Table II. Immunohistochemical analysis of cell cycle gene expression in advanced serous epithelial ovarian cancer.

	Cyclin D1	pRb	$p16^{Ink4a}$	p53	$p27^{Kip1}$	$p21^{Waf1/Cip1}$	Cyclin E
Range of staining (% positive)	0-50	0-90	0-90	0-90	0-80	0-70	0-90
Median staining (%)	10	50	80	60	50	5	50
Cutoff value (%) (positive staining)	>20	>50	>50	>40	>40	>5	>70
Positive tumors (%)	11 (16.7)	26 (39.4)	42 (63.6)	37 (56.1)	37 (56.1)	20 (30.3)	11 (16.7)

Range of staining indicates the proportion of positive nuclear staining within representative areas of the tumor samples. Cut-off value is based on published reports. Number of specimens showing positive staining is provided in the bottom row.

staining within representative areas of the tumor sample. The percentage score above, whose staining is considered representative of overexpression, is based on published reports (11). The range and the median percentage of immunostaining, percentage values regarded as overexpression (cutoff value) and the numbers of specimens displaying positive staining are shown in Table II. Representative photomicrographs of tumor tissue showing positive and negative staining for the specific antigens are presented in Fig. 1.

Statistical analysis. The associations between clinicopathological parameters and the immunostaining scores were analyzed. The correlations between the expression of each gene and the clinicopathological parameters were analyzed using the Chi-square test. $p \leq 0.05$ was considered to be statistically significant. For survival analysis, event time distributions were evaluated using the Kaplan-Meier method, and differences in survival rates were compared using the log-rank test for univariate analysis and Cox proportional hazards regression model for multivariate analysis. PFS was calculated from the date of primary surgery to the date of disease progression. The duration of OS was defined from the date of primary surgery to the date the patient succumbed to the disease or to the date of last follow-up. The treatment-free interval (TFI) was defined as being from the last date of first-line chemotherapy to the date of recurrence or last follow-up without recurrence.

Results

Expression of G1-S phase-regulatory proteins and the association with clinicopathological parameters. The expression of G1-S phase-regulatory proteins was analyzed by immunohistochemistry in advanced serous EOC. Overexpression of cyclin D1, pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and cyclin E was detected with incidences of 16.7, 39.4, 63.6, 56.1, 56.1, 30.3 and 16.7%, respectively. Associations of the expression of each protein and clinicopathological parameters are shown in Table III. The volume of postoperative residual disease and the presence of ascites were not correlated with the expression pattern of any of the studied proteins. The expression of p53 appeared to be positively correlated with that of $p16^{Ink4a}$ (Table III). No other significant association among the gene expressions was observed.

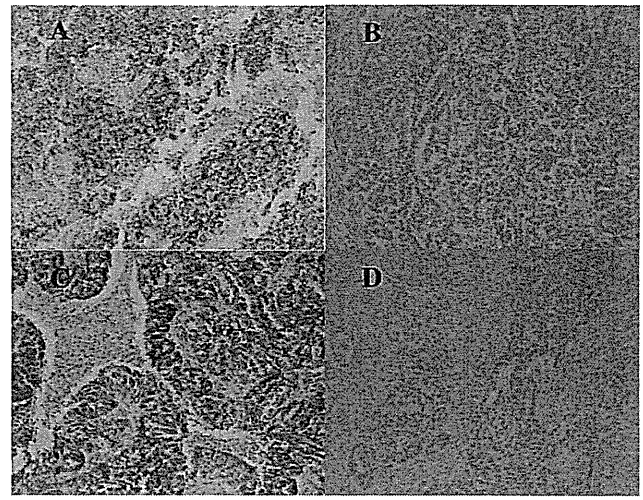

Figure 1. Immunohistochemistry on primary tumor tissue. Representative stainings for cyclin D1 and $p27^{Kip1}$ expression are shown. Magnification, x100. (A) Cyclin D1-positive staining. (B) Cyclin D1-negative staining. (C) $p27^{Kip1}$-positive staining. (D) $p27^{Kip1}$-negative staining.

Correlation between G1-S phase-regulatory protein expression and patient outcome in advanced serous epithelial ovarian cancer. The relationship between gene expression and patient prognosis was assessed. Upon univariate analysis, the clinicopathological determinants of reduced OS included age and volume of residual disease >2 cm (Table IV). A molecular marker predictive of reduced OS upon univariate analysis was overexpression of cyclin D1 (p=0.00037, RR=0.28, 95% CI 0.044-0.40). Reduced expression of $p27^{Kip1}$ had a trend of association with a shorter OS (p=0.064, RR=1.88, 95% CI 0.97-4.21). Regarding PFS, overexpression of cyclin D1 (p=0.00063, RR=0.34, 95% CI 0.054-0.43) was significantly correlated with reduced PFS, but reduced expression of $p27^{Kip1}$ had no statistically significant correlation with PFS. The CA125 level, volume of intraoperative ascites, pRb, p16, p53, $p21^{Waf1/Cip1}$ and cyclin E expression exhibited no statistically significant correlation with either OS or PFS. Kaplan-Meier curves and log-rank p-values according to cyclin D1 expression, $p27^{Kip1}$ expression and residual tumor volume are shown in Fig. 2.

In the multivariate analysis using the Cox proportional hazards model, overexpression of cyclin D1 was identified

Table III. Association of gene expression and clinicopathological parameters in serous epithelial ovarian cancer.

	Cyclin D1	pRb	$p16^{Ink4a}$	p53	$p27^{Kip1}$	$p21^{Waf/Cip1}$	Cyclin E
Residual disease	0.15	0.99	0.14	0.7300	0.097	0.17	0.91
Ascites	0.82	0.10	0.12	0.3000	0.310	0.81	0.82
Cyclin D1		0.43	0.30	0.6600	0.820	0.19	0.77
pRb			0.45	0.0820	0.420	0.95	0.14
$p16^{Ink4a}$				**0.0049**	0.190	0.34	0.73
p53					0.380	0.51	0.37
$p27^{Kip1}$						0.67	0.82
$p21^{Waf/Cip1}$							0.90

Significant p-values are indicated in boldface type.

Table IV. Univariate analysis for the association of clinicopathological parameters and gene expression with clinical outcome in serous epithelial ovarian cancer.

Parameters	PFS			OS		
	RR	95% CI	p-value	RR	95% CI	p-value
Age (≤65 vs. >65 years)	0.71	0.300-1.46	0.32000	0.42	0.100-0.87	**0.02900**
Residual disease (≤2 vs. >2 cm)	0.62	0.360-1.04	0.07800	0.27	0.150-0.60	**0.00087**
CA125 (≤500 vs. >500)	0.83	0.440-1.54	0.56000	1.10	0.470-2.65	0.81000
Cyclin D1 (≤20 vs. >20%)	0.34	0.054-0.43	**0.00063**	0.28	0.044-0.40	**0.00037**
pRb (≤50 vs. >50%)	1.00	0.580-1.73	0.99000	0.90	0.440-1.81	0.76000
$p16^{Ink4a}$ (≤50 vs. >50%)	1.13	0.650-2.00	0.65000	0.97	0.480-1.96	0.93000
p53 (≤40 vs. >40%)	1.43	0.850-2.59	0.17000	1.38	0.690-2.83	0.35000
$p27^{Kip1}$ (≤40 vs. >40%)	1.08	0.630-1.87	0.78000	1.88	0.970-4.21	0.06400
$p21^{Waf1/Cip1}$ (≤5 vs. >5%)	0.82	0.440-1.45	0.48000	1.48	0.710-3.00	0.31000
Cyclin E (≤70 vs. >70%)	0.97	0.460-2.04	0.94000	0.86	0.330-2.19	0.75000
Ascites (≤500 vs. >500 ml)	0.81	0.470-1.37	0.43000	0.63	0.320-1.28	0.21000

Data census was at 75 months. Significant p-values are indicated in boldface type. PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval.

as the key determinant of OS (p=0.0019, RR=3.61, 95% CI 1.61-8.12) and PFS (p=0.0052, RR=2.70, 95% CI 1.35-5.41) (Table V). The volume of residual disease and reduced expression of $p27^{Kip1}$ were found to be independent predictors of OS (p=0.0092 and p=0.042, respectively), but not of PFS when incorporated into a multivariate model (Table V).

