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Diagnostic clinical application of two-color fluorescence *in situ* hybridization that detects chromosome 1 and 17 alterations to direct touch smear and liquid-based thin-layer cytologic preparations of endometrial cancers

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Abstract. Susumu N, Aoki D, Noda T, Nagashima Y, Hirao T, Tamada Y, Banno K, Suzuki A, Suzuki N, Tsuda H, Inazawa J, Nozawa S. Diagnostic clinical application of two-color fluorescence *in situ* hybridization that detects chromosome 1 and 17 alterations to direct touch smear and liquid-based thin-layer cytologic preparations of endometrial cancers. *Int J Gynecol Cancer* 2005;15:70–80.

We performed two-color fluorescence *in situ* hybridization (FISH) on direct touch smears and liquid-based thin-layer (ThinPrep) cytological preparations of endometrial tumors to detect alterations of chromosome 1 and 17 that present with high incidence in endometrial cancers. The DNA probes used for two-color FISH analysis were a combination of the probes designed for 17cen (cCI 17-321) and 17p13.3 (*D17S34*), and a combination of the probes designed for 1q12 (*D1Z1*) and 1p36 (cCI1-5335). Numerical or structural alterations of chromosome 1 and/or 17 were detected in 95% (19 of 20 cases) of the direct touch smears obtained from endometrial cancer, while these alterations were also detected in 93% (12 of 13 cases) of samples obtained from grade 1 endometrioid adenocarcinoma cases, including three cases that could not be diagnosed as positive by conventional Papanicolaou cytopathologic staining. Using ThinPrep cytopathologic preparations, numerical or structural abnormalities were found in 26 (90%) and five (100%) cases, respectively, of samples obtained transcervically from 29 endometrial cancer and five atypical endometrial hyperplasia cases. Therefore, two-color FISH may be a useful diagnostic method for endometrial adenocarcinoma and premalignant lesions that demonstrate only slight cellular atypia in conventional cytopathologic preparations.

KEYWORDS: cytological diagnosis, endometrial cancer, liquid-based cytology, two-color FISH.

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In Japan, endometrial cytology testing has been widely performed for women who present with irregular genital bleeding to test for the possibility of endometrial cancers. Compared to endometrial tissue

biopsies, cytological screening is less painful during patient sampling, and diagnostic results are obtained in a shorter time[1,2]. It has been shown that endometrial cytology examinations are useful for the early diagnosis of endometrial neoplasias[3] and that its sensitivity for endometrial cancers is 70–95%, if both cytologically suspicious cases and positive cases are combined and compared with histologically true-positive cases[1,4–6]. A major drawback in cytology examinations, however, is the difficulty in the diagnosis of grade 1 endometrioid adenocarcinoma cases that present with only slight nuclear atypia and cellular overlapping[1]. Thus, equivocal cases, defined as cases in which the differentiation between benign and malignant is difficult, are relatively common. In addition, interinstitutional differences in the sensitivity of cytological diagnosis, due to the subjective nature of cytological diagnosis, are often cited as problem points in the implementation of cytological screening examinations.

We aimed to develop a new objective method to diagnose endometrial cytology specimens based on alterations in specific chromosomes in cell specimens. Several cytogenetic studies have shown that chromosome 1 is often highly altered[7–9] and that the 1p36 region is often deleted, as detected by two-color FISH[10] in endometrial cancers. In addition, 17p has also been reported to be deleted in 35% of endometrial cancers[11]. Interestingly, p73, a tumor-suppressor gene (p53 homologue)[12], is located on 1p36, while the tumor-suppressor gene p53[13,14] and the HIC-1 (hypermethylation in cancer) gene [15] are located on the 17p13 region. We demonstrate in the current study that two-color fluorescence *in situ* hybridization (FISH) using DNA probes of a centromeric region and of a specific locus in the same chromosome can detect both numerical and structural chromosomal alterations.

In the present study, our initial objective was to detect numerical and structural alterations in chromosome 1 and chromosome 17 in endometrial cancers by two-color FISH. This method was applied to direct imprinted specimens to demonstrate the frequency of these alterations in tumors. Next, this two-color FISH method was applied to endometrial cytology specimens obtained by liquid-based thin-layer ThinPrep method (the ThinPrep slide preparation system, Cytyc Corporation, Boxborough, MA)[16], a method widely used for cervical cancer screening in the United States[17,18]. Fixation in liquid fixative was incorporated in this investigation due to its usefulness in reducing overlap of glandular cells[19].

Materials and methods

Tissue samples

Endometrial tumor tissues were obtained from 45 women who underwent hysterectomies (30 patients) or dilation and curettage (15 patients) at Keio University Hospital, Tokyo, Japan. With respect to histology, the tissue specimens consisted of 30 cases of well-differentiated grade 1 endometrioid adenocarcinoma, six cases of moderately differentiated grade 2 endometrioid adenocarcinoma, and four cases of poorly differentiated grade 3 endometrioid adenocarcinoma in addition to five cases of atypical endometrial hyperplasia complex, a precancerous lesion. All samples were pathologically diagnosed using surgical specimens. As controls, 10 cases of benign endometrial tissue were obtained from women who underwent hysterectomies due to a diagnosis of myoma uteri in addition to 10 cases of benign endometrial tissue obtained by curettage from outpatients. Pathological diagnoses were based on WHO criteria[20]. All tissues were sampled following informed consent from the patients.

As endometrial cancer tissue samples, FISH cytology slides were prepared by direct touch smear of tissue samples from 20 hysterectomy patients followed by fixation with Carnoy's solution and drying. Cytology slides were also prepared by transcervical sampling from nine of these patients just before hysterectomy with a Uterobrush[2] (Medscand, Malmö, Sweden and Teikoku Hormone, Tokyo, Japan), followed by a liquid-based cell suspension thin-layer slide technique. In brief, endometrial cells were collected using a Uterobrush and dispersed in preservation fluid (PreserveCyt, Cytyc Corporation)[18], following removal of debris and red blood. Cells were again dispersed before cell collection onto a filter by negative pressure. The cells on the filter were then transferred to a glass slide by positive pressure using a ThinPrep processor (Cytyc Corporation). In addition, FISH cytology slides were made from transcervical samplings from 10 patients with endometrial cancer undergoing hysterectomy and from 10 patients with endometrial cancers who underwent only dilation and curettages before conservative progestin therapy. In total, 20 direct touch smears and 29 liquid-based smear samples were used as specimens. The majority (>90%) of the constituent cells in each sample were confirmed to be tumor cells by microscopic observations. Similarly, with atypical endometrial hyperplasia complex samples, specimens were obtained from patients undergoing dilation and curettage before conservative

progestin therapy. With benign endometrial tissue samples, cytology slides were prepared by direct touch smears before hysterectomy and by transcervical preparation with liquid-based cytological methods.

DNA probes

The DNA probes used were as follows: plasmid clones pUC1.77 (*D1Z1;* 1q12)[21], p144D6 (*D17S34;* 17p13.3)[22], and cosmid clones cCI-1-5335 (1p36)[23], cCI-17-321 (17 centromere)[24]. Two satellite DNA probes (pUC1.77 and cCI 17-321) were labeled with digoxigenin-11-dUTP, and two locus-specific DNA probes (cCI 1-5335 and p144D6) were labeled with biotin-16-dUTP using a nick translation kit (Boehringer Mannheim, Mannheim, Germany). These labeled DNA probes were precipitated in ethanol with 20 μg each of salmon testis DNA (Sigma Chemical Co., St. Louis, MO) and *Escherichia coli* tRNA (Sigma), dried, and dissolved in 20 μl of formamide.

Procedure for two-color FISH

The combinations of probe DNAs used for two-color FISH are presented in Table 1. The cell samples on glass slides were digested with 0.2–0.5 μg/ml of proteinase K (Merck, Darmstadt, Germany) for 30 min at 37 °C, fixed with 4% paraformaldehyde for 15 min, denatured in 70% formamide/×2 standard saline citrate (SSC) (0.3 mol/l NaCl, 0.03 mol/l sodium citrate) at 75 °C for 2 min, cooled, and dehydrated through an ethanol series. A total of 1 μg of the two DNA probes (Table 1) was mixed at a predetermined ratio with 2.5 μg of Cot-1 DNA (Gibco BRL, Grand Island, NY), denatured at 70 °C for 10 min, cooled on ice, mixed with an equal volume of ×2 SSC/20% dextran sulfate, incubated at 37 °C for 15 min, and hybridized with the samples on glass slides at 37 °C for 12–16 h. After washing, the slides were reacted with avidin-fluorescein isothiocyanate (FITC) and antidigoxigenin rhodamine (Boehringer Mannheim); following a second washing, the slides were counterstained with 4,6-diamidino-2-phenylindole and mounted in antifade solutions[25].

