A New Method for Isolating Colonocytes From Naturally Evacuated Feces and Its Clinical Application to Colorectal Cancer Diagnosis

HISAYUKI MATSUSHITA,* YASUHIRO MATSUMURA,* YOSHIHIRO MORIYA,† TAKAYUKI AKASU,† SHIN FUJITA,† SEIICHIRO YAMAMOTO,† SHIGEKI ONOUCHI,† NORIO SAITO,§ MASANORI SUGITO,§ MASAAKI ITO,§ TAKAHIRO KOZU,¶ TAKASHI MINOWA,‖ SAYURI NOMURA,‖ HIROYUKI TSUNODA,# and TADAO KAKIZOE**

*Investigative Treatment Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa; †Department of Surgery, National Cancer Center Hospital, Tokyo; §Department of Surgery, National Cancer Center Hospital East, Kashiwa; ¶Cancer Screening Division, National Cancer Center Research Center for Cancer Prevention and Screening, Tokyo; ‖Hitachi, Ltd., Life Science Group, Saitama; #Hitachi, Ltd., Advanced Research Laboratory, Tokyo; and **National Cancer Center, Tokyo, Japan

Reprinted from
GASTROENTEROLOGY
Volume 129, Number 6, December 2005

GASTROENTEROLOGY 2005;129:1918–1927

A New Method for Isolating Colonocytes From Naturally Evacuated Feces and Its Clinical Application to Colorectal Cancer Diagnosis

HISAYUKI MATSUSHITA,* YASUHIRO MATSUMURA,* YOSHIHIRO MORIYA,† TAKAYUKI AKASU,† SHIN FUJITA,† SEIICHIRO YAMAMOTO,† SHIGEKI ONOUCHI,† NORIO SAITO,§ MASANORI SUGITO,§ MASAAKI ITO,§ TAKAHIRO KOZU,¶ TAKASHI MINOWA,‖ SAYURI NOMURA,‖ HIROYUKI TSUNODA,# and TADAO KAKIZOE**

*Investigative Treatment Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa; †Department of Surgery, National Cancer Center Hospital, Tokyo; §Department of Surgery, National Cancer Center Hospital East, Kashiwa; ¶Cancer Screening Division, National Cancer Center Research Center for Cancer Prevention and Screening, Tokyo; ‖Hitachi, Ltd., Life Science Group, Saitama; #Hitachi, Ltd., Advanced Research Laboratory, Tokyo; and **National Cancer Center, Tokyo, Japan

Background & Aims: **The early detection of colorectal cancer is desired because this cancer can be cured surgically if diagnosed early. The purpose of the present study was to determine the feasibility of a new methodology for isolating colonocytes from naturally evacuated feces, followed by cytology or molecular biology of the colonocytes to detect colorectal cancer originating from any part of the colorectum.** *Methods:* **Several simulation studies were conducted to establish the optimal methods for retrieving colonocytes from any portion of feces. Colonocytes exfoliated into feces, which had been retrieved from 116 patients with colorectal cancer and 83 healthy volunteers, were analyzed. Part of the exfoliated colonocytes was examined cytologically, whereas the remainder was subjected to DNA analysis. The extracted DNA was examined for mutations of the APC, K-*ras*, and p53 genes using direct sequence analysis and was also subjected to microsatellite instability (MSI) analysis.** *Results:* **In the DNA analysis, the overall sensitivity and specificity were 71% (82 of 116) of patients with colorectal cancer and 88% (73 of 83) of healthy volunteers. The sensitivity for Dukes A and B was 72% (44 of 61). Furthermore, the sensitivity for cancers on the right side of the colon was 57% (20 of 35). The detection rate for genetic alterations using our methodology was 86% (80 of 93) when the analysis was limited to cases in which genetic alterations were present in the cancer tissue.** *Conclusions:* **We have developed a new methodology for isolating colonocytes from feces. The present study describes a promising procedure for future clinical evaluations and the early detection of colorectal cancers, including right-side colon cancer.**