Association between chemosensitivity and G1-S phase-regulatory protein expression. In order to assess whether the clinicopathological parameters reflect the chemosensitivity, the cohort was divided into two groups: patients who relapsed within 6 months after the last date of first-line chemotherapy; and patients who had no disease progression within 6 months after the last date of first-line chemotherapy. Using the Chi-square test, overexpression of cyclin D1 (p=0.011) as well as residual tumor volume >2 cm (p=0.006) were found to be significantly associated with TFI, suggesting that these parameters are correlated with first-line chemosensitivity (Table VI). In contrast, expression of pRb, p16, p53, $p27^{Kip1}$, $p21^{Waf1/Cip1}$ and cyclin E had no statistical correlation with chemosensitivity.

Discussion

Various studies exist concerning the association between G1-S phase-related genes and EOC prognosis, however, the results are conflicting. Amplification of cyclin E in high-resolution oligonucleotide microarrays was previously found to be associated with poor response to primary treatment in serous ovarian cancer (10), but in the present study, cyclin E expression in immunohistochemical analysis revealed no significant correlation with patient outcome of advanced serous EOC. It is considered that the variety of histological types of EOC, different tumor stages, tumor heterogeneity, racial backgrounds of patients, research methodologies and sample sizes may contribute to inconsistent results. In this study, we focused on advanced serous cases (limited to stage

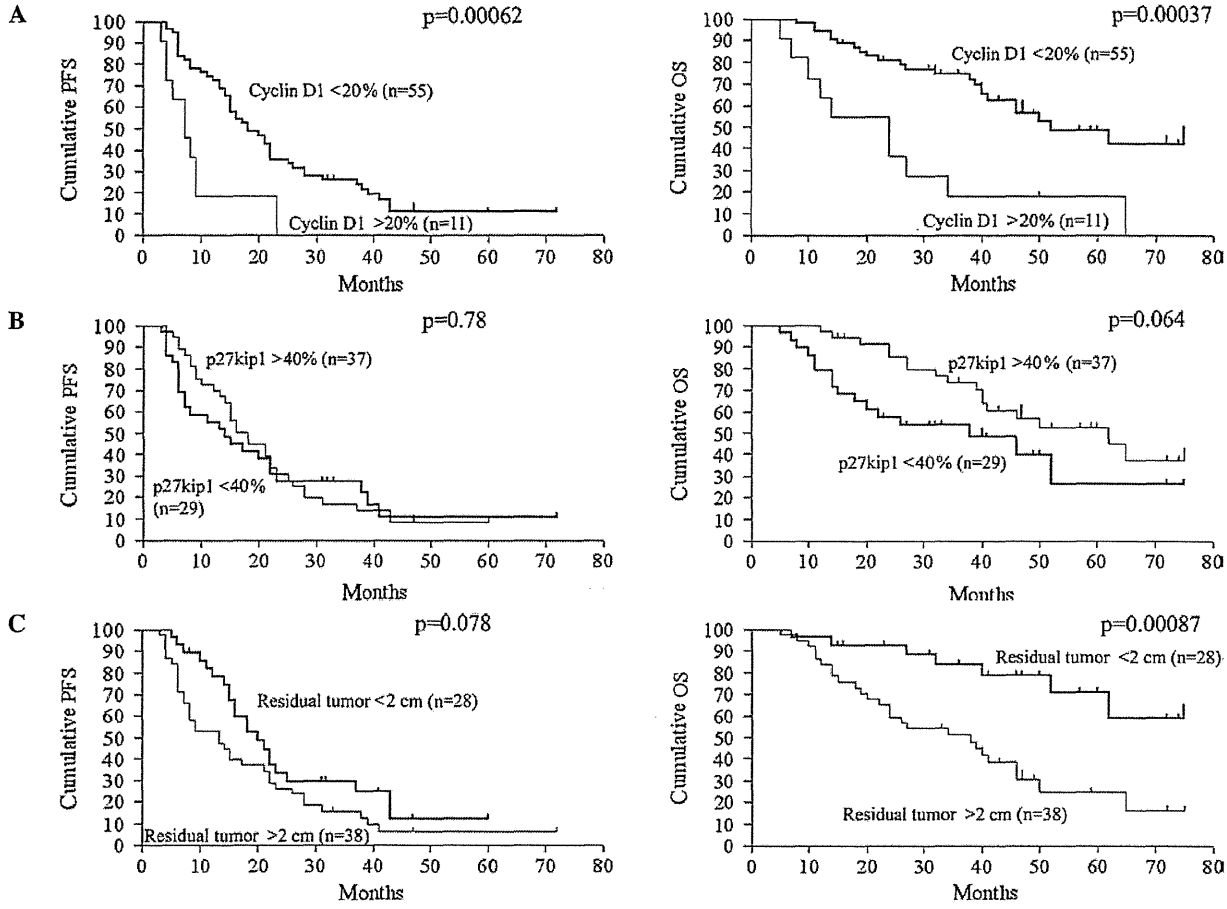

Figure 2. Kaplan-Meier curve. Kaplan-Meier curves and log-rank p-values for 5-year PFS and OS in the context of cyclin D1 expression (A), $p27^{Kipl}$ expression (B) and residual tumor volume (C) are shown. PFS, progression-free survival; OS, overall survival.

Table V. Multivariate Cox regression analysis of PFS and OS of patients with serous EOC.

Clinical parameter	PFS			OS		
	RR	95% CI	p-value	RR	95% CI	p-value
Residual disease (≤2 vs. >2 cm)	1.53	0.89-2.64	0.1200	3.06	1.32-7.12	**0.0093**
Cyclin D1 (≤20 vs. >20%)	2.70	1.35-5.41	**0.0052**	3.61	1.61-8.12	**0.0019**
$p27^{Kipl}$ (≤40 vs. >40%)	1.01	0.59-1.72	0.9700	2.15	1.03-4.51	**0.0420**

Significant p-values are indicated in boldface type. EOC, epithelial ovarian cancer; PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval.

III/IV cases) at a single institution with similar surgical and chemotherapeutic procedures administered in order to eliminate such bias.

Cyclin D1, a regulatory kinase subunit that is selectively associated with cyclin-dependent kinase 4 (CDK4), is a crucial modulator of G1 progression in the cell cycle (16). In our analysis, overexpression of cyclin D1 was detected in 11% of the cases (Table II). The overexpression of cyclin D1 was previously observed in 14-89% of EOC cases (11,17-19), but the underlying mechanism has yet to be elucidated. Amplification of cyclin D1 in ovarian tumors occurs infrequently (20). Furthermore, the mostly small cyclin D1 copy gains are not associated with an increase in detectable cyclin D1 protein by immunohistochemistry (21). These findings suggest that the post-transcriptional regulation of cyclin D1 protein production is complex. Recently, Jiang *et al* performed a systematic validation of the predicted microRNAs for cyclin D1 and revealed that microRNAs suppressed the endogenous cyclin D1 protein and mRNA levels *in vitro* (22). microRNAs may aid in determining the mechanism of cyclin D1 expression.

Barbieri *et al* reported that cyclin D1 overexpression significantly influenced the clinical outcome in advanced EOC

Table VI. Association of the TFI and clinicopathological parameters in serous EOC.