Interpretation of signals in interphase FISH images

Figure 1 shows a simplified schematic interpretation of two-color FISH signals in interphase images in relation to metaphase images. In this schematic representation, a red signal indicates the centromere and a green signal indicates the telomeric locus-specific signal on the short arm. As described previously[26], we categorized the signal patterns of each tumor cell as follows: disomy (D) presents with two centromeric signals and two telomeric signals; disomy with loss of the short arm (D + L) presents with two centromeric signals and one telomeric signal; and polysomy (P) presents with equal numbers of three or more centromeric and telomeric signals. In addition, polysomy with loss of the short arm (P + L) presents with three or more centromeric signals and a lesser number of telomeric signals (eg, 3 to 1, 3 to 2, or 4 to 2). Monosomy (M) presents with one centromeric signal and one telomeric signal, and gain of the short arm (G) presents with more telomeric signals than centromeric signals. We interpreted disomy with loss, monosomy, and polysomy with loss to be deletions of the short arm. The 1p36 or 17p13.3 region was interpreted to be deleted when the sum of the ratios of the tumor cells with a signal pattern of D + L, M, or P + L exceeded the mean plus 3SD value of the sum of the ratios of control endometrial cells with a signal pattern of D + L, M, or P + L. If two or more of the deletions, polisomies, and/or gains of the short arm were interpreted to coexist as significant clones, the category of the highest percentage was assigned to represent the pattern of chromosome 1 or chromosome 17 of the tumor. Overlapping nuclei were excluded from the analysis. Scattered signals or two small neighboring signals were interpreted as a single signal, and any yellow signals were reconfirmed with a single-band pass filter to determine whether it consisted of an overlapping green signal and red signal. The fluorescent signals of 100–200 nuclei were counted through a triple-band pass (D-F-T) filter, a dual-band pass (F-T) filter, an fluorescein isothiocyanate (FITC) filter, and a rhodamine filter (Chroma, Brattleboro, VT) using a fluorescence microscope (model BX50, Nikon, Tokyo, Japan).

Results

Ten benign endometrial samples were used as two-color FISH controls. Table 1 summarizes the percentages of abnormal categories in these benign endometrial glandular cells. Based on the above criteria, the cut-off values were estimated to be 26.5% for the loss of 1p36 and 36.7% for the loss of 17p13.3.

Figure 2 shows representative images of two-color FISH in metaphase spreads of normal lymphocytes after short-term culture. Figure 2A shows two pairs of signals for 1p13.6 (FITC signals) and 1 centromere (rhodamine signals), and Figure 2B shows a single pair of signals for 17p13.3 (an FITC signal) and 17

Table 1. Combinations of DNA probes used for two-color fluorescence *in situ* hybridization (FISH) analyses and their cut-off values calculated from the mean and standard deviation of 10 control specimens

	Chromosome 1 (cCI-1-5335, pUC1.77)							Chromosome 17 (p144D6, cCI-17-321)						
		Percentage of signals							Percentage of signals					
Categories of signal pattern	Number of counted cells	D+L	M	P	P+L	G	Deletion (D+L, M, P+L)	Number of counted cells	D+L	M	P	P+L	G	Deletion (D+L, M, P+L)
1	191	4.2	2.1	2.1	9.4	3.7	15.7	180	11.7	4.4	4.4	15.0	2.8	31.1
2	190	4.2	4.2	6.3	11.6	1.1	20.0	194	10.8	1.5	9.3	16.5	2.1	28.9
3	108	5.6	1.9	6.5	5.6	6.5	13.0	110	6.4	6.4	8.2	9.1	1.8	21.8
4	105	4.8	2.9	6.7	6.7	1.9	14.3	104	8.7	4.8	3.8	4.8	4.8	18.3
5	198	3.0	2.0	7.1	9.1	7.1	14.1	115	7.8	2.6	3.5	7.0	8.7	17.4
6	102	3.9	3.9	9.8	4.9	9.8	12.7	196	8.2	4.1	4.1	6.1	5.1	18.4
7	150	5.3	2.7	3.3	6.7	2.7	14.7	111	11.7	2.7	4.5	13.5	1.8	27.9
8	177	7.3	3.4	6.8	11.9	3.4	22.6	150	12.7	2.7	8.0	2.7	5.7	18.0
9	203	3.9	3.0	6.9	7.9	2.0	14.8	163	9.8	2.5	4.3	11.0	2.0	23.3
10	160	6.3	3.1	6.3	8.1	3.1	17.5	177	7.3	2.3	10.2	5.1	7.0	14.9
Mean		4.9	3.0	6.2	8.1	4.2	16.1		9.8	3.7	6.0	9.1	4.3	21.6
SD		1.18	0.75	2.18	2.49	2.90	3.47		2.70	1.38	2.63	5.62	2.58	5.03
Mean+3SD (cut-off value)		8.4	5.2	12.7	15.6	12.9	26.5		17.9	7.8	13.9	26.0	12.0	36.7

The two-color FISH signals correspond to the patterns of signals shown in Fig. 1. D+L (disomy with loss of the short arm) represents two centromeric signals and one telomeric signal; M (monosomy) represents one centromeric signal and one telomeric signal; P (polysomy) represents equal numbers of three of more centromeric and telomeric signals; P+L (polysomy with loss of the short arm) represents three or more centromeric signals and a lesser number of telomeric signals (eg, 3 to 1, 3 to 2, and 4 to 2); G (gain) represents three or more telomeric signals and a lesser number of centromeric signals (eg, 1 to 3, 2 to 3, and 2 to 4).

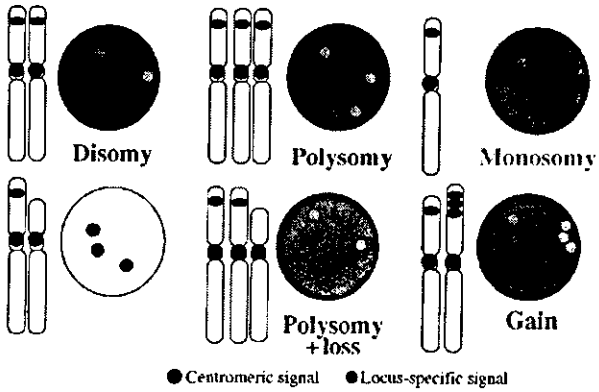

Fig. 1. Interpretation of two-color fluorescence *in situ* hybridization signals in interphase images of endometrial materials in relation to metaphase images.

centromere (a rhodamine signal). The signals were specific, and no cross-reaction was observed.

Figure 3A shows the interphase images of two-color FISH using direct touch smear preparations of normal endometrial tissues hybridized with the chromosome 1 probes. In 10 cases of normal endometrial tissue, no abnormal categories or deletions were detected based on the criteria described in Figure 1 by counting nuclei hybridized with chromosome 1 probes or chromosome 17 probes. In endometrial adenocarcinomas samples, cells with a P+L pattern in specimens hybridized with chromosome 1 probes (Fig. 3B) and cells with a P+L pattern in specimens hybridized with chromosome 17 probes (Fig. 3C) were detected. In specimens hybridized with chromosome 17 probes, cells with a D+L pattern were detected (Fig. 3D).

Analysis of the 20 adenocarcinoma cases demonstrated interpretable FISH data for chromosome 1 in 19 cases with alterations of chromosome 1 in 16 cases (84.2%). Deletions of 1p36 were detected in 11 cases, gains of 1p36 were detected in four cases, and polysomy of chromosome 1 was detected in one case. FISH

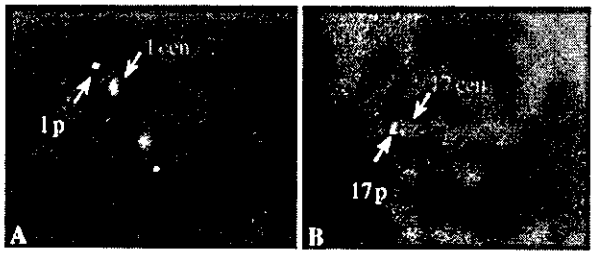

Fig. 2. Metaphase image of two-color fluorescence *in situ* hybridization (FISH). (A) FISH image of a normal lymphocyte hybridized with chromosome 1 probes (original magnification ×4000). (B) FISH image of a normal lymphocyte hybridized with chromosome 17 probes (original magnification ×5000).

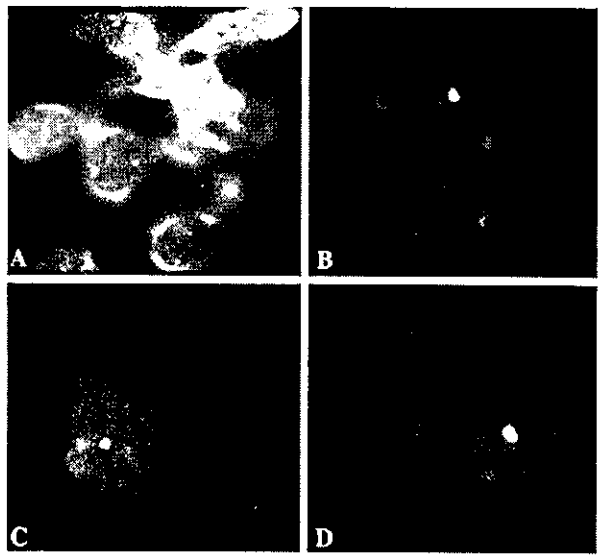

Fig. 3. Interphase images of two-color fluorescence *in situ* hybridization (FISH). (A) FISH image of a normal endometrial cell hybridized with chromosome 1 probes (original magnification ×1000). (B) FISH image of a well-differentiated endometrioid adenocarcinoma cell hybridized with chromosome 1 probes. Three fluorescein isothiocyanate (FITC) signals for the locus on 1p and four rhodamine signals for the centromere of chromosome 1 can be seen (original magnification ×3000). (C) FISH image of a well-differentiated endometrioid adenocarcinoma cell hybridized with chromosome 17 probes. One FITC signal for the locus on 17p and three rhodamine signals for the centromere of chromosome 17 can be seen (original magnification ×3000). (D) FISH image of a well-differentiated endometrioid adenocarcinoma cell hybridized with chromosome 17 probes. One FITC signal for the locus on 17p and two rhodamine signals for the centromere of chromosome 17 can be seen (original magnification ×3000).

data on chromosome 17 were interpretable for 16 cases, with alterations of chromosome 17 in 10 cases (62.5%). Deletions of 17p13.3 were detected in nine cases, and monosomy of chromosome 17 was detected in one case.