Colorectal cancer is one of the most common malignancies worldwide. In Japan, colorectal cancer is the third and second leading cause of death from cancer in men and women, respectively.[1] However, colorectal cancer is curable by surgical resection if diagnosed at a sufficiently early stage. This incentive has prompted investigators to develop new methods enabling the early diagnosis of colorectal cancer and has led to the introduction of cancer screening programs in many countries. For mass cancer screenings, a simple, economic, and noninvasive method of cancer detection is desired. The Hemoccult test is currently used in many countries for this purpose.[2–6] However, this test is nonspecific and is not sufficiently sensitive to detect early stage colorectal cancer, although a higher sensitivity has been reported for advanced-stage colorectal cancer.[7] Radioimmunoassays using tumor markers, such as carcinoembryonic antigen, also are not suitable for the detection of early cancer, although such tests can be used to monitor patients for an increasing tumor burden or tumor recurrence. Diagnosis by barium enema study and fiberoptic colonoscopy is accurate but time-consuming, expensive, and invasive. Therefore, an urgent need exists to establish a sensitive, reliable, and noninvasive method for the detection of colorectal cancer at an early stage.

To date, several screening methods for colorectal cancer based on the detection of mutated DNA in feces have been reported.[8–20] These methods, however, are time-consuming and are not sufficiently sensitive. The major reason for this inaccuracy is the fact that

Abbreviations used in this paper: APC, adenomatous polyposis coli; MSI, microsatellite instability; OMIM, Online Mendelian Inheritance in Man.

0016-5085/05/$30.00
doi:10.1053/j.gastro.2005.10.007

nucleic acids in feces are derived from an enormous number and variety of bacteria and normal cells. Accordingly, the proportion of genes derived from cancer cells in feces is as low as 1%, at most.[9] This makes the application of gene-detecting methods difficult in clinical practice.

We previously reported that the expression of CD44 variants in exfoliated colonocytes isolated from feces according to the Percoll centrifugation method could serve as a noninvasive diagnostic marker for early colorectal cancer.[21] However, the repetition of the Percoll centrifugation method was found to distort the morphology of the exfoliated colonocytes. Accordingly, the sensitivity of this method also appeared to be unsatisfactory because of the low retrieval rate of the exfoliated colonocytes. Another study described a processing method that involved scraping or washing the stool's surface with a buffer to collect exfoliated colonocytes.[22] In the ascending colon, however, the feces remains unformed. Therefore, most cancer cells exfoliated from the walls of the ascending colon would be incorporated into the inner core of the feces during the course of its formation. Thus, recovering cancer cells that originated from the ascending colon might be difficult using methods that involve scraping or washing solid feces.

Under these circumstances, we succeeded in developing a new, very effective methodology that allows the simple isolation of exfoliated colonocytes from not only the surface but also the central portion of feces while maintaining the colonocytes' initial morphology. Currently, we are attempting to apply a molecular biologic tool to purified colonocytes exfoliated into feces to detect cells from early colorectal cancers, including right-side colon cancer.

Materials and Methods

Study Design

This was a prospective study conducted between December 2002 and August 2004. The study protocol was reviewed and approved by the Institutional Review Board of the National Cancer Center, Japan. Written informed consent was obtained from all patients and healthy volunteers. No modifications to the protocol procedures were made during the course of the study.

Study Population

A total of 116 patients with histologically confirmed colorectal cancer and 83 healthy volunteers were enrolled. The healthy volunteers consisted of 37 men and 46 women with no apparent abnormalities, such as adenoma or carcinoma (including hyperplastic polyps), found during a total colonoscopy performed at the National Cancer Center Research Center for Cancer Prevention and Screening. The median age of these volunteers was 58.4 years (range, 40–70 years). The characteristics of the patients and healthy volunteers are summarized in Table 1. All the patients with colorectal cancer had undergone surgical resection of their primary tumor at the National Cancer Center Hospital, Tsukiji, or at Hospital East, Kashiwa, Japan. The median age of the patients was 62.0 years (range, 32–82 years). There were 69 men and 47 women patients. The primary tumors were located in the following sites: rectum in 53 patients, sigmoid colon in 21 patients, descending colon in 7 patients, transverse colon in 6 patients, ascending colon in 23 patients, and cecum in 6 patients. The clinical stage of the patients according to Dukes' classification was as follows: Dukes' stage A in 30 patients, stage B in 31 patients, stage C in 53 patients, and stage D in 2 patients.