	TFI <6 months, n (%)	TFI ≥6 months, n (%)	p-value
Residual disease			
≤2 cm (n=28)	4 (6.0)	24 (36.4)	**0.006**
>2 cm (n=38)	19 (28.8)	19 (28.8)	
Cyclin D1			
≤20% (n=55)	15 (22.7)	40 (60.6)	**0.011**
>20% (n=11)	8 (12.1)	3 (4.6)	
pRB			
≤50% (n=40)	12 (18.2)	28 (42.4)	0.305
>50% (n=26)	11 (16.7)	15 (22.7)	
$p16^{Ink4a}$			
≤50% (n=24)	7 (10.6)	17 (25.8)	0.464
>50% (n=42)	16 (24.3)	26 (39.4)	
p53			
≤40% (n=29)	11 (16.7)	18 (27.3)	0.642
>40% (n=37)	12 (18.2)	25 (37.9)	
$p27^{Kipl}$			
≤40% (n=29)	13 (19.7)	16 (24.3)	0.132
>40% (n=37)	10 (15.2)	27 (40.9)	
$p21^{Waf/Cipl}$			
≤5% (n=46)	19 (28.8)	27 (40.9)	0.165
>5% (n=20)	4 (6.0)	16 (24.3)	
Cyclin E			
≤70% (n=55)	18 (27.3)	37 (56.1)	0.644
>70% (n=11)	5 (7.6)	6 (9.1)	

p-values are for TFI <6 months vs. TFI ≥6 months (Chi-square test, Yates correlation). Significant p-values are indicated in boldface type. EOC, epithelial ovarian cancer; TFI, treatment-free interval.

cases with residual disease greater than 2 cm. They identified cyclin D1 overexpression as an independent prognostic factor in multivariate analysis (5). Similarly, Bali *et al* identified cyclin D1 overexpression as an independent prognostic factor in the multivariate analysis of 134 serous EOC cases (11). In our study, overexpression of cyclin D1 was significantly correlated with reduced OS and PFS in both univariate and multivariate analyses, suggesting that overexpression of cyclin D1 actually contributes to the prognosis of advanced serous EOCs; therefore, its application to clinical practice is expected.

We found that both overexpression of cyclin D1 and residual tumor volume were significantly associated with TFI, suggesting that these parameters are correlated with first-line chemosensitivity (Table VI). Zhou *et al* showed that inhibition of cyclin D1 expression by siRNA in oral squamous cell carcinoma cells resulted in a decrease in cisplatin IC_{50} level. *In vivo* transplantation models also confirmed a cisplatin-sensitizing effect of cyclin D1 knockdown in these cell lines (23). In addition, it was reported that overexpression of cyclin D1 was associated with reduced chemosensitivity and a higher survival rate upon cisplatin administration in a pancreatic cancer model (24). Moreover, inhibition of cyclin D1 expression rendered cells more susceptible to cisplatin-mediated apoptosis in the same model (24). Taken together, these findings indicate that cyclin D1 expression may contribute to chemoresistance in a number of cancers, although further investigation is required. Therefore, we speculate that overexpression of cyclin D1 contributes to poor prognosis, which may in part be mediated by chemoresistance in ovarian cancer.

$p27^{Kipl}$ is a cyclin-dependent kinase (cdk) inhibitor that regulates cell cycle progression from the G1 to S-phase. In non-cycling cells, $p27^{Kipl}$ binds to cyclin E-cdk2 complexes and inhibits their activation. In contrast, $p27^{Kipl}$ binding to catalytically active cyclin D-cdk4/6 complexes results in $p27^{Kipl}$ degradation and the subsequent release of cdk2 from inhibition in proliferating cells (12,25). Therefore, $p27^{Kipl}$ helps to coordinate a balance between proliferation and arrest (12,25). In the present study, reduced expression of $p27^{Kipl}$ was detected in 43.9% of the cases. In previous immunohistochemical studies of ovarian tumors, 36.2-100% exibited low expression of $p27^{Kipl}$ (26). We found that reduced expression of $p27^{Kipl}$ was associated with shorter OS (p=0.064), but had no statistically significant correlation with PFS (p=0.78). When incorporated into a multivariate model, reduced expression of $p27^{Kipl}$ was found to be an independent predictor of OS (p=0.042) (Table V). The relationship between $p27^{Kipl}$ expression levels and prognosis is controversial. Conflicting data regarding the possible prognostic role of $p27^{Kipl}$ status also exist for ovarian cancer. Psyrri *et al* evaluated subcellular

localization and protein levels of $p27^{Kip1}$ in 150 advanced EOCs and found that low nuclear $p27^{Kip1}$ expression was associated with improved prognosis, suggesting its potential as a strong predictor of outcome in advanced EOCs (12). On the other hand, Shigemasa *et al* reported that negative $p27^{Kip1}$ expression was significantly correlated with poor survival in serous EOC patients, suggesting that the underexpression of $p27^{Kip1}$ caused by a post-translational mechanism may contribute to development and progression and may result in poor prognosis of serous EOCs (27). It is hypothesized that different methodologies of immunohistochemical grading may account for these discrepancies. Our result suggests that $p27^{Kip1}$ is associated with the prognosis of this disease, as previously reported.

In conclusion, overexpression of cyclin D1 contributed markedly to poor prognosis in advanced serous EOC; this may in part be mediated by chemoresistance. Cyclin D1 may be a target for overcoming the refractory nature of advanced serous EOC.

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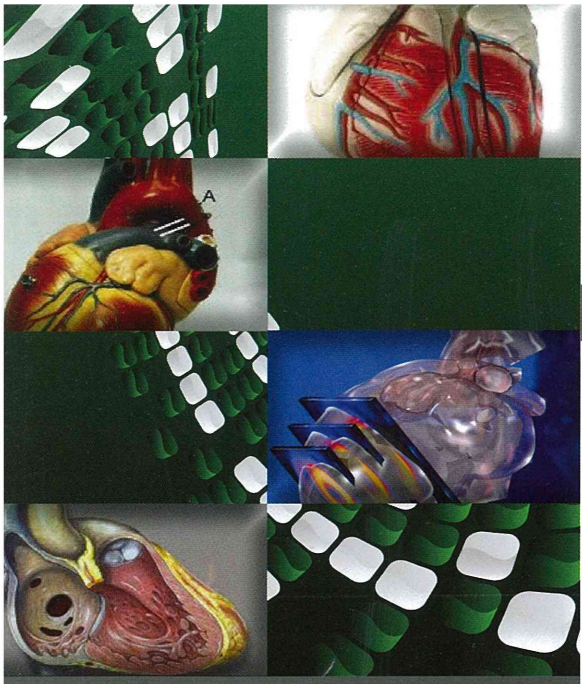

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Comparison of Two Sampling Procedures for Diagnosing Endometrial Carcinoma and Hyperplasia: Outpatient Tissue Biopsy Versus Cytologic Examination

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Abstract

Background: We compared the sensitivity of 2 diagnostic procedures—tissue biopsy and cytologic examination—for detecting endometrial carcinoma and hyperplasia in outpatients. The patients' degree of acceptance of these methods was also evaluated.

Methods: The study included 124 women who had been diagnosed with carcinoma and hyperplasia by histological examination in private clinics or were suspected to have endometrial carcinoma and hyperplasia—for example, those presenting with uterine bleeding and/or abnormal endometrial morphology on cytologic examination—at Jikei University Hospital, University of Yamanashi Hospital and National Hospital Organization Kure Medical Center from January 28, 1999, to August 28, 2006. Both cytologic examination (using Endocyte®) and tissue biopsy (using Suresample™) of the endometrium were performed before complete curettage and/or hysterectomy. The diagnosis made using these two outpatient procedures was compared to the final diagnosis made using curettage and/or hysterectomy. McNemar's chi-square test was used to evaluate the statistical significance.

Results: The sensitivity of tissue biopsy for detecting endometrial carcinoma and hyperplasia was 84% and 91%, respectively, and that of cytologic examination was 78% and 55%, respectively. There was a significant difference in the sensitivity of the 2 methods for detecting hyperplasia ($p = 0.045$). No patients complained of severe pain, and no other complication occurred during both methods. Both methods were well tolerated by the patients.

Conclusion: Our data indicate a certain diagnostic superiority of tissue biopsy over cytologic examination.