Table 2 summarizes the data for chromosome 1 and chromosome 17 alterations in 20 direct touch smear samples of endometrial adenocarcinomas. The two-color FISH signal patterns correspond to the categories of signals shown in the schematic representation in Figure 1. The main abnormal patterns were polysomy with loss of 1p and disomy with loss of 17p. Thus, in 19 of 20 endometrial adenocarcinoma cases (95.0%), either chromosome 1 or 17 showed alterations.

These chromosomal alterations were detected by this two-color FISH method in 12 of 13 grade 1 endometrioid adenocarcinoma cases (92.3%), in all three grade 2 endometrioid adenocarcinoma cases (100%), and in all four grade 3 endometrioid adenocarcinoma

Table 2. The results of two-color fluorescence *in situ* hybridization (FISH) using direct touch smear preparations of endometrial cancer tissues

			Chromosome 1				Chromosome 17			
				Percentage of signals				Percentage of signals		
Case	Grade	Cytology	Number of counted cells	Abnormal pattern	Disomy pattern	Deletion	Number of counted cells	Abnormal pattern	Disomy pattern	Deletion
1	G1	Suspicious	101		D (75)	− (25)	107		D (72)	− (20)
2	G1	Positive	132		D (81)	− (10)	122	M (13)	D (66)	− (19)
3	G2	Positive	120	G (34)	D (53)	− (8)			Not done	
4	G3	Positive	100	G (41)	D (46)	− (8)			Not done	
5	G1	Positive	106	P (42)	D (46)	− (10)			Not done	
6	G2	Positive	104	G (21), P (19)	D (60)	− (1)	121	D+L (50)	D (46)	+ (54)
7	G1	Positive	102		D (78)	− (10)	112	D+L (42)	D (43)	+ (50)
8	G1	Positive	102	G (18),.P (15)	D (62)	− (6)	111	D+L (30)	D (58)	+ (42)
9	G2	Positive	Not done				124	P+L (28)	D (20)	+ (76)
10	G1	Positive	135	D+L (20)	D (52)	+ (33)	102	D+L (19)	D (66)	− (28)
11	G3	Positive	110	D+L (35)	D (52)	+ (42)	110	D+L (21)	D (65)	− (27)
12	G1	Negative	102	P+L (23)	D (74)	+ (27)	130		D (58)	− (25)
13	G1	Positive	148	P+L (89)	D (9)	+ (89)	106	D+L (21)	D (63)	− (26)
14	G3	Positive	147	P+L (26)	D (61)	+ (29)	146		D (72)	− (24)
15	G3	Positive	100	P+L (27)	D (52)	+ (27)	105	D+L (26)	D (66)	− (33)
16	G1	Suspicious	109	P+L (27)	D (63)	+ (27)	120		D (68)	− (31)
17	G1	Positive	120	P+L (39)	D (59)	+ (38)	Not done			
18	G1	Positive	148	P+L (81)	D (5)	+ (88)	120	D+L (29)	D (47)	+ (40)
19	G1	Suspicious	102	P+L (73)	D (26)	+ (74)	102	D+L (37)	D (53)	+ (48)
20	G1	Positive	103	P+L (50)	D (40)	+ (53)	118	D+L (31)	D (49)	+ (47)

The two-color FISH signals correspond to the patterns of signals shown in Fig. 1. D, disomy; P, polysomy; M, monosomy; D+L, disomy with loss of the short arm; P+L, polysomy with loss of the short arm; G, gain; G1, endometrioid adenocarcinoma grade 1; G2, endometrioid adenocarcinoma grade 2; G3, endometrioid adenocarcinoma grade 3.

cases (100%). These results suggest that two-color FISH is a very useful method to diagnose endometrial cancers, showing high sensitivity (95.0%) in direct touch smears.

Table 3 summarizes the results of two-color FISH on ThinPrep specimens obtained by transcervical sampling of endometrial cancers. In all of the 29 adenocarcinomas, interpretable FISH data on chromosome 1 and 17 were obtained. Alterations of chromosome 1 were detected in 22 cases (75.9%), and deletions of 1p36 were detected in 13 cases (44.8%). The main abnormal patterns for 29 cases of adenocarcinoma were polysomy with loss of 1p36 (detected in 12 of 29 cases, 41.4%), gain (G) of 1p36 (detected in seven of 29 cases), and polysomy(P) of chromosome 1 (detected in five of 29 cases). Alterations of chromosome 17 were detected in 24 cases (82.8%), and deletions of 17p13.3 were detected in 17 cases (58.6%). The main abnormal patterns for 29 cases of adenocarcinoma were disomy with loss of 17p13.3 (detected in 22 of 29 cases, 75.9%) and polysomy with loss of 17p13.3 (P+L) (detected in three of 29 cases). Thus, in 26 of the 29 adenocarcinoma cases (89.7%), either chromosome 1 or 17 demonstrated alterations. By grade of differentiation, two-color FISH detected chromosome alterations in 22 of 25 grade 1 adenocarcinoma cases and in all four cases of grade 2 adenocarcinomas. Table 4 summarizes the frequencies of deletion or abnormal signal patterns detected by two-color FISH in chromosome 1 or 17.

Table 5 summarizes the frequency of alterations of chromosome 1 or 17 in ThinPrep specimens from cases that presented with either negative or suspicious cytologic diagnosis by Papanicolaou staining. All specimens were grade 1 endometrioid adenocarcinomas. All the four negative cases demonstrated a D+L pattern of chromosome 17 as summarized in Table 3. Among 10 suspicious cases, seven demonstrated abnormal patterns; these included P+L of chromosome 1 in five cases, D+L of chromosome 17 in seven cases, G of chromosome 1 in one case, and polysomy of chromosome 1 in two cases. Moreover, these chromosomal alterations were detected by two-color FISH in 11 of 14 cases for which cytological diagnosis was rendered as false-negative or equivocal. In atypical endometrial hyperplasia complex cases, alterations of 1p or 17p were detected in all five cases. The main abnormal patterns were P+L of

Table 3. The results of two-color fluorescence *in situ* hybridization (FISH) using transcervical sampling smear preparations of endometrial cancer tissues

			Chromosome 1				Chromosome 17			
				Percentage of signals				Percentage of signals		
Case	Histology and grade	Cytology	Number of counted cells	Abnormal pattern	Disomy pattern	Deletion	Number of counted cells	Abnormal pattern	Disomy pattern	Deletion
1	AEH	Suspicious	120		D (68)	− (16)	115	D+L (18)	D (62)	− (30)
2	AEH	Suspicious	105	P+L (21)	D (53)	− (21)	108	D+L (20)	D (49)	− (30)
3	AEH	Suspicious	110	P+L (20)	D (55)	− (26)	114	D+L (24)	D (53)	+ (40)
4	AEH	Suspicious	121	P+L (26), P (17)	D (51)	+ (32)	103	D+L (26)	D (62)	− (33)
5	AEH	Suspicious	131	P+L (30)	D (53)	+ (42)	120	D+L (22)	D (43)	+ (39)
6	G1	Suspicious	102		D (78)	− (17)	104		D (85)	− (15)
7	G1	Suspicious	103		D (78)	− (7)	116		D (68)	− (30)
8	G1	Suspicious	111		D (85)	− (6)	131		D (79)	− (10)
9	G2	Positive	147	G (45)	D (54)	− (1)	150		D (88)	− (8)
10	G1	Suspicious	110	D+L (9)	D (71)	− (23)	105	D+L (18)	D (71)	− (22)
11	G1	Positive	120	P (21)	D (60)	− (19)	105	D+L (19)	D (71)	− (19)
12	G1	Negative	115		D (85)	− (6)	121	D+L (35)	D (52)	+ (45)
13	G1	Negative	145		D (70)	− (17)	152	D+L (34)	D (42)	+ (41)
14	G1	Negative	104		D (68)	− (22)	108	D+L (30)	D (50)	+ (42)
15	G1	Positive	104		D (59)	− (23)	115	P+L (30)	D (55)	+ (40)
16	G1	Positive	160	G (38)	D (38)	− (24)	117	P+L (35)	D (52)	+ (42)
17	G1	Suspicious	156	G (32)	D (59)	− (6)	141	D+L (28)	D (52)	+ (40)
18	G1	Positive	158	G (22)	D (63)	− (11)	144	D+L (21)	D (55)	+ (40)
19	G2	Positive	122	G (35)	D (51)	− (6)	126	D+L (40)	D (51)	+ (49)
20	G1	Positive	104	G (32)	D (63)	− (4)	107	D+L (28)	D (54)	+ (46)
21	G2	Positive	100	G (30)	D (60)	− (6)	105	D+L (51)	D (29)	+ (71)
22	G2	Positive	145	P+L (27)	D (62)	+ (27)	147		D (82)	− (7)
23	G1	Positive	104	D+L (28)	D (58)	+ (32)	103	D+L (22)	D (68)	− (31)
24	G1	Positive	132	P+L (28)	D (58)	+ (35)	128	D+L (26)	D (65)	− (28)
25	G1	Suspicious	166	P+L (23)	D (60)	+ (31)	145	D+L (23)	D (56)	− (29)
26	G1	Positive	133	P+L (32)	D (55)	+ (40)	148	D+L (21)	D (69)	− (25)
27	G1	Suspicious	122	P+L (25), P (13)	D (56)	+ (31)	119	D+L (26)	D (60)	− (32)
28	G1	Positive	108	P+L (36)	D (56)	+ (42)	103	D+L (23)	D (57)	+ (38)
29	G1	Positive	102	P+L (30)	D (64)	+ (35)	147	D+L (28)	D (54)	+ (39)
30	G1	Suspicious	125	P+L (23)	D (50)	+ (30)	105	D+L (35)	D (55)	+ (41)
31	G1	Negative	133	P+L (23), P (20)	D (45)	+ (32)	109	D+L (30)	D (57)	+ (40)
32	G1	Positive	110	P+L (28), P (13)	D (50)	+ (35)	131	D+L (34)	D (51)	+ (42)
33	G1	Suspicious	122	P+L (23), P (17)	D (53)	+ (27)	128	D+L (30)	D (58)	+ (39)
34	G1	Suspicious	101	P+L (28)	D (53)	+ (40)	109	P+L (27), D+L (18)	D (45)	+ (52)