Table 1. Characteristics of Patients and Healthy Volunteers

Characteristic	Patient (N = 116)	Healthy volunteer (N = 83)
Age, *y*		
Mean	62.0	58.4
Range	32–82	40–70
Sex, no (%)		
Male	69 (59.5)	37 (44.6)
Female	47 (40.5)	46 (55.4)
DNA, ng/gram of stool		
Mean	570.8	175.3
Range	2.0–7462.8	0.2–1907.5
Tumor location, no (%)		
Cecum	6 (5.2)	
Ascending colon	23 (19.8)	
Transverse colon	6 (5.2)	
Descending colon	7 (6.0)	
Sigmoid colon	21 (18.1)	
Rectum	53 (45.7)	
Size, mm		
Mean	40.0	
Range	4.0–120.0	
Histology, no (%)		
W/D	55 (47.4)	
M/D	56 (48.3)	
P/D	2 (1.7)	
Mucinous carcinoma	2 (1.7)	
Carcinoid tumor	1 (0.9)	
Depth, no (%)		
T1	10 (8.6)	
T2	32 (27.6)	
T3	71 (61.2)	
T4	3 (2.6)	
Dukes' stage, no (%)		
A	30 (25.9)	
B	31 (26.7)	
C	53 (45.7)	
D	2 (1.7)	

W/D, Well-differentiated adenocarcinoma; M/D, moderately differentiated adenocarcinoma; P/D, poorly differentiated adenocarcinoma.

Stool Samples

Before surgical resection, stool samples were obtained from 116 patients with colorectal cancer. Stool sam-

ples were also obtained from 83 healthy volunteers a few weeks after they had undergone a total colonoscopy. Naturally evacuated feces from subjects who had not taken laxatives were used as stool samples. Each patient was instructed to evacuate into a polystyrene disposable tray (AS one, Osaka, Japan) measuring 5 × 10 cm in size at home and bring the sample to the reception counter at the outpatient clinic or the Cancer Prevention and Screening Center of the National Cancer Center. The samples were collected and transferred to a laboratory at which they were allowed to stand at room temperature. Preparation of the stool samples for examination was conducted within 1–6 hours after the evacuation.

Magnetic Beads

Dynabeads Epithelial Enrich are uniform, superparamagnetic, polystyrene beads (4.5-μm diameter) coated with a mouse IgG1 monoclonal antibody (mAb Ber-EP4) specific for the glycopolypeptide membrane antigen Ep-CAM, which is expressed on most normal and neoplastic human epithelial tissues (Dynal, Oslo, Norway). Ep-CAM is widely expressed in the highly proliferative cells of the intestinal epithelium, from the basal cells to cells throughout the crypts at the basolateral membranes, and only the apical membrane facing the lumen is negative. The development of adenomas has been reported to be associated with increased Ep-CAM expression, and Ep-CAM over expression (mAb GA733) has frequently been demonstrated in colorectal carcinomas.[23–25]

Simulation Studies

A series of simulation studies were conducted to establish the optimal conditions for retrieving HT-29 colorectal cancer cells from feces. Feces from healthy volunteers were divided into several portions, each of which was seeded with 100 μL HT-29 cells (1 × 10^6/approximately 5 g feces). The cells were retrieved under several different conditions as follows: use of a Hank's solution and 25 mmol/L Hepes buffer (pH 7.35); processed feces of 5, 10, or 30 g volume; filter with a pore size of 48, 96, 512, or 1000 μm; incubation of homogenized solution with magnetic beads at 4°C or room temperature; application of 20, 40, 80, 200, or 400 μL magnetic beads; incubation of homogenized solution with magnetic beads under gentle rolling at 15 rounds/minute in a mixer for 10, 20, 30, or 40 minutes; and the reaction time between the cell-magnetic bead complexes and a magnet on a shaking platform for 0, 2, 10, 20, 30, 40, 50, or 60 minutes. Finally, the cell retrieval rate calculated for the magnetic beads method under the conditions determined to be the most suitable for this simulation study was compared with that calculated for the Percoll centrifugation method. The retrieval rate was calculated by dividing the number of cells that bound to the retrieved beads by the number of cells initially added to the feces. The cells were counted using a NucleoCounter (ChemoMetec A/S, Allerød, Denmark).

Isolation of Exfoliated Cells From Feces

The procedure was conducted using the most suitable and optimal conditions determined by the simulation study (Figure 1). Approximately 5–10 g of naturally evacuated feces were used to isolate exfoliated cells. Feces were collected into Stomacher Lab Blender bags (Seward, Thetford, United Kingdom). The stool samples were homogenized with a buffer (200 mL) consisting of Hank's solution, 10% fetal bovine serum (FBS), and 25 mmol/L Hepes buffer (pH 7.35) at 200 rpm for 1 minute using a Stomacher (Seward). The homogenates were then filtered through a nylon filter (pore size, 512 μm), followed by division into 5 portions (40 mL each). Subsequently, 40 μL of magnetic beads were added to each homogenized solution portion, and the mixtures were incubated for 30 minutes under gentle rolling in a mixer at room temperature. The samples on the magnet were then incubated on a shaking platform for 15 minutes at room temperature. Colonocytes isolated from 5 tubes were smeared onto slides and then stained using the Papanicolaou method. The remainder of the samples was centrifuged, and the sediments were stored at −80°C until DNA extraction.