Keywords: Endocyte®; Suresample™; Endometrial carcinoma; Hyperplasia; Diagnostic procedure

Introduction

Each year, there are about 142,000 new cases of endometrial carcinoma worldwide, and an estimated 42,000 women die because of this type of cancer [1]. The surgical stage, determined according to the criteria of the International Federation of Gynecology and Obstetrics, reflects the 5-year survival, which is around 85% for stage I, 75% for stage II, 45% for stage III, and 25% for stage IV disease [1]. Endometrial cancer is often preceded by endometrial hyperplasia, which is a spectrum of morphologic and biologic alterations of the endometrial glands and stroma and is often secondary to hyperestrogenism. It has been shown that progression to carcinoma occurs in 1% of patients with simple hyperplasia, 3% of patients with complex hyperplasia, 8% of patients with simple hyperplasia with atypia, and 29% of patients with complex hyperplasia with atypia [2].

The Japanese Ministry of Health and Welfare investigated the effectiveness of mass endometrial carcinoma screening. During the 9-year study, 126 cases were detected by mass screening and 1,069 cases were diagnosed in outpatient clinics. Early-stage cases were significantly more frequent in the screening group ($p < 0.001$): 88.1% of the patients in the screening group had stage I disease, as compared to 65.3% of the patients in the outpatient group. The 5-year survival rate was also significantly higher in the screening group than in the outpatient group (94.7% vs 84.3%; $p = 0.041$) [3]. These statistics suggest that early detection of endometrial carcinoma and hyperplasia is necessary to improve the prognosis of these diseases.

Outpatient endometrial sampling is now replacing complete curettage as the method of choice for diagnosing endometrial disease. This procedure is easy to perform, associated with minimal patient discomfort, and reported to be highly sensitive in detecting endometrial carcinoma [4-14]. The Pipelle de Cornier® device (Laboratoire CCD, Paris, France) is an endometrial biopsy sampler that is seemingly better tolerated by patients than most other endometrial biopsy devices [15,16]. However, we cannot use this device because it is not available in Japan. Instead, we collect endometrial tissue by using the Suresample™ (Smith Medical International Ltd., Kent, UK) endometrial sampler, which is similar to the Pipelle® device. This endometrial sampler has an aperture not only on the side near the distal tip, similar to the Pipelle® device, but also at the distal tip and is expected to collect a larger sample. However, in Japan, cytologic examination is often used initially to detect endometrial carcinoma and its precursor stages, as stipulated by a 1987 health insurance law for the elderly. During this cytologic

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examination, endometrial cells are collected using the Endocyte® sampler (Laboratoire CCD, Paris, France). The cell processing technique is similar to that used for a cervical cytology smear and is thus relatively inexpensive.

A few studies have compared the 2 above-mentioned sampling procedures. However, in these studies, the diagnostic sensitivity was not sufficiently evaluated because of the small number of carcinoma and hyperplasia cases used for the investigation [13,17]. In the present study, in order to determine the optimal technique for detection of endometrial carcinoma and hyperplasia, we compared the diagnostic sensitivity of cytologic examination using Endocyte® and tissue biopsy using Suresample™; we also estimated the degree of patient acceptance for both these procedures.

Materials and Methods

This study included 124 patients who had been diagnosed with carcinoma and hyperplasia by histological examination in private clinics or were suspected of having carcinoma and hyperplasia—for example, those presenting with uterine bleeding and/or abnormal endometrial morphology on cytologic examination—at Jikei University Hospital, University of Yamanashi Hospital and National Hospital Organization Kure Medical Center from January 28, 1999, to August 28, 2006. Patients with complications such as pregnancy, acute pelvic infection, infection of the uterine cervix, and coagulation disorder were excluded. Both cytologic examination and tissue biopsy were performed for all the patients; the former was performed before the latter. This study was approved by the hospital ethics committees, and informed consent was obtained from all the patients.

Cytologic materials were obtained using Endocyte®. The Endocyte® sampler is composed of flexible plastic, is presterilized, and measures 21 cm in length; its greatest external diameter is 2.6 mm. Along its length are graduation marks that guide the operator in introducing the device into the endometrial cavity, as described by Byrne [8]. The collected cellular components were placed on a glass slide, crushed, and smeared using the regular pull-apart method. After fixation in 95% alcohol, Papanicolaou staining was performed. The cytologic findings were divided into different classes on the basis of structural abnormalities, such as papillary clusters, type A stroma, arborescent clusters, and back-to-back structures (Table 1) [18,19]. Outpatient tissue biopsy was performed using Suresample™. Suresample™ is a flexible, clear polypropylene suction curette containing an internal piston and measures 24 cm in length and 3.1 mm in external diameter. It has a round aperture with a diameter of 1.5 mm at the distal tip of its sheath and 2 oval apertures each measuring 5.9 × 1.5 mm at 3.2mm from the distal tip. In order to obtain a specimen, the device is inserted into the uterine cavity and negative pressure is then created within the sheath by withdrawing the piston. The device is rotated while also being moved back and forth several times within the uterine cavity. Suresample™ is then withdrawn, and the tissue sample is ejected into 10% buffered formalin by using the piston. The entire sample is histologically examined.

After collecting the sample for both procedures, the patient was asked to comment on the intensity of any pain experienced during the procedure. Pain or discomfort was subjectively graded as mild, moderate, or severe. Thereafter, in 93 patients, complete curettage and/or hysterectomy was performed. In the remaining 31 patients, these procedures were not performed because of the attending physician's decision or the patient's refusal. The final diagnosis was made on the basis of the histological findings of the samples obtained during complete curettage and/or hysterectomy. The diagnosis made using both outpatient procedures was then compared with the final diagnosis.

We estimated sensitivity for detecting endometrial carcinoma, sensitivity for detecting endometrial hyperplasia, and specificity of each procedure separately and reported them with 95% confidence intervals. Patients who were not diagnosed histologically were excluded from this analysis. McNemar's chi-square test was used to compare each measure of diagnostic accuracy. All reported p values for statistical tests are two-tailed, and $p < 0.05$ was taken to indicate statistical significance. Data management and statistical analysis were conducted at an independent academic data center, Translational Research Center, Kyoto University Hospital, using SAS version 9.2 (SAS Institute, Cary, NC).

Results

The median age of the patients was 54 years (range: 23-85 years). Of the 124 patients, 68 (55%) were postmenopausal, and 88 (71%) showed abnormal uterine bleeding.

Of the 93 patients who underwent complete curettage and/or hysterectomy, 69 were finally diagnosed with endometrial carcinoma, 11 with endometrial hyperplasia, 6 with other tumor, and 7 with normal endometrium. Of the 69 patients with endometrial carcinoma, 50 had endometrioid adenocarcinoma; 12, adenoacanthoma; 2, serous papillary adenocarcinoma; 2, clear cell adenocarcinoma; 1, mucinous adenocarcinoma; and 2, mixed carcinoma. Of the 50 endometrioid adenocarcinoma tumors, 33 were well differentiated, 12 were moderately differentiated, and 5 were poorly differentiated. Of the 11 patients with endometrial hyperplasia, 5 had complex hyperplasia with atypia, 3 had complex hyperplasia without atypia, and 3 had simple hyperplasia without atypia.

Of the 69 patients with endometrial carcinoma, cytological examination using Endocyte® revealed carcinoma (class V) in 54 patients, hyperplasia (class III or IV) in 7, and a normal endometrium (class II) in 5; in 3 patients, adequate samples could not be obtained.

Class	Findings
I	No abnormal findings.
II	Inflammatory findings or reactive changes because of an intrauterine device(IUD).
IIb	Papillary clusters with few structural abnormalities. Complex hyperplasia not fully suspected but follow-up necessary.
III	Papillary clusters accompanied by structural abnormalities. Complex hyperplasia suspected.
IV	Small number of arborescent clusters. Complex hyperplasia with atypia or worse suspected.
V	Clear glandular cavity with back-to-back structures and arborescent clusters. Endometrial cancer diagnosed.

