Not done	DOD (42)
12	Negative	2	>2/3	Absent	Spleen	24	Surgery	AWD (47)
13	Negative	3	>2/3	Absent	Bone	11	Not done	DOD (13)
14	Negative	3	>2/3	Absent	Lung	31	Unknown	DOD (40)

[a]Radiation therapy; [b]Dead of disease; [c]Alive with disease; [d]No evidence of disease.

three (1%) of the 280 patients received postoperative adjuvant therapy.

Positive cytology was not an adverse prognostic factor in endometrial carcinoma limited to the uterus, and it is unknown from where these cancer cells were derived. Although there are insufficient data to reach a conclusion about the source of the cancer cells in peritoneal washings, the following mechanisms may be deduced from the literature (McLellan *et al*, 1989; Lurain, 1992): (1) result of transtubal transport; (2) direct extension of tumour through the myometrium; (3) lymphatic metastasis to the peritoneal cavity; and (4) reflection of multifocal peritoneal occult spread. The transtubal transport theory seems to be the most popular. Hirai *et al* (2001) demonstrated by using a tube that was inserted into the abdomen during the operation for cytologic analysis, that positive peritoneal cytology usually disappeared within a short period of time after the operation (within 14 days) in patients with limited disease in comparison to patients with adnexal metastasis. Additionally, as for the failure site in the present series, peritoneal spread was found in only 20% of the patients with positive cytology who recurred, and in the remaining patients, the affected site was outside the peritoneal cavity. Another study (Lurain *et al*, 1989) showed that 17% of patients with stage I disease who had positive cytology suffered recurrence, and only 20% of these recurrences were within the abdomen. The above-mentioned findings suggest that malignant cells obtained by peritoneal washing may not reflect the potential of peritoneal spread in a significant proportion of endometrial carcinoma cases unless other extrauterine disease is present.

In most studies including the present study, peritoneal cytology was analysed by conventional cytopathologic techniques and morphologic findings. Although cytopathologic findings including adequate sampling are essential for analysing the prognostic value of peritoneal cytology, evaluating the objectivity of cytopathologic diagnosis is difficult. The available data indicated that among 3091 reported cases with clinical stage I disease, the overall incidence of positive cytology was 11.4% (range, 2.9–29.8%) (McLellan *et al*, 1989). If the positive rate in a study is rather high, the possibility that reactive mesothelial cells were confused with malignant cells must be considered. If the positive rate in a study is too low, sampling error should be considered. Szpak *et al* (1981) demonstrated that the presence of abundant malignant cells (greater than 1000 cells per 100 ml sample) significantly shortened the time to recurrence. Yanoh *et al* (1999) proposed that the findings of endometrial adenocarcinoma cells exhibiting high cellularity, scalloped edge of cell clusters and isolated cells in peritoneal cytology could be regarded as a risk factor for intra-abdominal recurrence. Luo *et al* (2001) reported that analysis of peritoneal washings with conventional and immunocytochemical (MOC-31) staining improved the diagnosis of peritoneal cytology in endometrial carcinoma, and positive combined cytology was a prognostic factor. The results of research on these morphological findings have not yet been widely accepted, and will be worthy of consideration in the future.

Currently, we believe that the presence of positive peritoneal cytology is not an independent prognostic factor, and that it does not seem to reflect the potential of peritoneal spread in patients with endometrial carcinoma confined to the uterus. Adjuvant therapy such as chemotherapy, radiation therapy, or progestins does not appear to be beneficial in these patients at present. Nonetheless, further investigation and prospective multiinstitutional prospective analyses are needed.

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REVIEW ARTICLE

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Association of HNPCC and endometrial cancers

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Abstract Hereditary nonpolyposis colorectal cancer (HNPCC) is among the representative familial cancers that are autosomally dominant inherited disorders. Because endometrial cancers develop at high rates in women with HNPCC, it is suggested that some endometrial cancers are familial cancers that are induced by mutations of the DNA mismatch repair (MMR) genes, as in HNPCC. To understand the clinical pathology of familial endometrial cancers that are associated with HNPCC, we surveyed the family histories of 385 patients with endometrial cancer and found that 0.5% of endometrial cancers met the new diagnostic criteria of HNPCC. From molecular and biological analyses, we found microsatellite instability in 30.8% of endometrial cancers and germline mutations of MMR genes in 8.3%. These results suggest a close relationship of MMR gene mutations to the development of endometrial cancers. For a better understanding of the clinical pathology of HNPCC-associated familial endometrial cancers, it is critical for gynecologists to perform a large multicenter study, including detailed family histories.

Key words HNPCC · Endometrial cancer · Revised Amsterdam Criteria · *hMLH1* · *hMSH2*

Introduction

Among gynecological malignant tumors, the incidence of endometrial cancers has increased in Japan in recent years (due to the westernization of our lifestyle and changes in the environment), rising to about 40% of all uterine cancers in Japan. As the number of cases diagnosed and the incidence have increased in Japan and are projected to continue to increase worldwide, the prevention of endometrial cancers is a most important issue for gynecologists. However, the mechanisms of their development and progression remain unclear. It has been assumed that risk factors, such as obesity and high estrogen states, play important roles in the development of endometrial cancers. On the other hand, it has been suggested that genetic factors are closely involved in their development, because multiple cancers occur in endometrial cancer patients, and there are many instances in which family members of endometrial cancer patients also develop cancer. Recently, it was found that endometrial cancer occurred at a high rate in women with hereditary nonpolyposis colorectal cancer (HNPCC; a representative familial tumor inherited as an autosomal dominant trait), showing the relation of familial endometrial cancers with HNPCC.