Extraction of DNA

Fresh tissue samples were obtained from the surgically resected specimens of 116 patients with colorectal cancer. The samples were snap frozen in liquid nitrogen within 20 minutes of their arrival at the pathologic specimen reception area and were stored in liquid nitrogen until analysis.

Genomic DNA was extracted from each tumor tissue specimen using a DNeasy kit (QIAGEN, Valencia, CA). Genomic DNA was also extracted from colonocytes isolated from feces using the SepaGene kit (Sanko-Junyaku, Tokyo, Japan).

Direct Sequence Analysis

Direct sequencing was conducted to identify mutations in the APC codon 1270–1594, in codons 12 and 13 of the K-*ras* gene, and in exons 5, 6, 7, and 8 of the p53 gene.

The PCR primers used in this study were as follows: APC (5′-AAACACCTCAAGTTCCAACCAC-3′, 5′-GGTAATTTTGAAGCAGTCTGGGC-3′); K-*ras* (5′-CTGGTGGAGTATTTGATAGTG-3′, 5′-CCCAAGGAAAGTAAAGTTC-3′); p53 exon 5 (5′-GCCGTCTTCCAGTTGCTTTAT-3′, 5′-CCAAATACTCCACACGCAAAT-3′); p53 exon 6 (5′-CATGAGCGCTGCTCAGATAG-3′, 5′-TGCACATCTCATGGGGTTATAG-3′); p53 exon 7 (5′-CTTGGGCCTGTGTTATCTCCTA-3′, 5′-AAGAAAACTGAGTGGGAGCAGT-3′); and p53 exon 8 (5′-ACCTCTTAACCTGTGGCTTC-3′, 5′-TACAACCAGGAGCCATTGTC-3′).

The sequence primers used in this study were as follows: APC (5′-CAAAAGGCTGCCACTTGCAAAG-3′, 5′-AAAATAAAGCACCTACTGCTG-3′, 5′-GAATCAGCCAGGCACAAAGC-3′); K-*ras* (5′-CTGGTGGAGTATTTGATAGTG-3′); p53 exon 5 (5′-CCAAATACTCCACACGCAAAT-3′); p53 exon 6 (5′-CATGAGCGCTGCTCAGATAG-3′); p53 exon 7 (5′-AAGAAAACTGAGTGGGAGCAGT-3′); and p53 exon 8 (5′-