Table 1: Different classes of cytologic findings of the endometrium.

	Final histological diagnosis					
Cytology (class)	Normal endometrium	EH (Atypical)	EMCA	Other tumor	Not performed	Total
I	3	0	0	0	6	9
II	4	5 (1)	5	1	13	28
III	0	5 (3)	6	1	4	16
IV	0	1 (1)	1	0	3	5
V	0	0	54	4	2	60
Inadequate	0	0	3	0	3	6
Total	7	11 (5)	69	6	31	124

EH: endometrial hyperplasia; EMCA: endometrial carcinoma

Table 2: Comparison between cytologic examination and the final histological study.

	Final histological diagnosis					
Biopsy	Normal endometrium	EH (Atypical)	EMCA	Other tumor	Not performed	Total
Normal endometrium	6	0	0	3	17	26
EH	1	10 (5)	6	0	4	21
EMCA	0	0	58	1	1	60
Other tumor	0	0	0	1	2	3
Inadequate	0	1	5	1	7	14
Total	7	11 (5)	69	6	31	124

EH: endometrial hyperplasia; EMCA: endometrial carcinoma

Table 3: Comparison between outpatient biopsy and the final histological study.

	Cytologic examination (95% Confidence interval) (%)		Tissue biopsy (95% Confidence interval) (%)		p*1
Sensitivity for detecting endometrial carcinoma	78.3	(66.7–87.3)	84.1	(73.3–91.8)	0.157
Sensitivity for detecting endometrial hyperplasia	54.5	(23.4–83.3)	90.9	(58.7–99.8)	0.045
Specificity	100.0	(59.0–100.0)	85.7	(42.1–99.6)	*2

*1McNemar's chi-square test
*2Not calculable due to specificity of 100%

Table 4: Comparison of diagnostic accuracy between cytologic examination and tissue biopsy.

Tissue biopsy using Suresample™ identified 58 cases of endometrial carcinoma, while 6 were misdiagnosed as endometrial hyperplasia. In 5 patients, adequate samples could not be obtained. Of the 11 patients with endometrial hyperplasia, cytologic examination using Endocyte® revealed hyperplasia (class III or IV) in 6 patients and a normal endometrium (class II) in 5. Tissue biopsy using Suresample™ helped to identify 10 cases of hyperplasia; in 1 patient, an adequate sample could not be obtained (Table 2 and Table 3). The sensitivity of Endocyte® and Suresample™ for detecting endometrial carcinoma was 78% and 84%, whereas the sensitivity for detecting endometrial hyperplasia was 55% and 91%, respectively. The specificity of Endocyte® and Suresample™ for detecting endometrial disease was 100% and 86%, respectively. These data suggest that as compared to cytologic examination using Endocyte®, outpatient endometrial tissue biopsy using Suresample™ has a significantly higher sensitivity for detecting endometrial hyperplasia ($p = 0.045$; Table 4).

Pain was reported to be nil by 42 (34%) and 49 (40%) patients, mild by 69 (56%) and 67 (54%) patients, and moderate by 13 (10%) and 8 (6%) patients during the insertion of Endocyte® and Suresample™, respectively. None of the patients complained of severe pain. Pain was reported to be nil by 30 (24%) and 42 (34%) patients, mild by 77 (62%) and 70 (56%) patients, and moderate by 17 (14%) and 12 (10%) patients during the collection of samples using Endocyte® and Suresample™, respectively. No patient complained of severe pain. In all patients, bloody discharge from the cervix after cell collection was either absent or minimal.

Discussion

To the best of our knowledge, this is the first study to investigate the diagnostic accuracy of outpatient endometrial tissue biopsy using Suresample™. However, there are several studies on the use of the Pipelle® device, which is similar to Suresample™ [4-7,13,15,16]. A meta-analysis revealed that the Pipelle® device has a sensitivity of 99.6% and 91% in postmenopausal and premenopausal patients, respectively [5]. Another review showed that the sensitivity of the Pipelle® device varies between 86% and 100% [4]. We found the sensitivity of Suresample™ to be 84%, which is lower than that of the Pipelle® device, as mentioned above. In the present study, the inadequate sample (no specimen obtained or insufficient specimen for adequate assessment for histological or cytological diagnosis) at the outpatient examination was regarded as 'negative' for the calculation of the sensitivity, in contrast to some previous studies where inadequate diagnoses were excluded from the calculations [4,8,10-12]. Had we calculated sensitivity by excluding inadequate samples, the sensitivity of Suresample™ for detecting endometrial carcinoma would be 91%, which is similar to the values reported in previous studies. Moreover, no patient with carcinoma was falsely diagnosed as having a normal endometrium by outpatient tissue biopsy. Other reports have showed that the sensitivity of cytologic examination for diagnosing endometrial carcinoma is 74.1–100% [8-14]. One study evaluated and compared the accuracy of sampling using Endopap® and Pipelle® for diagnosing postmenopausal disease. The sensitivity of Endopap® and Pipelle® for detecting endometrial disease was 56% and 51% and the specificity was 94% and 100%, respectively. The sensitivity for endometrial carcinoma was 80% for Endopap® and 100% for Pipelle®. The authors therefore favored Pipelle® for diagnosing endometrial disease in symptomatic postmenopausal women [13]. In the present study too, the sensitivity of cytologic examination for detecting carcinoma tended to be lower than that of outpatient tissue biopsy. Furthermore, 5 patients with carcinoma were falsely diagnosed as having a normal endometrium by cytologic examination. Such false negatives pose a grave risk for patients when screened for endometrial carcinoma.

Outpatient endometrial sampling also aims to detect endometrial hyperplasia, because of the supposed role of the latter as a precursor of endometrial carcinoma. However, detecting endometrial hyperplasia in the smears of endometrial samples is very difficult. Therefore, the diagnostic rate is not always high because of the lack of cellular atypia. In fact, it has been reported that hyperplasia can be detected in only 32.3–80.5% of cases [8-12,14]. A meta-analysis shows that the sensitivity of the Pipelle® device in detecting atypical hyperplasia is 81% [5]. In the present study, the sensitivity of cytologic examination for detecting hyperplasia was 55%, whereas that of outpatient tissue biopsy was 91%. Thus, the difference between the sensitivity of these 2 methods is significant ($p = 0.045$).

At the time of sampling, adverse effects such as severe pain and bloody discharge decrease the patient's acceptance of the collection method. The adverse effects of the Pipelle® device, used without anesthesia, have been evaluated by surveying 40 patients. Although 2 patients (5%) complained of severe pain, none of the biopsy attempts were prematurely terminated as a result of pain and no complications related to endometrial sampling occurred [6]. The incidence and intensity of pain during and after a cytologic procedure using Endocyte® have also been examined. The present pain intensity index developed by Melzack assesses the overall discomfort or pain experienced on a scale of 0-5. Pain was reported as 0 (no pain) by 60% patients, as 1 (mild) by 30%, and as 2 (discomfort) by 10% [17,20]. In the present study, the intensity of pain tend to be stronger during cytologic examination using Endocyte® than tissue biopsy using Suresample™, despite the larger diameter of the Suresample™ probe. As the cytologic examination performed before the tissue biopsy made the insertion of the Suresample™ probe easier, we cannot provide definitive conclusions on the superiority of Suresample™ with regard to patient acceptance. However, during both procedures, none of the patients complained of severe pain and no complications occurred. This suggests that both outpatient sampling procedures were well tolerated, which is a finding consistent with those of previous reports [6,17,20].