Hereditary nonpolyposis colorectal cancer (HNPCC) comprises a group of inherited diseases (with high risks of cancers in the colorectum) which are transmitted by autosomal dominant inheritance. In 1913, Wartin et al. reported for the first time the clustered occurrences of colorectal cancers within certain families. After a detailed analysis of the families, the disease concept, "cancer family syndrome", was proposed by Lynch and Krush in 1971.[1,2] In 1991, the International Collaborative Group (ICG) proposed HNPCC clinical diagnostic criteria, called the "Amsterdam Minimum 0Criteria":[3] (1) colorectal cancer (pathologically verified) is diagnosed in at least three relatives. (2) One is a first degree relative of the other two. (3) At least two successive generations are affected. (4) One colorectal cancer is diagnosed before the age of 50 years.

In 1993, it was discovered that HNPCC was inherited through the DNA mismatch repair genes, the so-called DNA mismatch repair (MMR) gene group. The Amsterdam Minimum Criteria were amended by the ICG-HNPCC in 1999 to include the diagnosis of endometrial cancers.[4] Thus, the association of endometrial cancers and

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Table 1. Hereditary nonpolyposis colon cancer (HNPCC) clinical criteria

Amsterdam Minimum Criteria (1991)
1. At least three relatives with colorectal cancer (pathologically verified)
2. One is a first-degree relative of the other two
3. At least two successive generations should be affected
4. One colorectal cancer is diagnosed before the age of 50 years
5. FAP should be excluded
New Amsterdam Criteria (1999)
1. At least three relatives with an HNPCC-associated cancer (cancer of colorectum, endometrium, small bowel, urethra, or renal pelvis)
2–5: Same as for Amsterdam Minimum Criteria

Four types of cancer, including uterine cancer, were added to the HNPCC-associated cancers in the revision made in 1999 (from Banno et al. [2003],[5] with permission)

HNPCC has been recognized internationally, suggesting that, like HNPCC, some endometrial cancers develop from mutations involving one of the MMR genes.

New clinical criteria of HNPCC

After the Amsterdam Minimum Criteria were proposed as the HNPCC clinical diagnostic criteria in 1991, several other clinical standards, such as the Japanese Criteria and the Bethesda Criteria, were established, inviting confusion in the clinical diagnosis of HNPCC from time to time. In 1999, the Amsterdam Minimum Criteria were modified, and the New Amsterdam Criteria were approved by the ICG-HNPCC[5] (Table 1). Of particular note in the New Amsterdam Criteria is that the old diagnostic criteria apply only to colorectal cancers, and the new ones extend to endometrial, intestinal, urethral, and renal cancers. With this revision, cases that do not meet the Amsterdam Minimum Criteria may meet the New Amsterdam Criteria, resulting in an increase in the number of cases that are diagnosed as HNPCC. In addition, it will become possible to identify cases of HNPCC by surveying the family histories of patients with endometrial cancer in detail. On the other hand, there remains a possibility that only a proportion of hereditary endometrial cancers will be identified by the New Amsterdam Criteria, because ovarian, breast, and gastric cancers, which are suggested to be associated with HNPCC, are not included.

Responsible genes in HNPCC

Mutations of DNA in cells are induced by external factors such as radiation and mutagens, as well as by errors during DNA replication. These DNA changes can result in serious consequences, including the carcinogenesis of cells. The DNA repair system provides a mechanism for removing these changes in DNA. One element is the MMR mechanism that detects and repairs errors during DNA, replication, which is carried out by the DNA MMR enzymes. This mechanism was originally studied using *Escherichia coli*, and the DNA MMR genes (*Mut S*, *Mut L*) were identified. MMR genes were found to be conserved among species, and six kinds of MMR genes (*hMSH2, hMLH1, hMSH3, hMSH6, hPMS1, and hPMS2*) have been identified. These MMR genes were found to be the genes responsible for the development of HNPCC. It is suggested that these MMR genes function in a multisubunit complex in human. During DNA replication, hMSH2 recognizes a mismatch and repairs it in a complex with other MMR proteins. Abnormalities in one to two bases are recognized by the complex of hMSH2 and hMSH6, and two to four base defects and insertions are recognized by the complex of hMSH2 and hMSH3, followed by repair in concert with hMLH1 and hPMS2 further recruited into the complex. These repair mechanisms remain to be defined further.[5]

DNA mismatch repair (MMR) genes and microsatellite instability (MSI)

When there are abnormalities in the MMR genes, mismatch bases generated during DNA replication cannot be corrected, generating DNA chains with different lengths. This phenomenon tends to occur particularly in regions with repeated sequences of several bases in the human genome and is called microsatellite instability (MSI). In the presence of MSI, the incidence of genetic abnormalities in the genes involved in carcinogenesis increases. MSI has been identified in about 10% of all colorectal cancers. It is suggested that about 25% of MSI-positive colorectal cancers are varieties of HNPCC and that MSI analysis could be an effective screening method for HNPCC. Among the six MMR genes, germline mutations of the *hMLH1* gene on chromosome 3 and the *hMSH2* gene on chromosome 2 are suggested to contribute to the majority (about 60%) of HNPCC cases. It remains to be determined if similar mechanisms or genetic abnormalities are involved in hereditary forms of uterine cancer. *TGF-typeIIR*, which is involved in the regulation of cell proliferation, and *BAX*, which is involved in the induction of apoptosis, are reported as candidates for target genes in the MMR mechanism.[6,7] Other candidates for target genes include the *E2F* gene[8,9] and *TCF-4* gene.[10] Abnormalities in these genes occur very infrequently in endometrial cancers, suggesting that target genes that cause an abnormality in the MMR mechanism differ in each organ, and that other specific target genes may be responsible for endometrial cancers.