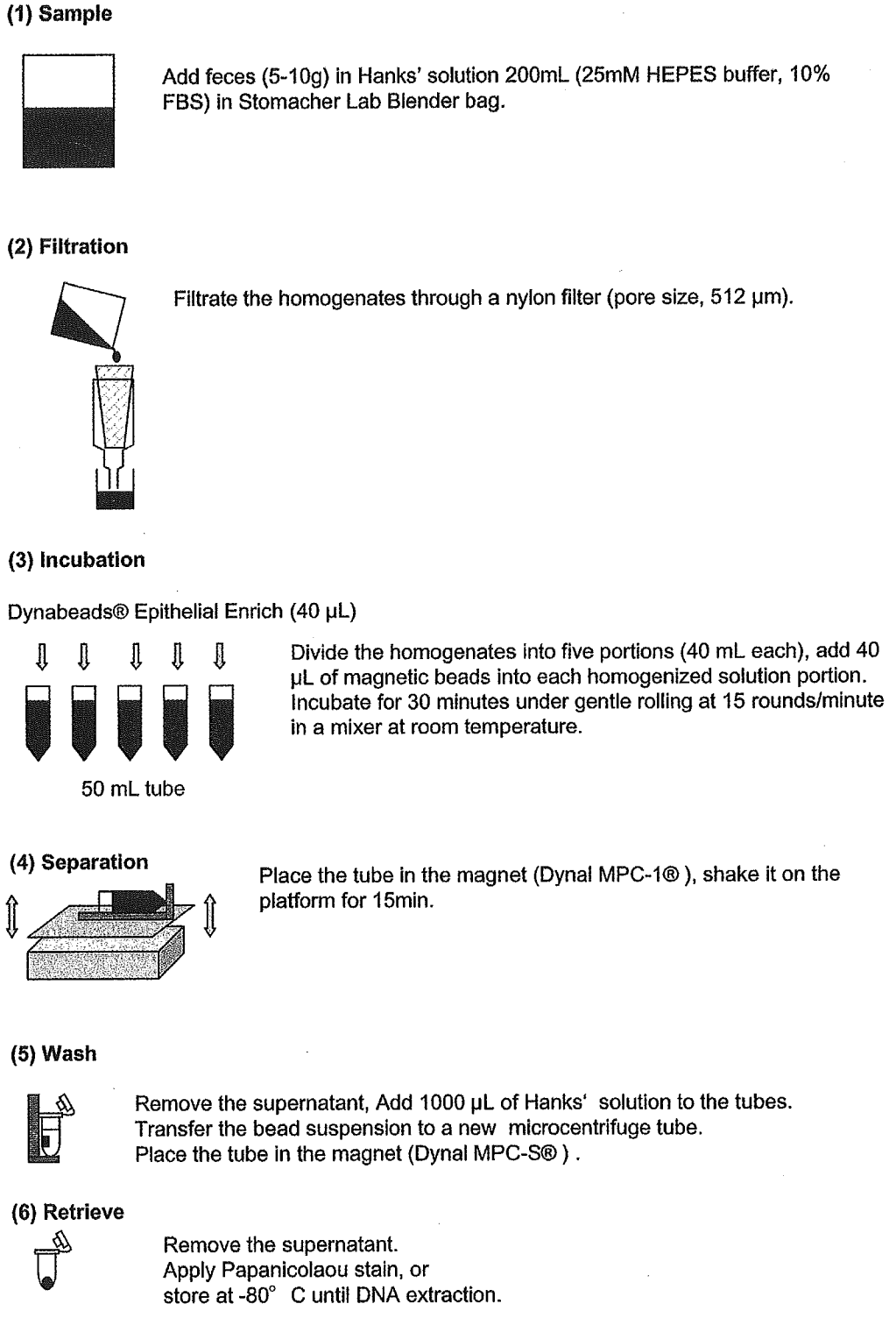

Figure 1. Schematic of procedure for isolating colonocytes from feces.

ACCTCTTAACCTGTGGCTTC-3′). Each fragment was sequenced by direct sequencing using the Big Dye Terminator v 3.1/1.1 cycle kit (Applied Biosystems, Forester City, CA).

All obtained sequences were aligned with previously published sequences (National Center for Biotechnology Information [NCBI] Genbank accession No. M74088 [APC], M54968 [K-*ras*], and X54156 [p53]) for each of the target genes and were analyzed using Phred/Phrp/DNASIS pro (Hitachi Software Engineering, Tokyo, Japan). The presence and nature of each mutation were confirmed by repeated PCR and sequencing.

BAT26

The BAT26 gene, an indicator of microsatellite instability (MSI), was amplified by PCR. Each fragment was elec-