Our data indicated a certain diagnostic superiority of outpatient tissue biopsy to cytologic examination. Because of the small number of patients with a normal endometrium, we could not sufficiently evaluate the specificity for detecting endometrial disease. Furthermore, this study did not compare the two methods in terms of cost effectiveness. Therefore, cytologic examination for detecting endometrial carcinoma and hyperplasia cannot be completely disregarded. Yet, our data suggest that the use of tissue biopsy in an endometrial carcinoma screening program might improve the detection rate of endometrial carcinoma and hyperplasia. For example, in the cases of patients with normal endometrial morphology on cytologic examination, who show strongly suspicious symptoms such as abnormal endometrium thickness on the ultrasonography and/or continuous genital bleeding, reexamination using tissue biopsy should be considered. Diagnostic superiority of outpatient tissue biopsy to cytologic examination is most likely because cytologic examination cannot provide the architectural detail. Recently, liquid-based cytology and cell block preparation were reported as the methods that had more excellent architectural preservation than conventional cytologic examination. Several reports suggest that those methods are useful for diagnosing endometrial disease [21-23]. Further studies focusing on the effectiveness of various methods including liquid-based cytology and cell block preparation are necessary.

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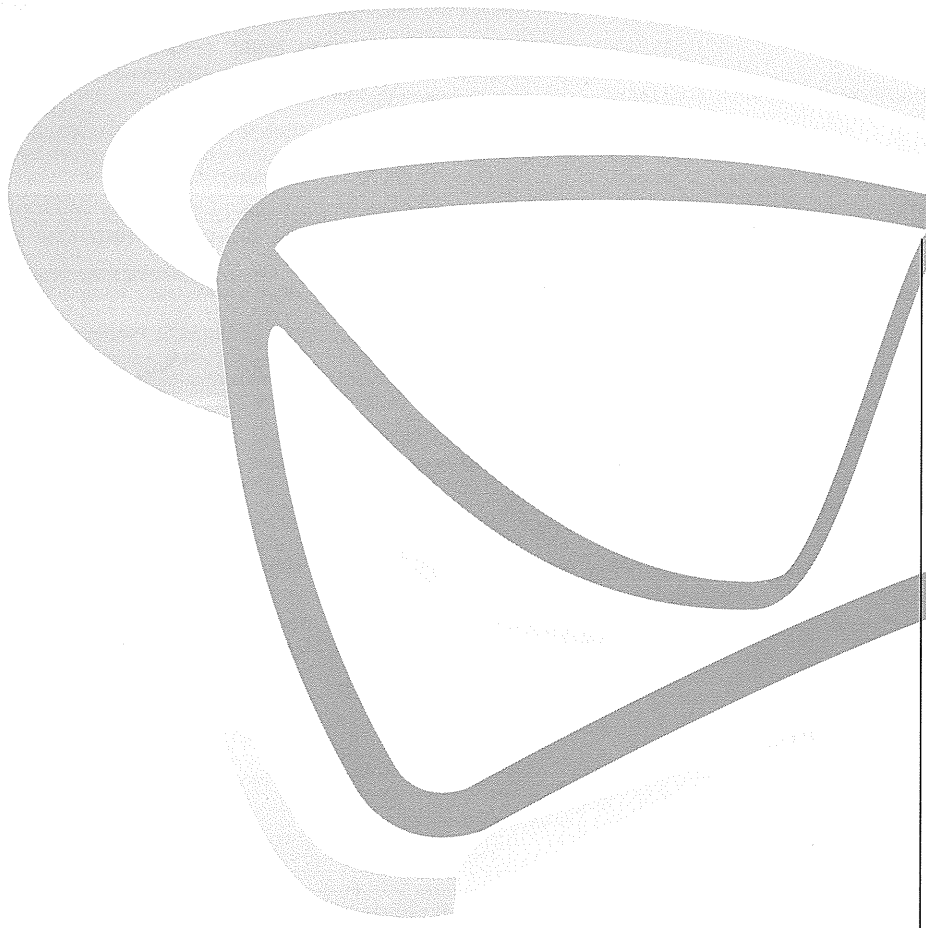

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Expression of the Wild Type Rearranged during Transfection Protooncogene in Ovarian Cancer

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ABSTRACT

Background : The rearranged during transfection (RET) protooncogene is expressed in a variety of cancers. The pathogenesis of ovarian cancer is poorly understood. The aim of this study was to determine whether the RET protooncogene is expressed in ovarian cancer.

Materials and Methods : The reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical methods were used to confirm the expression of the RET protooncogene in two ovarian cancer cell lines and ovarian tumor samples.

Results : The PCR products of the RET protooncogene were 300 bp in both ovarian cancer cell lines. On immunohistochemical analysis using an anti-RET polyclonal antibody, positive signals were observed in 59 of 82 cases of ovarian cancer (72.0%). The rates of RET expression in ovarian cystadenomas and ovarian cystadenomas with borderline malignancy were 20.7% and 53.3%, respectively.

Conclusion : The wild type RET protooncogene is expressed in ovarian cancer. This result suggests that the RET protooncogene is involved in the pathogenesis of ovarian cancer.
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Key words : RET protooncogene, ovarian cancer, immunohistochemistry

INTRODUCTION

Ovarian cancer is a leading cause of death among gynecologic malignancies. Although survival rates have increased somewhat owing to adjuvant chemotherapy with paclitaxel and carboplatin, the overall survival rate in patients with ovarian cancer remains poor[1], because ovarian cancer is diagnosed at an advanced stage in most patients and because effective therapies are not available to prevent recurrence in patients who have shown a complete response to chemotherapy. Recently, new therapeutic approaches, such as targeted therapy, have been explored to improve the prognosis of patients with ovarian cancer. To develop a targeted therapy for ovarian cancer, a tumor-specific antigen must be identified.

The rearranged during transfection (RET) protooncogene encodes a receptor tyrosine kinase. The receptor tyrosine kinase controls cell growth and differentiation. It is also known to be activated as oncogenes in human tumors. In addition, RET is a characteristic protooncogene found in several hereditary and nonhereditary diseases, such as multiple endocrine neoplasia (MEN) 2A, 2B,

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Hirschsprung's disease, and papillary thyroid carcinoma[2-4]. In our previous study RET was expressed in all neuroblastomas developed from 11 tumor cell lines and 29 fresh tumor cells[5]. Glial cell line-derived neurotrophic factor (GDNF) knock-out mice show phenotypes similar to those of RET knock-out mice[6-8], implying that RET is a functional receptor for GDNF. Because the GDNF receptor itself has no cytoplasmic domain serving as a signal transducer, the tyrosine kinase domain of RET has been proposed to function as a transmitter of the biological signals triggered by GDNF[9].

Moreover, we already obtained a monoclonal antibody to the RET extracellular domain (NBL-1)[10]. For tumor-cell targeting, monoclonal antibodies are frequently used. In addition, several novel peptides that can bind specific molecules and cells have been identified[11-13].

Park et al. have reported that a rationally designed anti-HER2/neu peptide can inhibit the growth of breast cancer cells *in vitro* and *in vivo*[13], suggesting that targeting peptides can mimic the activities of monoclonal antibodies. In the treatment of breast cancer trastuzumab (trade name : Herceptin) is a humanized recombinant monoclonal antibody that recognizes the extracellular domain of the HER2 transmembrane protein. Trastuzumab was the first clinically applied immunologic target specific drug. Its development represents a model for integrating new agents[14,15]. Therefore, the identification of tumor-associated cell surface antigens is important for the development of tumor-targeted antibody therapy[15].

The aim of this study was to confirm the expression of the RET protooncogene in patients with ovarian cancer and to establish the effective ovarian tumor targeting diagnosis and therapy using the anti-RET monoclonal antibody.

MATERIALS AND METHODS

Cell lines

Two cell lines derived from human ovarian cancer were used. The 2008 cell line[16] was kindly provided by Dr. Howell, and the A2780 cell line[17] was provided by Dr. Ozds. Both NB-39-nu cells (human neuroblastoma cells)[18] and HL60 cells (human promyelocytic leukemia cells) were used as RET positive controls.

Tissue samples

Ovarian cancer tissue samples were resected from 82 patients (mean age, 48±11 years) who had been admitted to The Jikei University School of Medicine from 1989 through 1999.