Clinical characteristics of HNPCC

The exact occurrence rate of HNPCC is unclear due to differences in reports, but it is suggested to be about 5% among all colorectal cancers.[11–17] The clinico-pathological characteristics of HNPCC are reported to include: (1) autosomal dominant inheritance; (2) approximately 85% of

gene penetrance by age 80; (3) young onset; (4) a higher occurrence on the right side of the colorectum; (5) a high frequency of cases of mucinous or poorly differentiated carcinoma; (6) diploidy on cytometric analysis; (7) significant lymphocyte infiltration; (8) MSI positive; (9) high risk of endometrial, urethral, and intestinal cancers; and (10) a favorable prognosis.[18] It is not understood why the prognosis is favorable even though there are many cases of mucinous and poorly differentiated carcinomas, but lymphocyte infiltration into the tumors and a low rate of lymphatic metastasis are suggested to contribute to the favorable prognosis. It has been reported recently that abnormalities of the MMR genes are associated with a decrease in sensitivity to chemotherapy such as cisplatin.[19]

HNPCC-associated gynecological cancers

In regard to patients with HNPCC, the incidence of other cancers in first-degree relatives is highest for endometrial cancers, at 9%–19%, followed by 6%–14% for gastric cancers, and 5%–7% for ovarian cancers.[20] A relation between HNPCC and ovarian cancers is suggested, although it is not as strong as that for endometrial cancers. Recently, a relation has also been suggested for breast cancers. Muir-Torre syndrome, a hereditary disease associated with sebaceous tumors (adenoma, epithelioma, and carcinoma) and malignant tumors of internal organs, is assumed to be transmitted by autosomal dominant inheritance. Among malignant tumors of internal organs, the occurrence of colorectal cancers and urethral/genital organ cancers is high, followed by breast cancers and hematological malignancies. The probability of developing breast cancer is about 12%. Mutations of the *hMSH2* gene, which is an MMR gene and a gene responsible for HNPCC, were reported in 2 two patients with Muir-Torre syndrome.[21,22] It is suggested that this disease is one of the hereditary breast cancers and a subtype of HNPCC.

Molecular epidemiological analysis of HNPCC-associated endometrial cancers

To study the status and clinicopathological characteristics of familial endometrial cancers, the family history and disease history in 385 patients who received treatments for endometrial cancer at our clinic between 1994 and 2002 were studied. After obtaining informed consent from the patients, MSI analysis was performed in 39 of these patients. From the study of family history, 2 of the 385 patients met the New Amsterdam Criteria of HNPCC, and, therefore, about 0.5% of all endometrial cancers were HNPCC-associated tumors[23] (Figs. 1 and 2). The familial cancer clustering in the relatives (890 persons; 439 male and 451 female) of the 39 patients with endometrial cancers was examined. The occurrence of endometrial, colorectal, and ovarian cancers, in which the incidence of HNPCC is high, appeared to be high, suggesting possible involvement of common genetic factors between endometrial cancers and HNPCC.

In MSI analysis using one to five microsatellite markers (Table 2), MSI was identified with at least one marker in 12 of the 39 patients with endometrial cancer (30.8%; Fig. 3).[23] Thus, the occurrence of MSI was significantly