The two-color FISH signals correspond to the patterns of signals shown in Fig. 1. D, disomy; P, polysomy; M, monosomy; D+L, disomy with loss of the short arm; P+L, polysomy with loss of the short arm; G, gain; AEH, atypical endometrial hyperplasia complex; G1, endometrioid adenocarcinoma grade 1; G2, endometrioid adenocarcinoma grade 2.

Table 4. Frequency of deletion or abnormal signal patterns detected by two-color fluorescence *in situ* hybridization with ThinPrep method obtained by transcervical sampling of endometrial carcinomas

	1p	17p	1p or 17p
Deletion	13/29 (44.8%)	17/29 (58.6%)	23/29 (79.3%)
Expression of abnormal categories	22/29 (75.9%)	24/29 (82.8%)	26/29 (89.7%)

Table 5. Relation between deletion or abnormal signal patterns in 1p or 17p detected by two-color fluorescence *in situ* hybridization and cytological diagnosis of endometrial cancers

	Endometrial cytology		
	Negative	Suspicious	Positive
Deletion	4/4 (100%)	6/10 (60%)	13/15 (87%)
Expression of abnormal categories	4/4 (100%)	7/10 (70%)	15/15 (100%)

1p36 (detected in four of five cases) and D+L of 17p13.3 (detected in five of five cases).

Discussion

The pathological differentiation of atypical endometrial hyperplasia complex from well-differentiated (grade 1) endometrioid adenocarcinoma is often confusing and lacks consensus in diagnostic criteria even among pathologists who specialize in the field of gynecological malignancies. Some pathologists emphasize nuclear atypia, such as enlarged nuclei, increased nuclear chromatin, enlarged nucleoli, irregular thickening of the nuclear membrane, irregular intranuclear distribution of chromatin, and frequent mitotic figures, as well as other nuclear features, whereas other pathologists place greater emphasis on architectural abnormalities such as the degree of crowded glands, irregular contour of glands, high degree of pseudostratification of glandular cells, as well as other architectural features. The criteria advocated by Blaustein[27] places the greatest diagnostic emphasis on stromal invasion such as cribriform pattern growth in the differential diagnosis between grade 1 endometrioid adenocarcinomas and atypical endometrial hyperplasia complex. However, uniform diagnostic criteria to cytologically discriminate the two diseases have not been firmly established based on conventional Papanicolaou staining.

Recently, novel methods for distinguishing endometrial carcinoma from nonmalignant endometrial lesions have been reported. Langer *et al.*[28] reported that transvaginal ultrasonography had 90% sensitivity for detecting endometrial cancer, but that the specificity was only 48% and that some difficulties remained concerning the discrimination of endometrial cancer and endometrial hyperplasia using various cut-off criteria. Simil3ar attempts to define diagnostic methods to detect endometrial cancer have failed to achieve high sensitivity (>80%) and high specificity (>80%). Therefore, we aimed to develop a new, less-invasive diagnostic method for detecting endometrial cancer with both high sensitivity and high specificity. We objectively evaluated the significance of chromosomal alterations in endometrial adenocarcinoma by two-color fluorescence *in situ* hybridization (FISH) using liquid-based thin-layer cytological preparations.

There have been few reports on cytogenetic analysis methods for endometrial cancers. Numerical and structural rearrangements have been reported in chromosomes 1, 3, 6, 7, 10, 12, 17, and the X chromosome. Fujita *et al.*[7] demonstrated alterations of chromosome 1 in five of six cases, while Milatovich *et al.*[9] demonstrated chromosome 1 alterations in 10 of 14 stage I tumors with deletion of 1p36 or translocated regions involving 1p36 in four of 10 cases. Recently, using comparative genomic hybridization (CGH), Kiechle *et al.*[29] reported a high incidence of loss of 1p36-pter region in endometrial hyperplasia and suggested that loss of putative tumor-suppressor genes located within this region may be involved in the initiation and progression of endometrial hyperplasia complex. Based on restriction fragment length polymorphisms (RFLP) analysis, Jones *et al.*[11] reported frequent loss of heterozygosity on 17p, 3p, 19q, and 10q. Similarly, using CGH, Pere *et al.*[30] demonstrated that gains of 8q, especially 8q23-qter, are associated with aggressive phenotypes such as lymph node metastasis and adnexal tissue involvement in

endometrial carcinomas. In contrast, Hirasawa *et al.*[31] demonstrated no obvious difference in the frequency of 8q gain among different tumor grades. Based on a comprehensive overview of all available evidence, we determined that specific analyses of chromosomes 1 and 17 by two-color FISH would be an effective tool to detect chromosomal alterations in endometrial carcinomas.

The specific detection of large fluorescent signals by fluorescent microscopy requires selection of probe DNA fragments with a highly repeated sequence in a localized region of the sample DNA. Variable number tandem repeat DNA (VNTR DNA) is commonly used as probe DNA for detection in fluorescent techniques. Thus, we used VNTR DNA probes for chromosome 1 and chromosome 17, more specifically p144D6 (*D17S34;* 17p13.3) and cosmid cCI-1-5335(1p36) clones.

With regard to the clonality of normal endometrial glandular cells, the altered chromosomal patterns are random and they are derived from inevitable technical problems. DNA probes, avidin-FITC, and anti-digoxigenin rhodamine cannot always equally penetrate through the nuclear membrane of all examined cells treated with proteinase K solution. In addition, due to the different composition of the nucleic acids of centromere DNA probe and the locus-specific DNA probe, the hybridization states are subtly different. Furthermore, the fluorescent signals are often overlapped, and the number of FITC signals or rhodamine signals is often underestimated. Owing to these inevitable technical problems, normal endometrial glandular cells do not always reveal a disomy pattern. To avoid diagnosing these random chromosomal alterations as significant clonal chromosomal alteration, the alterations were analyzed using cut-off values of mean + 3SD values, values that have often been adopted in many FISH analyses.

Two-color FISH using the ThinPrep method with Uterobrush sampling detected numerical or structural alterations in either 1p or 17p in 89.7% of endometrial carcinoma cases, in 100% of atypical endometrial hyperplasia cases, and in 0% of 10 normal endometrium cases. In this study, the cut-off level was set at mean + 3SD, a standard that has been frequently adopted for the detection of genomic alterations by two-color FISH in various solid tumors[26,32–36]. Using this cut-off level, our method demonstrated a sensitivity of 89.7% and a specificity of 100%, which as a diagnostic method is one of the highest values reported for endometrial cytology screening[1,5,6]. However, the number of examined cases is still small, and further study is required for further validation of this method. In general, the low sensitivity in the detection of endometrial adenocarcinoma is believed to be due to difficulties associated with objectively distinguishing the slight nuclear atypia in well-differentiated grade 1 endometrioid adenocarcinoma from benign nuclear atypia due to inflammatory change. This is clinically important as the latter nuclear atypia often occurs with the repetitive samplings necessary to determine the cause of abnormal uterine bleeding. Based on the sensitivity and specificity of this method, our results suggest that this cut-off setting was appropriate for detecting these chromosomal alterations in endometrial malignancies.