The clinicopathologic diagnoses of ovarian tumors were ovarian cystadenoma (87 cases ; mean age, 38±13 years) and ovarian cystadenomas with borderline malignancy (15 cases ; mean age, 40±15 years). All histological diagnoses were reviewed according to established morphological classification criteria of the World Health Organization. Overall survival was defined by the interval from the first surgery to death.

Reverse transcriptase-polymerase chain reaction

Total tissue RNA was isolated from 2 ovarian cancer cell lines by using the RNeasy Mini kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. Two micrograms of each RNA sample was subjected to complementary DNA synthesis with an Omniscript RT kit (Qiagen) according to the manufacturer's protocol. The polymerase chain reaction (PCR) was subsequently performed to analyze the expression of RET by using 5′<AGATCCTGGAGGATCCAAAG<3′ and 5′<GTATTTGGCGTACTCCACGA<3′ as forward and reverse primers, respectively. The PCR was performed using 1 μl of template, 0.5 μl of each 10 μM primer, 0.1 μl of Taq DNA polymerase (TaKaRa, Tokyo, Japan), 0.5 μl of 10 mM deoxyribonucleoside triphosphate, 2.5 μl of 10×buffer, and 19.9 μl of purified and ionized water (MilliQ, Millipore Corp., Billerica, MA, USA), in a total volume of 25 μl. The thermal cycler conditions used were 94°C for 5 minutes followed by 40 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The PCR products were visualized with ethidium bromide staining after separation over a 1% agarose gel.

Characterization of the RET protooncogene in ovarian cancer cell lines

Exons 10, 11, and 13 to 16 of the RET protooncogene, which are the most common mutation sites of the RET protooncogene in other diseases, were analyzed in cells of the 2008 cell line[19]. Briefly, each exon was amplified with PCR and reported primers[20] and then cloned into the pTOPO

vector (Invitrogen, Tokyo, Japan). Several independent clones were subjected to sequence analysis with an automated sequencer (Prism 370, Applied Biosystems, Foster City, CA, USA).

Immunohistochemistry

Immunohistochemical staining was performed as follows. The tissues were fixed immediately with buffered 10% formalin for 24 to 72 hours, dehydrated, and embedded in paraffin. Four-micrometer-thick sections were cut and deparaffinized in ethanol and xylene. Immunohistochemical staining was performed with an immunoperoxidase avidin-biotin conjugate system, with diaminobenzidine and hydrogen peroxide as the substrate and with hematoxylin as the counterstain. Slides were rinsed in a phosphate buffer (pH 7.4) for examination. The sections were incubated with a primary polyclonal anti-RET antibody[21]. All primary antibodies were titrated by dilution (1 : 500 with anti-RET polyclonal antibodies) to obtain optimal intensity of specific staining with minimal nonspecific background reactivity. The secondary antibody was a biotinylated horse anti-mouse immunoglobulin (Zymed, South San Francisco, CA, USA) for use with primary polyclonal antibodies. Negative controls initially consisted of tissue processed without inclusion of the primary antibody. The slides were examined with light microscopy and the intensity of immunostaining was evaluated. The intensity of immunostaining in all slides was classified into 4 levels : level 0 for negative staining, level 1 for lower intensity, level 2 for moderate intensity, and level 3 for the highest intensity. To simplify the results, intensity levels 0 and 1 were defined as negative staining, and levels 2 and 3 were defined as positive staining, except for cases in which the number of stained cells in levels 2 and 3 were less than 50% of all cells in a slide.

Statistical analysis

Fisher's exact test were used to compare the RET-positivity rate among patients with different histologic diagnoses (cystadenoma, cystadenoma with borderline malignancy, and cancer). All statistical analyses were performed with a statistical software program (SAS version 9.1, SAS Institute, Cary, NC, USA). A p value of <0.05 was considered to indicate significance.

Results

Expression and characterization of the RET protooncogene in ovarian cancer cell lines

Synthesized complementary DNAs from 2 cell lines

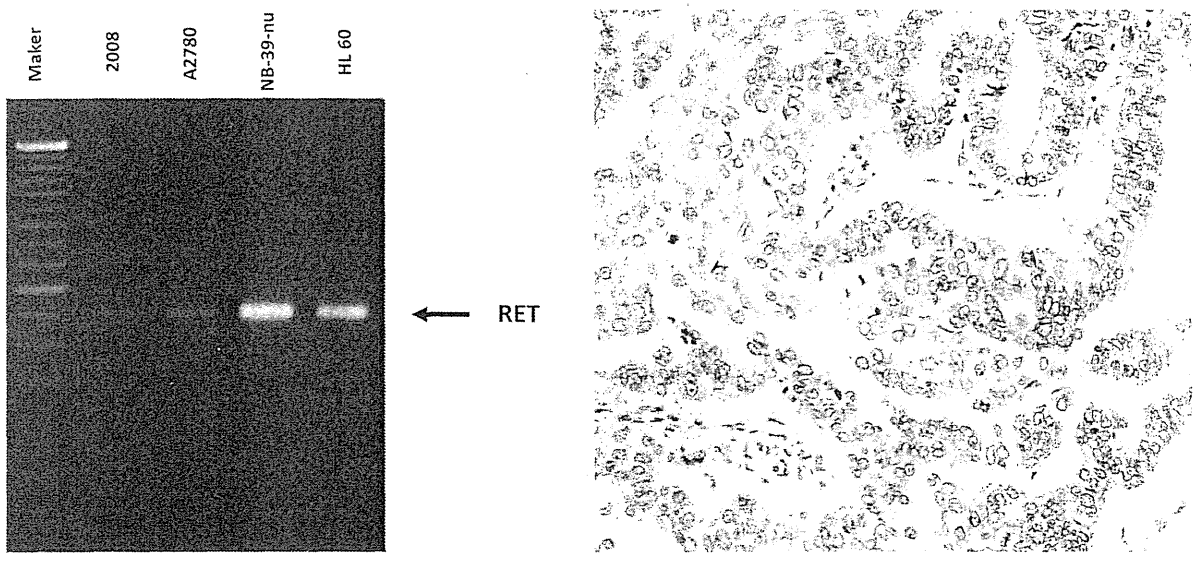

Fig. 1. RET protooncogene expression in ovarian cancer. A, Reverse trascriptase-PCR with primers RET yielded an expected band of 300 bp in 2008 cells and A2780 cells. Both NB-39-nu cells and HL 60 cells served as positive controls. B, Immunohistochemical analysis of ovarian cancer showing cytoplasmic staining for RET.

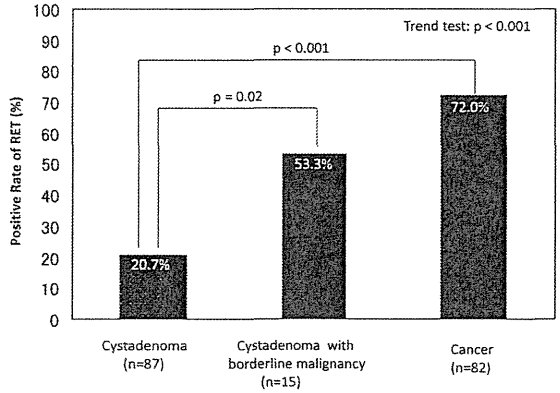

Fig. 2. RET expression in relation to histological type. The rates of RET staining in 3 histologic types : ovarian cystadenoma, cystadenoma with borderline malignancy, and ovarian cancer.

Table 1. Correlation between RET expression and clinicopathologic features in Ovarian Cancer (n=82)

Clinicopathologic Parameters	No. of Patients	RET positive rate
FIGO stage		
I	45 (54.9%)	84.4%
II	7 (8.5%)	71.4%
III	26 (31.7%)	53.9%
IV	4 (4.9%)	50.0%
Histologic type		
Serous	25 (30.5%)	48.0%
Endometrioid	10 (12.2%)	70.0%
Mucinous	18 (21.9%)	100%
Clear cell	29 (35.4%)	75.9%
Grade		
1	24 (29.3%)	79.2%
2	46 (56.1%)	71.7%
3	12 (14.6%)	58.3%
Age		
50 >	45 (54.9%)	77.8%
50 ≦	37 (45.1%)	64.9%

(2008 cells, A2780 cells) were successfully amplified with RET primers. The size of PCR products was 300 bp (Fig. 1A). To characterize the RET protooncogene in 2008 cells, PCR amplification followed by nucleotide sequencing was peformed. Analysis of exons 10, 11, and 13 to 16 revealed no mutations (data not shown).