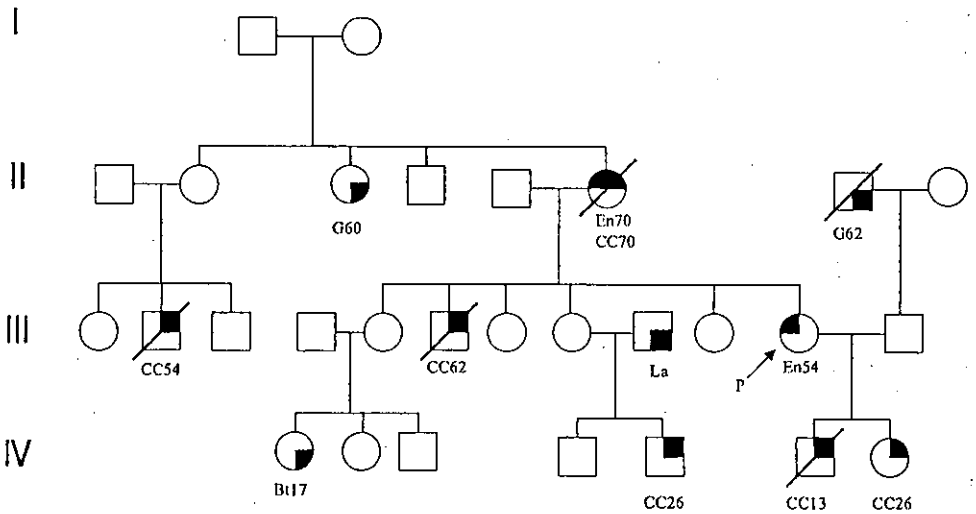

Fig. 1. Pedigree of case A, which meets the New Amsterdam Criteria. The *arrow* shows the proband (*p*) with uterine cancer. ○, female; □, male. ◐ and *En* are uterine cancers. ◔ and *CC* are large-bowel cancers. ◕, other cancers. (*G*, *Bt*, and *La*, respectively, show gastric cancer, brain tumor, and laryngeal cancer.) The *numbers* show the age at the time of diagnosis. The *diagonal lines* show deceased family members. The *Roman numerals on the left* show the generation numbers. Five cases of hereditary nonpolyposis colon cancer (HNPCC)-associated tumors, including the original carrier, were found within the first-degree relatives. The mother of the original carrier had multiple cancers (uterine and large-bowel cancers) (from Banno [2004],[23] with permission)

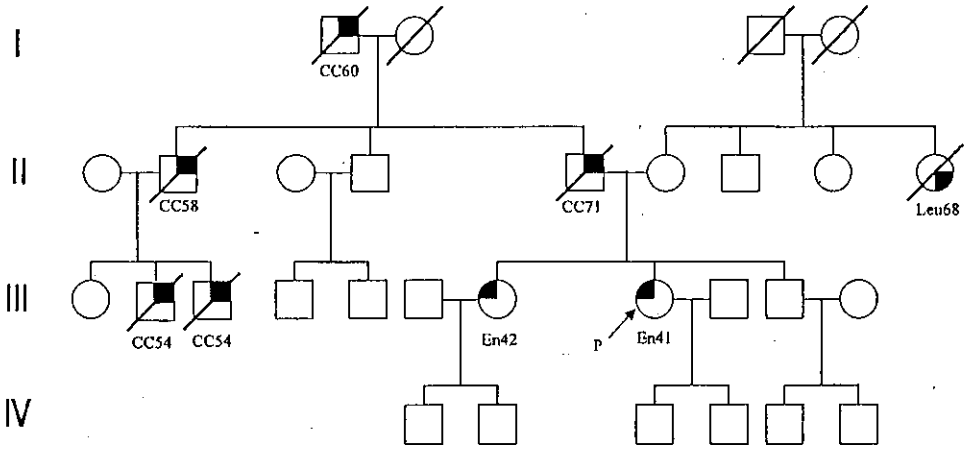

Fig. 2. Pedigree of case B, which meets the New Amsterdam Criteria. The *arrow* is the proband (*p*) with uterine cancer. ○, female; □, male. ◐ and *En* are uterine cancers. ◔ and *CC* are large-bowel cancers. ◑ is other cancers (*Leu*, leukemia). The *numbers* show the age at the time of diagnosis. The *diagonal lines* show deceased family members. The *Roman numerals on the left* show the generation numbers. The original carrier developed uterine cancer at the relatively young age of 41 years. Three cases, including the original carrier of HNPCC-associated tumors, were found within the first-degree relatives. (from Banno et al. [2004],[23] with permission)

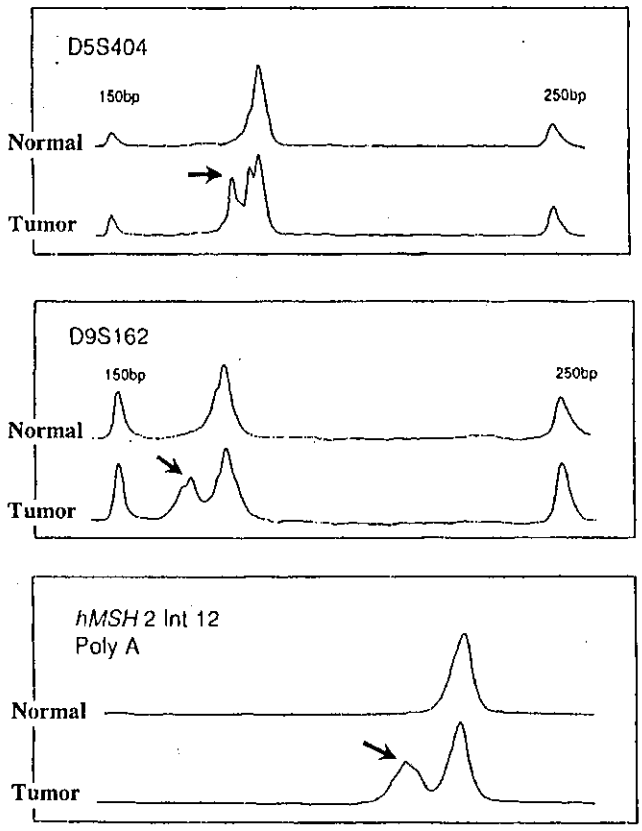

Fig. 3. Microsatellite instability (MSI) analysis in case 1. The *arrow* shows an abnormal wave pattern observed in the tumor-derived DNA. Five areas at the most were analyzed with microsatellite markers. MSI was judged positive when an abnormal wave pattern due to replication error was observed in one area or more *Int, intron* (from Banno et al. [2003],[24] with permission)

higher than that in cancers of other organs, proving that abnormal MMR genes play important roles in the development of endometrial cancers. Statistical analysis of the relationship between MSI-positive endometrial cancers and clinopathological characteristics failed to show a correlation with early onset and multiple cancers, although this was generally observed in familial cancers. On the other hand, histopathological association with tumor differentiation was shown, as in HNPCC, by the significant number of poorly differentiated G2 and G3 adenocarcinomas in MSI-positive endometrial cancers (Table 3). There was no difference in the prognosis of MSI-positive and MSI-negative patients.