Numerical or structural chromosomal alterations were frequently found in endometrial adenocarcinoma ThinPrep cytologic preparations. In 11 of 14 grade 1 adenocarcinoma cases with an initial negative or equivocal cytological diagnosis, alterations of chromosome 1 and/or 17 were objectively detected by two-color FISH. These 11 cases included four negative cases and seven suspicious but not positive cases by cytological diagnosis with Papanicolaou staining. These results suggest that two-color FISH may be useful as a supplemental diagnostic method in the detection of endometrial precancerous lesions or low-grade endometrial cancers.

With regard to the clonality of tumor cells with abnormal chromosomal alterations, the majority of examined cases demonstrated clonal expression of abnormal chromosomal alterations in addition to normal disomy patterns in chromosome 1 or 17, but a number of cases demonstrated simultaneous expressions of two different categories of abnormal chromosomal alterations. For example, cases 6 and 8 in Table 2 demonstrated both polysomy and gain of chromosome 1, while cases 4, 27, 31, 32, and 33 in Table 3 demonstrated both polysomy and polysomy with loss of short arm in chromosome 1, and case 34 demonstrated both polysomy with loss of short arm and disomy with loss of short arm in chromosome 17. Based on the present data, it is not possible to determine whether the coexpression of abnormal chromosomal alterations is derived from two different clonal expansions or random chromosomal alterations. Further studies of clonality analysis[37] will be necessary to address this problem.

Further studies are also necessary to elucidate the biological significance of the numerical and structural alterations in chromosome 1 and chromosome 17. The p73 tumor-suppressor gene, a p53 homologue[12], is located on 1p36. p73 has been reported to be deleted with high incidence in various brain tumors[38,39]. Tumor-suppressor genes such as p53 and HIC

(hypermethylation in cancer)-1 are located in the 17p13 region. p53 is a very well-characterized tumor-suppressor gene that has been reported to be deleted and/or mutated in approximately half of all malignant tumors[13,14]. Likewise, HIC-1 is a tumor-suppressor gene located on 17p13.3, distal to p53 on 17p13.1 that has been reported to be deleted in breast cancer[15,40,41]. However, the relation between the tumorigenesis and progression of endometrial adenocarcinoma and the frequent deletions of 1p or 17p in endometrial cancers and precancerous lesions are still unclear and will remain the focus of investigation in the future.

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Clinical Characteristics of Prognostic Factors in Poorly Differentiated (G3) Endometrioid Adenocarcinoma in Japan

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Background: It has been reported that prognosis is less favorable in poorly (G3) differentiated endometrioid adenocarcinoma than in well (G1) or moderately (G2) differentiated endometrioid adenocarcinoma. The goal of this study is therefore to analyze the prognosis of G3 endometrioid adenocarcinoma and various factors that may predict a favorable prognosis.
Method: This study included 699 Japanese cases of endometrioid adenocarcinoma at the International Federation of Gynaecology and Obstetrics (FIGO) surgical stages I–IV (including 74 G3 cases). We investigated the G1–G3 survival rates of endometrioid adenocarcinoma cases and the G2 and G3 disease-free periods. We also examined the clinicopathological characteristics of G3 endometrioid adenocarcinoma.
Result: The prognosis was poor in stages III and IV in G3 and in G2 cases, but recurrence was observed more frequently in G3 cases than in G2 cases. Adnexal metastasis and high pre-surgery CA602 values showed significantly low *P*-values for survival.
Conclusions: We suggest that the risk of late recurrence is higher in G3 than in G2 cases. The absence of adnexal metastasis and low pre-surgery CA19-9 values may suggest a relatively favorable prognosis in G3 endometrioid adenocarcinoma.

Key words: poorly differentiated type – G3 – endometrioid adenocarcinoma

INTRODUCTION

Endometrial carcinoma has a high morbidity in the advanced countries of Western Europe and the USA and also in Japan, where its morbidity has increased in recent years. In 1970, endometrial carcinoma constituted ~3% of total uterine cancers in Japan, but the ratio increased to ~40% in 1998. Therefore, it has become increasingly important to understand the oncogenic mechanisms and prognostic factors in endometrial cancer.

It was previously reported that grade of differentiation is one of the critical prognostic factors in endometrial carcinoma (1–4). Creasman et al. (5) reported that the 5-year survival rate was 92.0% for G1 endometrial carcinoma cases and 86.9% and 74.0%, respectively, for G2 and G3 cases. This suggested a significantly poorer prognosis for carcinomas of lower differentiation grades. Delaloye et al. (6) investigated the rates of local recurrence, metastasis, disease-free survival and overall survival according to differentiation grade for stage I endometrial adenocarcinoma cases, and showed that the lower the grade was, the higher the metastasis rate was and the lower the disease-free survival rate and overall survival rate were.

It has been suggested that there are two types of endometrial cancer based on oncogenic pathology. One type develops in women with signs of high-estrogen conditions such as obesity, hyperlipidemia, anovular bleeding, infertility, delayed menopause and proliferation of the ovarian stroma or endometrium. Another type develops in women without these signs. Many cases of the former type have the G1 or G2 differentiation grade with shallow muscle invasion, a high sensitivity to hormone therapy and a relatively favorable prognosis (7–9). The latter group, in many cases, has the G3 differentiation grade, with deep muscle invasion, high probability of lymph node metastasis, and shows a poor sensitivity to hormone therapy and a poor prognosis (8). Therefore, it is important to examine clinical characteristics of G3 endometrial carcinoma cases separately from highly differentiated cases.

Endometrioid adenocarcinoma constitutes 70% of endometrial carcinomas (5), and those with other tissue types such as clear cell adenocarcinoma and serous adenocarcinoma show a

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significantly poorer prognosis compared with endometrioid adenocarcinoma (5,10–14). Therefore, in this study, we limited the subjects to endometrioid adenocarcinoma patients. Specifically, we compared the prognosis of G3 endometrioid adenocarcinoma with G2 and examined the prognostic factors of G3.

SUBJECTS AND METHODS

Of the 890 endometrial carcinoma cases treated at the Keio University Hospital from 1975 to 2002, this study included 699 patients with endometrioid adenocarcinoma (including adenoacanthoma and adenosquamous cell carcinoma) for whom surgery had been performed. The breakdown was as follows: 405 G1 cases, 220 G2 cases and 74 G3 cases. The age at the start of treatment was 22–86 years (mean 54.8 years). The follow-up period was 1–302 months (mean 93.6 months). Patient backgrounds are summarized in Table 1. We had obtained informed consent to analyze prognostic factors from G3 endometrioid adenocarcinoma patients.

The standard surgical method in endometrial cancer in our department is modified radical hysterectomy for clinical stage I cases, radical hysterectomy for stage II cases, modified radical hysterectomy for stage III cases and total hysterectomy for stage IV cases. Pelvic lymphadenectomy is performed in all stages (I–IV). In modified radical hysterectomy, we dissect the anterior layer of the vesicouterine ligament, remove the ureter to the lateral side, dissect part of the posterior layer of vesicouterine ligament and part of the cardinal ligament and then deliver the uterus with about 1 cm of vaginal wall. Para-aortic lymphadenectomy is performed for: (i) patients with invasion to more than half of the myometrium; (ii) those with metastasis to the pelvic lymph nodes or the adnexas (diagnosed by the intraoperative frozen section); and (iii) those with G3 endometrioid adenocarcinoma (or specific pathological types such as serous adenocarcinoma and clear cell adenocarcinoma).

Table 1. Patients' backgrounds

Variables	Grade of differentiation		
	G1	G2	G3
Stage			
Stage I	307	140	32
Stage II	31	18	5
Stage III	62	54	29
Stage IV	5	8	8
Treatment			
Surgery alone	278	98	20
Surgery and radiotherapy	38	25	12
Surgery and chemotherapy	50	65	33
Surgery and MPA* treatment	17	16	1
Surgery and combination of multiple therapies#	22	16	8

*Methoxyprogesterone acetate.
#Radiotherapy, chemotherapy, and MPA treatment.

Adjuvant therapy after surgery is selected according to the protocol (the first to the fifth editions) of the Japan Gynecological Oncology Group (JGOG).

The G1–G3 survival rates of endometrioid adenocarcinoma cases and the G2–G3 disease-free periods (defined as the period from surgery to recurrence) were investigated. The survival rates and disease-free survival rates were calculated by the Kaplan–Meier method and statistical tests were performed with the log-rank method.

Univariate analysis was performed with the 5-year survival rate and disease-free survival rate of 74 cases of G3 endometrioid adenocarcinoma, to examine the relationships between clinicopathological factors and prognosis. The following 12 factors were examined: vessel permeation, muscle invasion (>1/3 versus ≤1/3), cervical involvement, lymph node metastasis, ascites cell analysis, parametrium invasion, adnexal metastasis, CA125 pre-surgery values (>35 U/ml versus ≤35 U/ml), CA602 pre-surgery values (>63 U/ml versus ≤63 U/ml), CA19-9 pre-surgery values (>37 U/ml versus ≤37 U/ml), the age at the start of the first treatment (age >60 versus age ≤60), and a family history of cancer or multiple cancers. This analysis was performed with the chi-square test. SAS Re16.12 TS060 was used for statistical analysis.