Immunohistochemical staining of ovarian tumors for RET

Immunohistochemical staining for RET proteins was found predominantly in the cytoplasm, but also in the nucleus (Fig. 1B). Among the 82 cases of ovarian cancer (Table 1), the frequency of RET expression was high in mucinous cystadenocarcinoma (18 of 18 cases ; 100%), clear cell adenocarcinoma (22 of 29 cases ; 75.9%), and endometrioid adenocarcinoma (7 of 10 cases ; 70.0%). According to disease stage, the frequency of RET-positive cases was 84.4% (38 of 45 cases) in stage I, 71.4% (5 of 7 cases) in stage II, 53.9% (14 of 26 cases) in stage III, and 50% (2 of 4 cases) in stage IV. According to histological grade, the rate of RET-positive staining was 79.2% in grade 1, 71.7% in grade 2, and 58.3% in grade 3. According to histological type, the rate of RET expression was 72.0% (59 of 82 patients) in ovarian cancer, 53.3% (8 of 15 patients) in ovarian cystadenoma with borderline malignancy, and 20.7% (18 of 87 patients) in ovarian cystadenoma (Fig. 2).

Correlation of RET expression with clinicopathologic characteristics

The rate of RET expression (Fig. 2) was significantly higher in ovarian cancer ($p<0.001$) or ovarian cystadenoma with borderline malignancy ($p=0.02$) than in ovarian cystadenoma.

DISCUSSION

The prognosis of advanced ovarian cancer is poor, with a 5-year survival rate of 30% to 35%, because most cases are not diagnosed until the advanced stage. Furthermore, if the first-line chemotherapy is not effective against ovarian cancer, cancer will continue to grow and spread. Mucinous cystadenocarcinoma and clear cell adenocarcinoma have been reported to show chemoresistance. Our present study has found high expression of the RET protooncogene in mucinous cystadenocarcinoma and clear cell adenocarcinoma. However, the relation between chemoresistance and expression of the RET protooncogene is unclear.

Several research efforts have focused on the identification of new biological markers of prognosis in ovarian cancer. Activation of oncogenes, such as HER-2, and inactivation of onco-suppressor genes, such as p53, have been used in an attempt to assess the prognosis of ovarian cancers[22]. Moreover, in vitro and in vivo studies have ob-

tained some evidence concerning genes related to cellular apoptosis in ovarian carcinogenesis, such as Bcl-2 and p53, and the resistance to chemotherapy in ovarian cancer[23,24]. Aberrations of the proteins produced by these genes are frequently observed in ovarian cancer. However, the degree of protein aberration does not correlate with prognosis.

Ovarian cancer, especially endometrioid adenocarcinoma and clear cell adenocarcinoma, often co-exist with endometriosis. The potential for carcinogenesis, such the change to endometrioid adenocarcinoma or clear cell adenocarcinoma, cannot be ruled out in any case of endometriosis, because no marker is available for identifying this change. The present study is, to our knowledge, the first to examine the expression of RET in a wide spectrum of ovarian tumors. In this study we have found that RET is frequently expressed in endometrioid adenocarcinoma (70%) and clear cell adenocarcinoma (75.9%). Thus, RET expression may be useful for assessing the potential for carcinogenesis and for deciding the follow-up period after surgery for endometriosis. We are studying a larger number of cases of endometriosis to further validate our findings. We hypothesized that RET expression would be a good maker to assess the malignant potential of ovarian tumor. This study suggests that, in the future, RET may be useful for recognizing whether a patient has an ovarian cystadenoma or a potential malignant tumor before operation. We hope that the findings of our study will also lead to new targeted therapies for ovarian cancer.

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Research Article Open Access

Use of Localization and Activity of Thymidine Phosphorylase in Human Gynecological Tumors for Predicting Sensitivity to Pyrimidine Antimetabolite Therapy: An Observational Study

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Abstract

Background: Thymidine phosphorylase (dThdPase) is the rate-limiting enzyme in the conversion of 5′-deoxy-5-fluorouridine (5′-dFUrd), an intermediate metabolite of capecitabine (Xeloda®), to 5-fluorouracil (5-FU). We investigated the correlation between dThdPase activity and immunohistochemical staining in gynecological carcinoma and adjacent normal tissues. We hypothesize that the differential dThdPase activity between tumors and adjacent tissue is predictive of response to treatment with pyrimidine antimetabolites.

Methods: In 45 samples of carcinoma tissue and 35 of adjacent normal tissue from 45 patients, we measured dThdPase activity as well as immunoreactivity using an anti-dThdPase monoclonal antibody and macrophage and histiocyte-specific antibodies.

Results: dThdPase activity in tumor tissue was significantly higher than that in the corresponding adjacent normal tissue in all samples tested (12 uterine cervical, 19 endometrial, and 4 ovarian tumors). Anti-dThdPase immunopositivity was observed in the epithelial tumor cells of 76.9% of uterine cervical cancer samples, 60.0% of endometrial cancer samples and 63.6% of ovarian cancer samples. In stromal tissue, 84.6% of uterine cervical tumors (11/13), 90.0% of endometrial tumors (18/20), and 81.8% of ovarian tumors (9/11) were immunopositive for anti-dThdPase in interstitial cells (mainly macrophages). Macrophages were also strongly reactive in the stromal tissues of uterine cervical, endometrial, and ovarian cancers. The correlation between dThdPase activity and intensity of immunohistochemical staining of epithelial tumor cells with anti-dThdPase monoclonal antibody was statistically significant in endometrial carcinoma ($P = 0.008$) but borderline in uterine cervical tumors ($P = 0.077$). We found a good correlation between dThdPase activity and staining of epithelial tumor cells, particularly in the case of endometrial cancer.

Conclusions: We show that gynecological carcinomas show increased dThdPase activity, and this activity correlates with dThdPase staining of tumor epithelial cells. Thus, dThdPase staining of biopsy specimens could be useful in predicting the outcome of therapy with pyrimidine metabolites.

Keywords: Thymidine phosphorylase; Gynecological carcinoma; Pyrimidine antimetabolite therapy

Abbreviations: dThdPase: thymidine phosphorylase; 5′-dFUrd: 5′-deoxy-5-fluorouridine; 5-FU: 5-fluorouracil; HPLC: High Performance Liquid Chromatography; mAb: monoclonal antibody; ABC: Avidin-biotin conjugate

Introduction

Capecitabine (Xeloda®) and 5′-deoxy-5-fluorouridine (5′-dFUrd, Furtulon®) are masked compounds derived from 5-fluorouracil (5-FU) [1]. Thymidine phosphorylase (dThdPase) is the rate-limiting enzyme in the conversion of 5′-deoxy-5-fluorouridine (5′-dFUrd), an intermediate metabolite of capecitabine (Xeloda®), to 5-fluorouracil (5-FU). Although cytostatically inactive by themselves, they exert cytotoxic activity *in vivo* after being converted into 5-FU by the action of pyrimidine nucleoside phosphorylases [2-4], predominantly uridine phosphorylase in mouse and thymidine phosphorylase (dThdPase), reportedly identical to platelet-derived endothelial cell growth factor, in human tumors [5,6]. In addition, 5′-dFUrd is an active intermediate metabolite of capecitabine, which was approved for breast and colorectal cancer in the United States and the European Union. Capecitabine is actively catabolized by dThdPase in humans. From their mechanism of action, the antitumor activity of 5′-dFUrd and

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