In 12 patients with MSI-positive endometrial cancer, germline mutations involving the *hMLH1* and *hMSH2* genes, both of which are DNA MMR genes, were analyzed, after approval by the institutional review board and obtaining informed patient consent (Fig. 4). The results showed germline mutations of the *hMLH1* and *hMSH2* genes in 3 of the 12 patients.[24] Mutation of one base was identified in the *hMSH2* gene in two cases, in which ATG was replaced by ATA at codon 688 (Met to Ile) and CTT was replaced by TTT at condon 390 (Leu to Phe). A nonsense mutation (Arg to stop codon) was identified in the other case, with CGA replaced by TGA at codon 100 of the *hMLH1* gene (Table 4). The mutations of one base in the *hMSH2* gene in two cases were not functional mutations but genetic polymorphisms in Japanese.

In the patient with a nonsense mutation in the *hMLH1* gene, a mucinous adenocarcinoma was present in the ileocecum, and hMLH1 protein expression was decreased in the endometrial and ileocecal cancers. A similar germline mutation was identified in a sister of the patient (Fig. 5). All these results clearly indicate that this is a functional germline mutation. It appears to be the case that germline mutations of the *hMLH1* gene disrupt the MMR mechanism, resulting in carcinogenesis of the endometrium

Table 2. Primer sequences of microsatellite markers used in the present study

Locus	Forward primer sequence	Reverse primer sequence	Size (bp)
D2S123	5′ AAACAGGATGCCTGCCTTTA 3′	5′ GGACTTTCCACCTATGGGAC 3′	197–227
D3S1284	5′ GGAATTACAGGCCACTGCTC 3′	5′ GGAATTACAGGCCACTGCTC 3′	155–177
D5S404	5′ GATCACCACATTCCACCTAAT 3′	5′ GATCACCACATTCCACCTAAT 3′	180–198
D9S162	5′ GCAATGACTTAAGGTTC 3′	5′ GCAATGACCAGTTAAGGTTC 3′	172–196
hMSH2 intron12	5′ GATGTTCCACATCATTACTG 3′	5′ GTGGTTCCACATCATTACTG 3′	182

The primer sequences and polymerase chain reaction (PCR) products are shown for the five different micro-satellite markers used in this study. The *hMSH2* intron 12 is a mononucleotide marker, and the other four markers are dinucleotide markers (from Banno et al. [2003],[24] with permission)

Table 3. Microsatellite instability (MSI) analysis in 39 patients with endometrial cancers

Case no.	Age at operation (years)	Histological type	Grade	Stage	Double cancer	D2	D3	D5	D9	IN
1	58	E	G2	IIIa	–	+	+	+	+	+
2	56	E	G1	Ia	–	+	+	–	+	+
3	55	AS	G2	IIIc	–	+	+	+	–	+
4	55	AS	G3	Ic	OC	+	+	+	+	–
5	60	E	G1	Ib	–	+	–	+	–	+
6	62	E	G2	IIIc	–	+	+	–	+	–
7	55	E	G3	IIIc	–	+	–	+	+	–
8	54	E	G1	Ia	CC	+	+	+	–	–
9	50	E	G1	IIb	OC	–	+	–	+	–
10	56	E	G2	Ic	–	–	–	–	–	+
11	66	E	G1	Ia	–	–	–	–	–	+
12	44	E	G2	IIIc	CC	+	ND	ND	ND	ND
13	59	E	G1	IIa	BC, OC	–	–	–	–	–
14	48	E	G2	Ib	–	–	–	–	–	–
15	58	E	G2	IIIc	–	–	–	–	–	–
16	65	E	G3	IIc	–	–	–	–	–	–
17	72	E	G2	IVb	–	–	–	–	–	–
18	55	E	G1	Ib	–	–	–	–	–	–
19	65	E	G1	Ic	–	–	–	–	–	–
20	56	E	G1	Ib	–	–	–	–	–	–
21	69	E	G1	Ia	–	–	–	–	–	–
22	68	E	G1	Ic	–	–	–	–	–	–
23	34	E	G1	Ib	–	–	–	–	–	–
24	50	E	G1	Ib	–	–	–	–	–	–
25	61	E	G1	Ic	–	–	–	–	–	–
26	57	E	G1	Ic	–	–	–	–	–	–
27	55	E	G1	Ia	BC	–	–	–	–	–
28	64	E	G3	IIIa	–	–	–	–	–	–
29	56	E	G1	Ic	–	–	–	–	–	–
30	44	E	G1	Ib	–	–	–	–	–	–
31	45	E	G1	Ib	OC	–	–	–	–	–
32	53	E	G1	Ib	–	–	–	–	–	–
33	69	E	G1	Ia	CC	–	–	–	–	–
34	81	E	G1	IIIa	–	–	–	–	–	–
35	58	E	G1	Ib	–	–	–	–	–	–
36	60	E	G3	Ic	–	–	–	–	–	–
37	64	E	G1	IIIc	–	–	–	–	–	–
38	66	S	G2	IIIa	–	–	–	–	–	–
39	62	S	G2	IIIa	–	–	–	–	–	–