RESULTS

We first investigated the survival rates separately for each differentiation grade (G1–G3) of endometrioid adenocarcinoma in our hospital. The 5-year survival rates were 97.0% for G1, 86.0% for G2 and 78.6% for G3, clearly showing the poorest prognosis in G3 cases. The 10-year survival rate was 95.1% for G1, 82.2% for G2 and 78.6% for G3. In G1 and G3, the survival rate decreased for 5 years and stabilized in the following 5 years whereas the survival rate appeared to decrease steadily for 10 years in G2 cases (Fig. 1).

When the survival rate was compared separately in each surgical stage of G2 and G3 cases, the 5-year survival rate was 93.9% for stage I, 86.9% for stage II, 71.9% for stage III

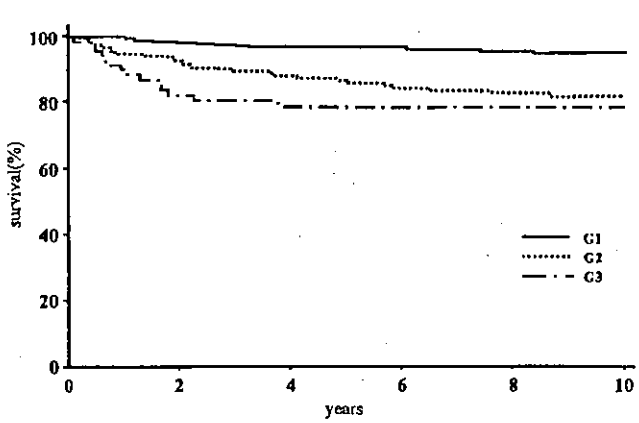

Figure 1. Survival by differentiation grade in 699 cases of endometrioid adenocarcinoma.

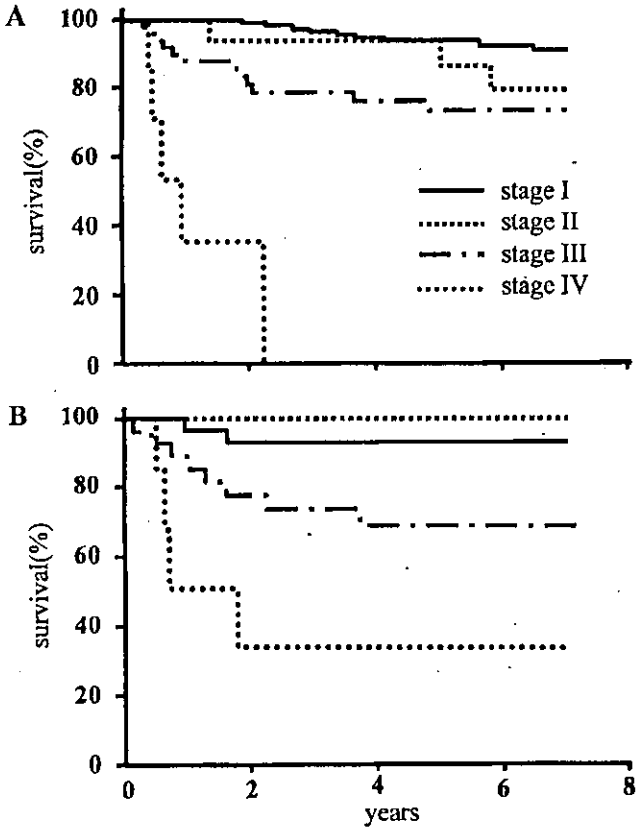

Figure 2. Survival by surgical stage for (A) G2 and (B) G3 endometrioid adenocarcinoma patients.

and 0% for stage IV G2 cases, and 93.2% for stage I, 100% for stage II, 68.9% for stage III and 34.3% for stage IV G3 cases. This clearly shows a poor prognosis in stages III and IV, even in G2 cases (Fig. 2).

When the G2–G3 disease-free periods were compared, there were recurrences in many cases within 5 years after surgery and some late recurrences after more than 10 years in G3 cases. In G2 cases, recurrences were observed steadily until 8 years after surgery, but not after 8 years (Fig. 3).

In the univariate analysis of the 5-year survival rate of 74 cases of G3 endometrioid adenocarcinoma, adnexal metastasis ($P = 0.0027$) and high pre-surgery CA19-9 values ($P = 0.020$) showed significantly low P-values for survival (Table 2). Cervical involvement ($P = 0.063$) and high pre-surgery CA602 values ($P = 0.070$) showed relatively low P-values, although they were not statistically significant. The 5-year survival rate, as analyzed separately by the presence or absence of these four factors, was 63.1% in the presence and 87.9% in the absence of cervical involvement, 61.9% in the presence or 87.9% in the absence of adnexal metastasis, 34.3% with high CA602 values and 87.5% with low CA602 values, and 50.8% with high CA19-9 values and 100% with low CA19-9 values (Fig. 4).

DISCUSSION

We examined the prognosis and the prognostic factors of G3 endometrioid adenocarcinoma in this study. First, we analyzed the 10-year survival rates for all grades. The rate decreased steadily for 5 years but remained steady without further

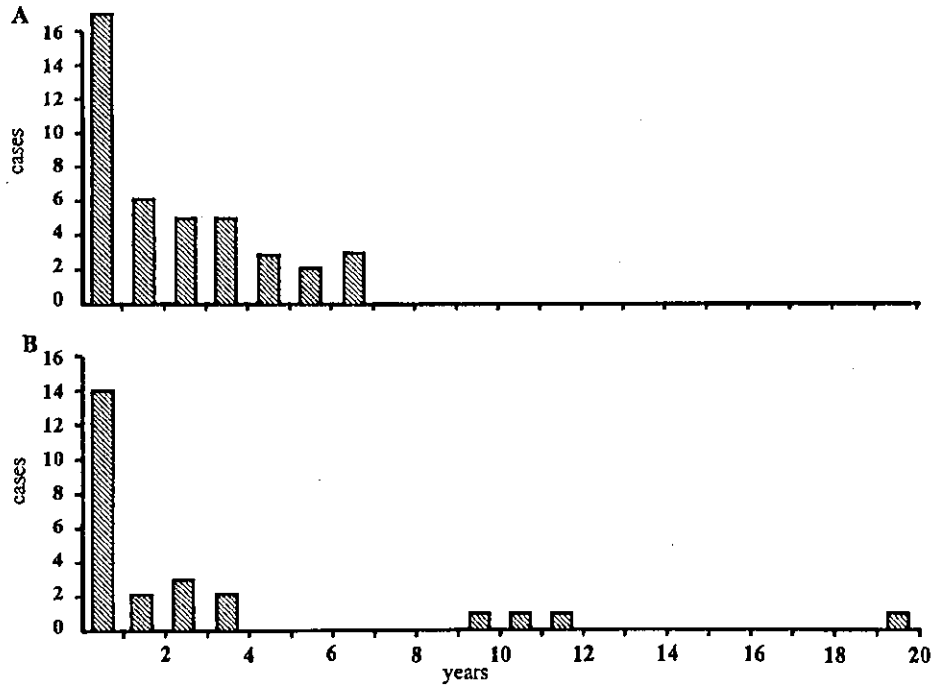

Figure 3. Disease-free periods for (A) G2 and (B) G3 endometrioid adenocarcinoma patients.

Table 2. Univariate analysis of G3 endometrioid adenocarcinoma

Clinicopathological factor	Number of cases (positive/negative or over/under)	5-year survival (*P*-value)	Disease-free survival (*P*-value)
Vessel permeation	51/20	0.39	0.058
Muscle invasion (>1/3 versus ≤1/3)	53/20	0.16	0.069
Cervical involvement	24/49	0.063	0.0058
Lymph node metastasis	7/42	0.48	0.20
Ascites cell analysis	20/8	0.11	0.25
Parametrium invasion	12/62	0.77	0.57
Adnexal metastasis	16/58	0.0027	0.00026
CA125 value (>35 U/ml versus ≤35 U/ml)	11/18	0.11	0.22
CA602 value (>63 U/ml versus ≤63 U/ml)	9/8	0.070	0.062
CA19-9 value (>37 U/ml versus ≤37 U/ml)	9/11	0.020	0.025
Age at start of the first treatment (age >60 versus ≤60)	64/10	0.20	0.072
Family history of cancer or multiple cancers	11/63	0.81	0.73