This table summarizes the results of MSI analysis and clinical pathological information (age at operation, clinical stage, differentiation grade, and presence or absence of multiple cancers) for 39 patients with uterine cancers. MSI-positive tumors were identified in 12 patients (30.8%) (from Banno et al. [2003],[24] with permission)
E, endometrioid adenocarcinoma; AS, adenosquamous carcinoma; S, serous adenocarcinoma; OC, ovarian cancer; BC, breast cancer; CC, colorectal cancer; D2, D2S123; D3, D3S1284; D5, D5S404; D9, D9S162; IN, *hMSH2* intron 12; ND, not done

and ileocecum. This case did not meet the New Amsterdam Criteria, but this case was part of a familial cancer cluster. The fact that this case did not meet the New Amsterdam Criteria suggests the possibility that the clinical criteria may identify only some HNPCC-associated endometrial cancers.

To examine the correlation between MSI and inactivation of one of the DNA MMR genes, immunohistochemical analyses were performed, using anti-hMLH1 antibody in 12 cases of MSI-positive endometrial cancer. A decrease in staining with anti-hMLH1 antibody, indicating a decrease of hMLH1 protein expression, was observed in 8 of 12 cases

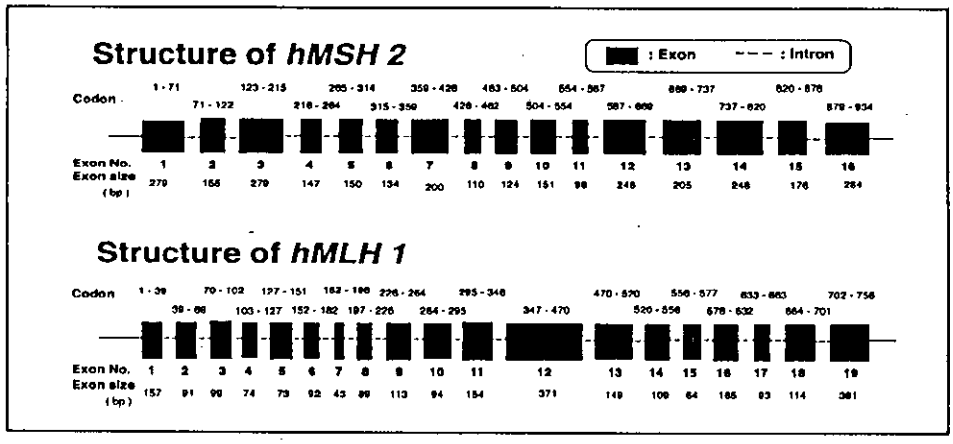

Fig. 4. DNA structures and primer sequences for *hMLH1* and *hMSH2* genes. The DNA structure and the location of the primers for DNA sequencing are shown in the *upper* and *lower pants*, respectively. The *hMSH2* and *hMLH1* genes are composed of 16 and 19 exons, respectively. *PCR*, polymerase chain reaction (from Banno et al. (2003),[24] with permission)

Primer sequences for *hMSH2*

	Sense primer sequence		Antisense primer sequence		Size of PCR products (bp)
Exon1	1a	5' TCGCGCATTTTCTTCAACC 3'	1b	5' GTCCCTCCCCAGCACCG 3'	285
Exon2	2a	5' TTGAACATGTAATATCTCAAATCTGT 3'	2b	5' AAAGGAAGATAATTACCTTATATGC 3'	220
Exon3	3a	5' TCAAGAGTTTGTTAAATTTTTAAAA 3'	3b	5' CTAGGCCTGGAATCTCCTCT 3'	363
Exon4	4a	5' TTCCTTTTCTCATAGTAGTTTAAAC 3'	4b	5' TTGTAATTCACATTTATAATCCATG 3'	216
Exon5	5a	5' CCAGATGGTATAGAAATCTTCG 3'	5b	5' CCATTCAACATTTTTAACCCTT 3'	240
Exon6	6a	5' GCTTGCCATTCTTTCTATTTTATT 3'	6b	5' GCAGGTTACATAAAACTAACGAAAG 3'	214
Exon7	7a	5' CATTAATTCAAGTTAATTTATTTCA 3'	7b	5' CATTAATTCAAGTTAATTTATTTCA 3'	246
Exon8	8a	5' TGAGATCTTTTTATTTGTTTGTTTT 3'	8b	5' TTTGCTTTTTAAAAATAACTACTGC 3'	200
Exon9	9a	5' GGATTTTGTCACTTTGTTCTGTT 3'	9b	5' TCCAACCTCCAATGACCCAT 3'	178
Exon10	10a	5' TGGAATACTTTTTCTTTTCTTCTT 3'	10b	5' GCATTTAGGGAATTAATAAAGGG 3'	235
Exon11	11a	5' ATAAAACTGTTATTTCGATTTGCA 3'	11b	5' CCAGGTGACATTCAGAACATT 3'	164
Exon12	12a	5' TTATTATTCAGTATTCCTGTGTACA 3'	12b	5' CCCACAAAGCCCAAAAACC 3'	325
Exon13	13a	5' ATAATTTGTTTTGTAGGCCCC 3'	13b	5' TTTCTATCTTCAAGGGACTAGGAG 3'	255
Exon14	14a	5' CCACATTTTATGTGATGGGAA 3'	14b	5' CCAATAGTACATACCTTTCTTCACC 3'	307
Exon15	15a	5' GTCCCCTCACGCTTCCC 3'	15b	5' AAACTATGAAAACAAACTGACAAAC 3'	232
Exon16	16a	5' AATGGGACATTCACATGTGTT 3'	16b	5' CCATGGGCACTGACAGTTAA 3'	306

Primer sequences for *hMLH1*

	Sense primer sequence		Antisense primer sequence		Size of PCR products (bp)
Exon1	1a	5' ACATCTAGACGTTTCCTTGG 3'	1b	5' AAGTCGTAGCCCTTAAGTGA 3'	195
Exon2	2a	5' TTTTCTGTTTGATTTGCCAG 3'	2b	5' GACTCTTCCATGAAGCGC 3'	162
Exon3	3a	5' TGGGAATTCAAAGAGATTTG 3'	3b	5' CAACAGGAGGATATTTTACAC 3'	211
Exon4	4a	5' GAAGCAGCAGTTCAGCTAAG 3'	4b	5' ATGAGTAAAAGAAGTCAGAC 3'	203
Exon5	5a	5' GGGATTAGTATCTATCTCTCTACTG 3'	5b	5' CAACAATTTACTCTCCATGTAC 3'	158
Exon6	6a	5' GTCAGTGCTTAGAACTGTGCTG 3'	6b	5' TCTCAGAGACCCACTCCCAG 3'	262
Exon7	7a	5' CTAGTGTGTGTTTTTGGCAAC 3'	7b	5' CCTTATCTCCACCAGCAAAC 3'	179
Exon8	8a	5' AATCCTTGTGTCTTCTGCTG 3'	8b	5' TAGGTTATCGACATACCGAC 3'	137
Exon9	9a	5' TTTTGTAATGTTTGAGTTTTGAGTA 3'	9b	5' GTTTCCTGTGAGTGGATTTC 3'	214
Exon10	10a	5' TCCTGAGGTGATTTCATGAC 3'	10b	5' CTGTTCCTTGTGAGTCTTGG 3'	232
Exon11	11a	5' TCCCACTATCTAAGGTAATTG 3'	11b	5' AGAAGTAGCTGGATGAGAAG 3'	231
Exon12	12a	5' CTTATTCTGAGTCTCTCC 3'	12c	5' GGTTTGCTCAGAGGCTGCAG 3'	474
	12b	5' CCAGATGGTTCGTACAGATTCC 3'	12d	5' GAGGTAGGCTGTACTTTTCC 3'	240,300
Exon13	13a	5' CACAGAGAAGTTGCTTGCTCC 3'	13b	5' TTGAGCCCTATCATCCCATG 3'	289
Exon14	14a	5' GGGTTGGTAGGATTCTATTAC 3'	14b	5' GGACCATTGTTGTAGTAGCTC 3'	214
Exon15	15a	5' CAACTGGTTGTATCTCAAGC 3'	15b	5' GAAACGATCAGTTGAAATTTC 3'	175
Exon16	16a	5' GCTTGCTCCTTCATGTTCTTG 3'	16b	5' GATTACAGCCATGAGCCACC 3'	278
Exon17	17a	5' GACAGCATTATTTCTTGTTCCC 3'	17b	5' CGAAATGCTTTTTAGTATCTGCTTG 3'	168
Exon18	18a	5' AATTCGGGGTACCTATTTTGAGG 3'	18b	5' ATTGTATAGGCCTGTCCTAG 3'	202
Exon19	19a	5' ACCAGTGTAGGTTGGGATGC 3'	19b	5' AAGAACACATCCCACAGTGC 3'	259

* First PCR : 12a + 12d , Second PCR : 12a + 12c or 12b + 12d

(66.7%) of MSI-positive endometrial cancer. Thus, MSI was strongly associated with negative staining with anti-hMLH1 antibody in endometrial cancers (Fig. 6).[5] These results showed that another mechanism of inactivation of the *hMLH1* gene, possibly including methylation, but not germline mutation, was involved in the MSI in many cases of endometrial cancer, and that immunohistochemical methods could take the place of MSI analysis.

Summary and future directions

It is important to understand the biological characteristics of endometrial cancers, for which the mechanism of carcinogenesis is not fully known. Some endometrial cancers are familial tumors, and abnormalities in the DNA MMR genes are significantly involved in their carcinogenesis. We believe it is very important to identify and analyze these