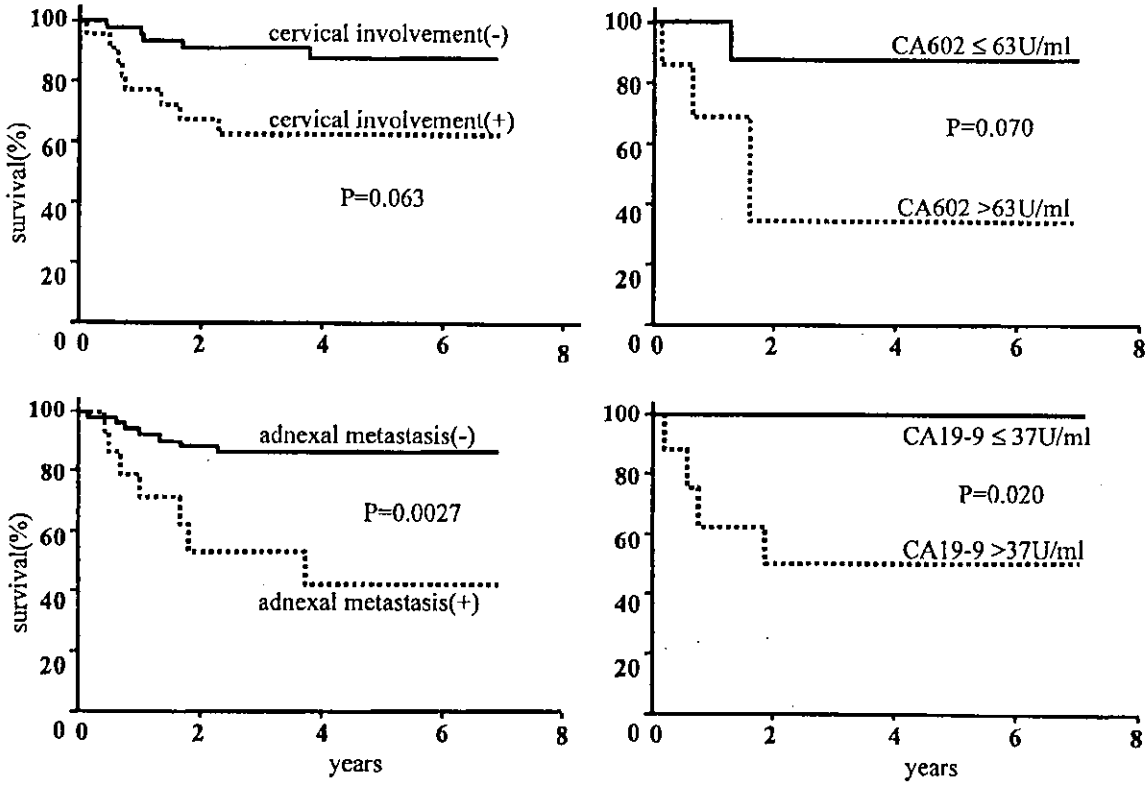

Figure 4. Survival by clinicopathological factors (cervical involvement, adnexal metastasis, and CA602 and CA19-9 pre-surgery values) in 74 cases of G3 endometrioid adenocarcinoma.

decreases in G1 and G3, suggesting that recurrence was rare during the 5–10-year period. In G2 cases, on the other hand, the survival rate decreased steadily for 10 years, but there were recurrences in many cases after 5 years. Compared with G1, both G2 and G3 showed poor prognosis, and the 10-year G2 survival rate was similar to that of G3.

Next, we analyzed the survival rate for each surgical stage of the G2 and G3 grades. Since the prognosis of G1 cases was much more favorable than that of G2 or G3 cases (as shown in Fig. 1), we limited the subjects to G2 and G3 cases. It was found that, in both G2 and G3 cases, prognosis was favorable in stages I and II, but poor in stages III and IV (Fig. 2). We

could not find any critical difference between G2 and G3 from this analysis.

We subsequently examined the disease-free period for G2 and G3 cases (Fig. 3), and showed that in G2 recurrences were observed steadily for 8 years after surgery, but there was no recurrence after 8 years. In G3, recurrences were often observed within 5 years after surgery and some late recurrences were also observed after 10 years. It was thus found that in G3 cases, recurrence occurred relatively early, quickly leading to death, but that late recurrences could also occur. We suggest that the high risk of late recurrence is one of the most significant features of G3 cases. Careful follow-up observation is important over a long period after surgery in G3 endometrioid adenocarcinoma.

In clinical practice we sometimes encounter G3 patients whose prognosis is rather favorable. In order to determine what factors might predict a favorable outcome, we analyzed 12 clinicopathological prognostic factors for G3 endometrioid adenocarcinoma. Most of the factors that we examined in this analysis proved to have a significant effect on the prognosis of endometrioid adenocarcinoma (1,15–17). However, we initially conjectured that the grade of differentiation might be so critical a prognostic factor that it could be expected that none of the other clinicopathological factors would be significant in our G3 case analysis. In fact, in the univariate analysis of clinicopathological prognostic factors in G3 endometrioid adenocarcinoma cases, adnexal metastasis and high pre-surgery CA19-9 values were the only factors that showed significantly low *P*-values for the 5-year survival. Of the other 10 factors, cervical involvement and high pre-surgery CA602 values showed relatively low *P*-values. Therefore, the absence of adnexal metastasis and cervical involvement, and low pre-surgery CA19-9 and CA602 values suggest relatively favorable prognosis in G3 endometrioid adenocarcinoma cases.

We examined the prognosis and the prognostic factors of G3 endometrioid adenocarcinoma. Although the prognosis of G2 and G3 cases was significantly poorer than that of G1, we could not find any critical difference between the G2 and G3 survival rates. We suggest that the high risk of late recurrence is one of the most significant features of G3 endometrioid adenocarcinoma. The univariate analysis of prognostic factors showed that the absence of adnexal metastasis and cervical involvement, and low pre-surgery CA19-9 and CA602 values had some favorable effect on the prognosis of G3 endometrioid adenocarcinoma.

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● 原 著 ●

進行子宮体癌に対するPaclitaxel, Doxorubicin, Cisplatin併用化学療法の臨床的検討

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〔*Jpn J Cancer Chemother* 31(4): 549-553, *April*, 2004〕

A Pilot Study of Combined Chemotherapy with Paclitaxel, Doxorubicin and Cisplatin for Endometrial Cancer: Hiroyuki Honma, Satoru Sagae, Katsuhiko Terasawa, Ryouichi Tanaka, Manabu Chida, Hisanobu Mizumoto, Shinichi Ishioka, Tsuyoshi Saito and Ryuichi Kudo (*Dept. of Obstetrics and Gynecology, School of Medicine, Sapporo Medical University*)

Summary

A pilot trial of combined chemotherapy with paclitaxel, doxorubicin and cisplatin was conducted in patients with advanced endometrial cancer. Between June 2000 and March 2002 8 patients were treated with combined chemotherapy, consisting of paclitaxel, 135mg/m^2; doxorubicin, 30mg/m^2; and cisplatin, 50mg/m^2 (TAP therapy). Patients received 3 to 5 courses of TAP therapy every 4 weeks. The major adverse effect was myelosuppression. All patients had grade 3 or 4 neutropenia, but did not have any severe infection with uncontrollable fever. Only 1 patient discontinued additional therapy due to grade 3 thrombocytopenia after 3 cycles. Grade 2 neurotoxicity occurred in 5 patients, but grade 3 was not observed. Among 5 patients with measurable tumors, 4 achieved partial response and 1 had no change of tumor size, indicating a response rate of 80.0%. We found that TAP therapy was feasible with G-CSF support and shows potential for high efficacy in advanced endometrial cancer.
Key words: Endometrial cancer, Paclitaxel, Doxorubicin, Cisplatin (*Received Feb. 26, 2003/Accepted Oct. 1, 2003*)

要旨 進行子宮体癌に対しpaclitaxel (PTX), doxorubicin (DXR), cisplatin (CDDP) 併用化学療法 (TAP療法) を行った。2000年6月から2002年3月までの間に当科で診断，治療した子宮体癌8症例を対象とした。組織型は類内膜腺癌5例，未分化型腺癌1例，漿液性腺癌1例，腺扁平上皮癌1例である。投与法はPTX 135 mg/m^2 (3時間)，CDDP 50 mg/m^2，DXR 30 mg/m^{2}をday 1に投与し4週ごとに3～5コース施行した。有害事象では顆粒球減少はgrade 3以上が全コースでみられたが，重篤な感染の合併はみられなかった。grade 3の血小板減少がみられた1例は3コースで治療中止となったが，残りの7症例については治療を完遂できた。また，PTX投与により生じる末梢神経障害はgrade 2が5例にみられた。抗腫瘍効果は，評価可能病変のある5例中4例でPR，漿液性腺癌の1例がNCであった。以上よりTAP療法は進行子宮体癌に対しG-CSF投与を必要とするが，安全に行うことが可能で，かつ効果も期待できる治療法と考えられた。今後，さらに症例を重ね多数の症例での臨床比較試験が必要である。

はじめに

子宮体癌は本邦において近年増加傾向にあり，早期癌は比較的予後良好といわれているが，進行癌は依然として予後不良である。そのため有用な寛解導入化学療法や術後補助化学療法が必要とされている。子宮体癌に対して単剤で有効とされている主な薬剤はcisplatin (CDDP), carboplatin, doxorubicin (DXR), epirubicin (EPI), paclitaxel (PTX) などがあり，多剤併用化学療法としてはcyclophosphamide (CPA), DXR, CDDPの3剤併用するCAP療法が最も広く行われており，31～56%と高い奏効率が確認されている[1]。しかし奏効期間が短く，生存期間の延長に必ずしも貢献しないなどの問題点も指摘されている[2]。

欧米では，術後の追加治療として化学療法よりも放射線療法が積極的に行われているが，どちらの治療がよいかという臨床的な疑問への明確な回答は得られていない。現在GOG #122[3]でIII, IV期進行子宮体癌に対する術