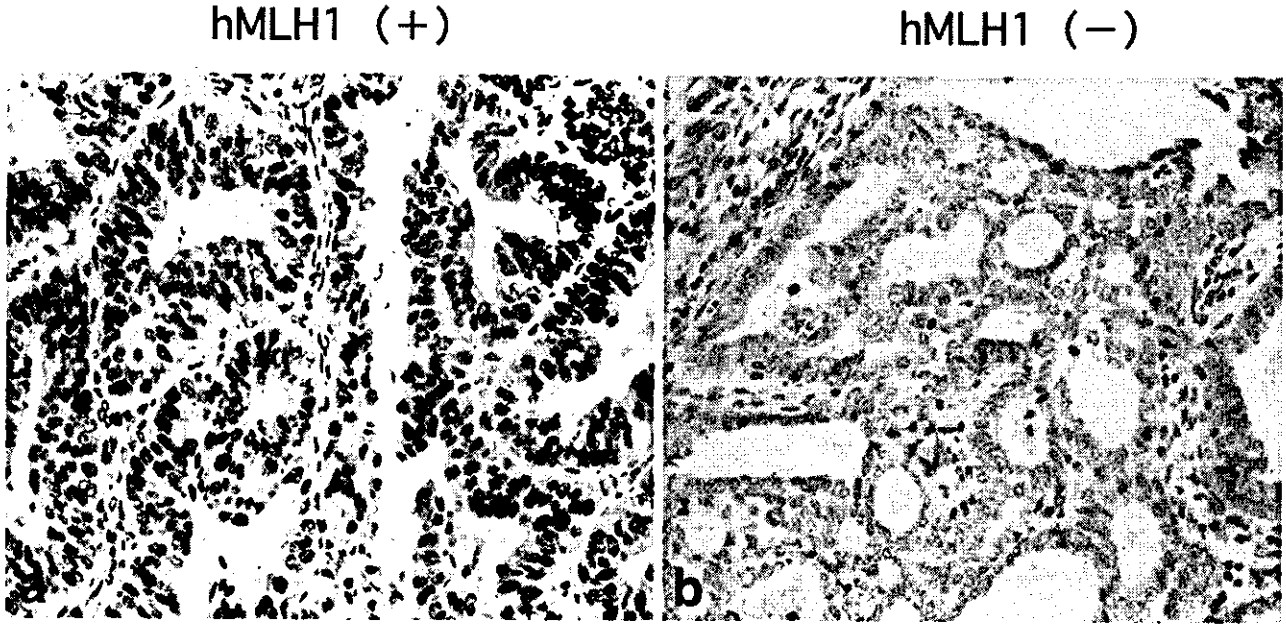

Fig. 5a,b. Reduced expression of hMLH1 protein in uterine cancers (endometrioid adenocarcinona). Immunohistochemical staining with anti-hMLH1 antibody is shown for an MSI-negative case (**a**; case 13; stage IIa, G1) and an MSI-positive case (**b**; case 5; stage Ib, G1). Staining with anti-hMLH1 antibody is absent in the nuclei of endometrial tumors in case 5 (**b**). In both cases, staining is positive in the normal area surrounding tumors. **a** and **b** ×100 (from Banno et al. [2003],[5] with permission)

Table 4. Germline mutation analysis of *hMLH1* and *hMSH2* genes

Case no.	Exon affected	Genomic DNA alteration	Predicted effects (codon)
1	Exon 13 (*hMSH2*)	ATG to ATA	Met (688) Ile
11	Exon 7 (*hMSH2*)	CTT to TTT	Leu (390) Phe
12	Exon 3 (*hMLH1*)	CGA to TGA	Arg (100) Stop

Germline mutation of an MMR gene was detected in 3 out of 12 patients with MSI-positive uterine cancers. Mutations of one base were identified at codon 688 of the *hMSH2* gene in case 1 and at codon 390 in case 11. Both cases were not functional mutations but genetic polymorphisms in Japanese. In case 12, a nonsense mutation was identified at codon 100 of the *hMLH1* gene (from Banno et al. [2003],[24] with permission)

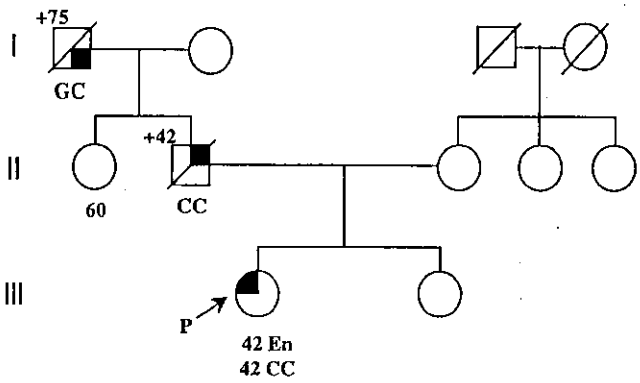

Fig. 6. Pedigree of case 12 with germline mutation in *hMLH1*. This is the family tree of case 12, with a germline gene mutation at codon 100 of the *hMLH1* gene. The *arrow* shows the original carrier of uterine cancer (*p*). ○, female; □, male. ◐ and *En* are uterine cancers, ◔ and *CC* are colorectal cancers. ◒ and *GC* are gastric cancer. The *diagonal lines* show deceased family members. *The numbers* are the ages at onset or death. *The Roman numerals on the left* show the generation numbers (from Banno et al. [2003],[5] with permission)

endometrial cancer cases. However, familial endometrial cancers constitute only about 0.5% of the total, and it is essential to examine family histories in detail. In addition, gynecologists must be accurately informed, and it is important to perform large-scale, multicenter studies nationwide and internationally. Furthermore, there is an urgent need for systems of genetic analysis and genetic counseling of patients. If the significance of surveillance methods and MSI could be understood from pathological studies of HNPCC-associated tumors, new diagnostic and therapeutic methods applicable to all endometrial cancers could be established in the future.

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