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表 1 症例

症例	年齢	Stage	手術	組織	Grade	転移
1	53	III a	MRH	Endometrioid	G 1	Ovary
2	47	IV b	TAH+BSO	Adenosquamous	G 3	Lung, liver
3	53	IV b	TAH+BSO	Undifferenciated	G 3	Vulva
4	72	IV b	MRH	Endometrioid	G 2	Liver
5	56	III c	MRH	Endometrioid	G 3	Pelvic lymphnode
6	43	III c	MRH	Serous papillary	G 3	Para-Aortic lymphnode
7	48	I b	RH	Endometrioid	G 3	—
8	53	III a	MRH	Endometrioid	G 3	Ovary

RH: radical hysterectomy, MRH: modified radical hysterectomy, TAH+BSO: total abdominal hysterectomy+bilateral salpingo-oophorectomy

後放射線療法（全骨盤照射）と化学療法（DXR 60 mg/m²+CDDP 50 mg/m²）についての無作為比較試験が行われており，この結果が待たれるところである（ASCO 2003 にて発表予定）。

近年，PTX を含む多剤併用化学療法が子宮体癌でもいくつか報告されている。藤田ら[4]は PTX+EPI+carboplatin が 5 例中 5 例に有効とし，Fleming ら[5]は PTX+DXR+CDDP（以下 TAP 療法）で 46.2%の奏効率を報告した。また，現在 GOG # 177[6]では III，IV期または再発子宮体癌症例において AP 群（DXR 60 mg/m²+CDDP 50 mg/m²）と TAP 群（DXR 60 mg/m²+CDDP 50 mg/m²+PTX 160 mg/m²）の比較が行われている。また，GOG # 184[7]では子宮全摘出術＋両側付属器摘出術を受けた III，IV期進行子宮体癌症例において，全骨盤照射後に AP 群（DXR 45 mg/m²+CDDP 50 mg/m²）または TAP 群（DXR 45 mg/m²+CDDP 50 mg/m²+PTX 160 mg/m²）を追加する第III相試験が進行中である。

以上の報告をもとに，われわれは進行子宮体癌症例に対し TAP 療法の pilot 試験を計画し，これまで 8 症例に施行した。今回，これらの症例に対する副作用と臨床的効果を報告する。

I．対象と方法

2000 年 6 月から 2002 年 3 月までの間に当科で診断，治療した子宮体癌 8 症例を対象とした。表 1 に全症例の特徴を示す。

わが国における婦人科癌化学療法共同研究会の子宮体癌に対する，5-FU と CAP 療法の第III相比較試験での CAP 療法の用量は CPA 500 mg/m²，DXR 30～40 mg/m²，CDDP 50～60 mg/m² と設定されている。また，GOG # 111[8]において CPA (750 mg/m²)＋CDDP と PTX (135 mg/m²)＋CDDP の第III相比較試験が行われた。以上の用量を参考に，今回われわれは PTX 135 mg/m²，DXR 30 mg/m²，CDDP 50 mg/m² と用量を設定した。症例の

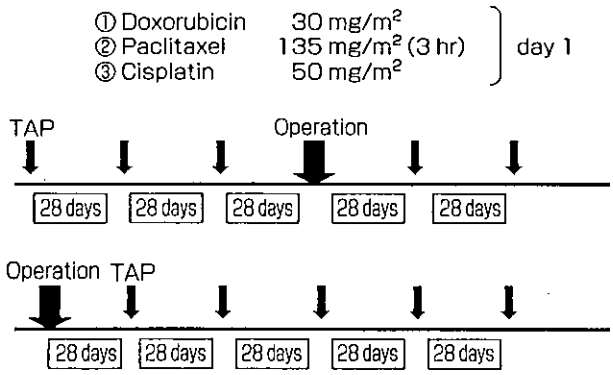

図 1 治療法

年齢は 43～72 歳（平均 51 歳），performance status はすべて 0，1 であり，いずれも肝，腎機能に異常なく，骨髄機能の低下なども認められていない。

手術による臨床進行期は I b 期 1 例，IIIa 期 2 例，IIIc 期 2 例，IVb 期 3 例であった。I b 期の 1 例は，子宮頸部腺癌の診断で TAP 療法を術前化学療法として 3 コース施行後手術を行い，術後病理組織診断にて子宮体部からの頸部浸潤と判断され子宮体癌の最終診断となった症例である。

組織型は類内膜腺癌が 5 例，未分化型腺癌が 1 例，漿液性腺癌が 1 例，腺扁平上皮癌 1 例だった。

1．治療内容

初回治療として手術的治療が困難な進行症例に対しては，術前化学療法として TAP 療法（PTX 135 mg/m²（3 時間），DXR 30 mg/m²，CDDP 50 mg/m² day 1）を 4 週ごとに 3 コース行い，術後 1 か月以内に追加治療としてさらに 1～2 コース行った。初回手術可能症例においては，術後 1 か月以内に TAP 療法を 3～5 コース行った（図 1）。

2．評価方法

治療効果の判定は，日本癌治療学会の婦人科がん化学療法の直接効果判定基準[9]に基づいて，副作用に関しては日本癌治療学会薬物有害判定基準に基づいて判定された。

表 2 抗腫瘍効果

症例	術後診断	治療回数	効果	測定可能病変-効果	全奏効期間（月）	転帰
2	pT3aN1M1	3(術前)+2(術後)	PR	Lung-CR, Liver-PR, Primary Carcinoma-NC	5	DOD
3	pT3aN1M1	3(術前)+2(術後)	PR	Vulva-CR, Primary Carcinoma-PR	8.3	DOD
4	pT2aN0M1	5 (術後)	PR	Liver-PR, Primary Carcinoma-NC	7.6	DOD
6	pT3aN1M0	5 (術後)	NC	Lymph node-NC, (CA 125-CR)	7.1*	AWD
7	pT1bN0M0	3(術前)+2(術後)	PR	Primary Carcinoma-PR	16.4+	NED

NED: no evidence of disease, AWD: alive with disease, DOD: die of disease, *: CA 125

表 3 副作用

	grade					grade≧3
Toxicity	0	1	2	3	4	%of patients
Vomitting	1	2	5	0	0	0%
Diarrhea	5	3	0	0	0	0%
Alopecia	0	2	6	—	—	0%
Fatigue	0	4	4	0	0	0%
Neurotoxicity	1	2	5	0	—	0%
Hemoglobin	0	3	3	2	—	25%
Granulocytes	0	0	0	3	5	100%
Platelets	6	1	0	1	0	13%
Infection	5	2	1	0	0	0%

II. 結　果

治療効果と転帰について表2に示す。全8症例のうち3症例は術後補助化学療法症例であり効果判定病変がなく，残りの5症例について治療効果判定を行った。その結果，測定可能病変を有し画像による治療効果判定可能な5例中4例がPRであり，1例がNCであり，奏効率は80.0%であった。ちなみにNCの1例の組織型は漿液性腺癌だった。また，全奏効期間は手術と化学療法を合わせての奏効期間を示した。このうち手術前投与症例を具体的に説明すると，以下のごとくである。

症例2は，入院時にみられた癌性胸膜炎，胸水，肺転移の所見もTAP療法2コース後にはほぼ消失した。肝転移も2コース終了時には50%以上の縮小を認め，3コース終了時にはほぼ消失，原発巣は3コース終了時にはMRI上65%程度の縮小を認め，以上の所見より総合的にPRと判断した。その後，3コース終了後に原発巣の縮小目的に腹式子宮全摘出術＋両側付属器摘出術施行。しかし，術後TAP療法1コース終了後に脳転移を来しそれが増悪したため，他院にてガンマナイフなどの治療を行ったが腫瘍のコントロールできず，TAP療法開始後8か月で亡くなった。

症例3は外陰部への転移を認めIVb期と診断された。TAP療法2コース終了時には外陰部の転移は消失，原発巣も50%以上縮小し，手術前よりPRの状態であった。3コース終了後に開腹手術にて原発巣が切除できたが，術後TAP療法2コース終了時に肝転移出現し，治療法を変更し化学療法を継続したが効果がみられず原癌死となった。

また，症例7は子宮頸部腺癌Ib2期，類内膜腺癌の診断にて，術前にTAP療法3コースを行った。2コース終了時にはMRI上原発巣の50%以上の縮小を認め，3コース終了時には画像上では腫瘍は消失したため，広汎子宮全摘術を施行したところ，術中所見では肉眼的に病変を認めなかった。しかし，術後病理組織診断にて子宮体癌の頸部浸潤と診断され，さらに子宮頸部間質内に15×5 mmの残存腫瘍を認めたためPRと判断した。

術後補助化学療法を施行した4症例については，stage IVbである症例4は原癌死となったが，残りの3症例については現在も再発兆候を認めていない。

次に副作用について表3に示す。主な副作用は骨髄抑制であり，他に脱毛，嘔吐などは必発であった。顆粒球減少については，全症例でG-CSF製剤を必要としたが重篤な感染症の合併はみられなかった。症例8ではコースごとのgrade 4の顆粒球減少に加え，grade 3の血小板減少も出現し，3コースで投与中止となった。表4に全症例におけるコースごとの顆粒球減少の推移を示す。術前投与例，術後投与例，ともに連続した化学療法による明らかな副作用の蓄積は認められないが，全37コース中でのgrade 3以上の副作用出現の割合は92%と高率に