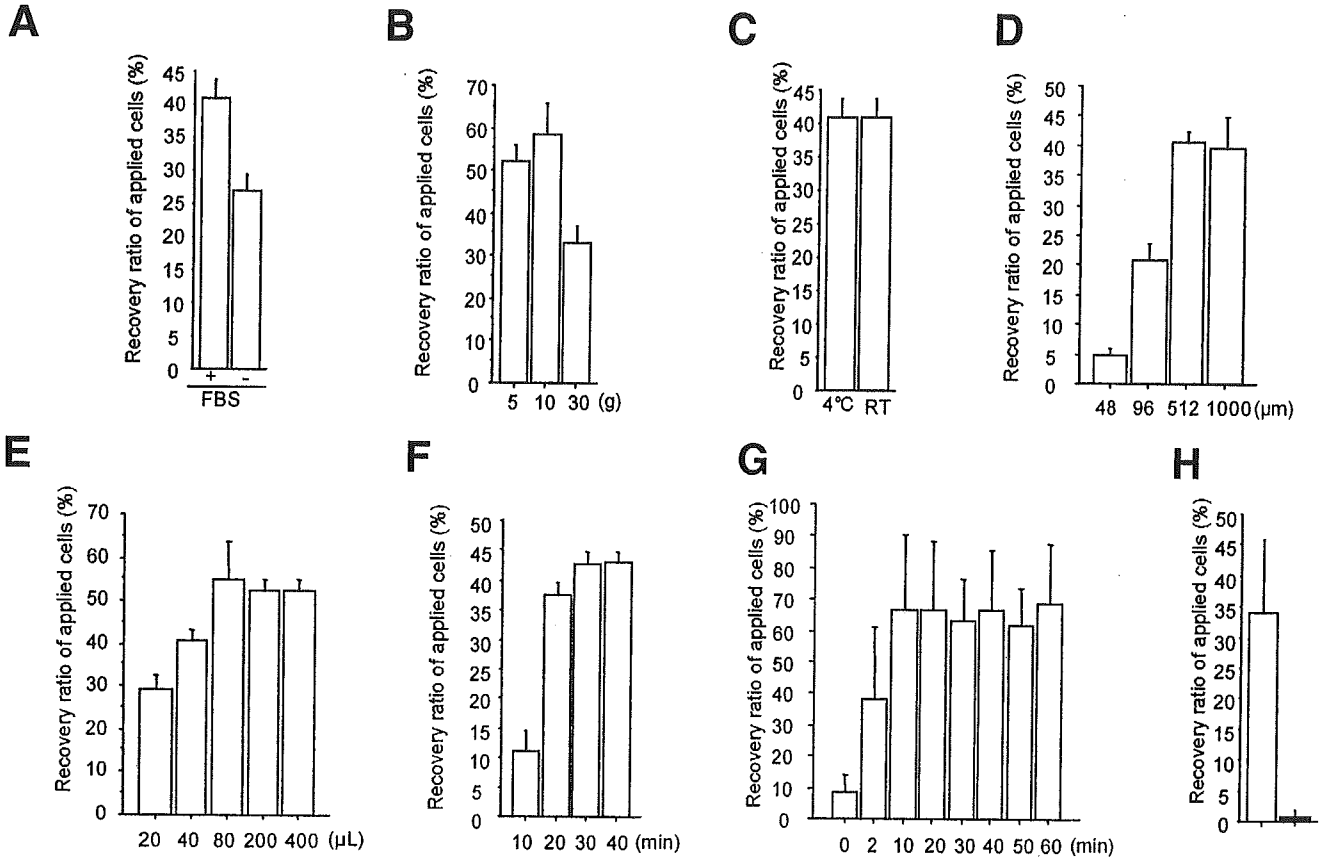

Figure 2. Simulation study to establish the optimal conditions for retrieving HT-29 colorectal cancer cells from feces and to compare the cell retrieval rates for the magnetic beads methods and the Percoll centrifugation method. Feces from healthy volunteers were divided into several portions, each of which was seeded with 100 μL HT-29 colorectal cancer cells (1×10^6/approximately 5 grams of feces). The procedure for retrieving the HT-29 cells was conducted under various conditions as follows: (*A*) homogenizing buffer with or without FBS; (*B*) stool weight (5, 10, or 30 g); (*C*) temperature during the cell-yielding procedure (4°C or room temperature); (*D*) filter pore size (48, 96, 512, or 1000 μm); (*E*) volume of applied magnetic beads (20, 40, 80, 200, or 400 μL); (*F*) incubation time of the homogenized solution with the magnetic beads under gentle rolling in a mixer (10, 20, 30, or 40 minutes); and (*G*) reaction time for the cells-magnetic bead complexes and the magnet on the shaking platform (0, 2, 10, 20, 30, 40, 50, or 60 minutes). The cell retrieval ratio (%) was calculated using the following formula: 100 × number of HT-29 cells retrieved/number of applied HT-29 cells. (*H*) Comparison of cell retrieval rates for the magnetic beads methods (*open column*) and the Percoll centrifugation method (*solid column*).

trophoresed using an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and then analyzed by GeneScan v 3.7 (Applied Biosystems). The PCR primers used in this study were 5′-TGACTACTTTTGACTTCAGCC-3′ and 5′-AACCATTCAACATTTTTAACCC-3′.

Cytology

Colonocytes isolated from feces were examined by 2 experienced cytotechnologists after Papanicolaou staining.

Study Blinding

We followed the guidelines of our medical institution for preparing blinded samples. Technicians processed the stool samples and prepared the slides for cytology and the cell pellets for DNA extraction. The samples were blinded to prevent the identification of individuals and the samples' origins. Two cytologists assessed the blinded samples, and the Life Science Group of Hitachi, Ltd, analyzed the DNA sequences.

Statistical Analysis

A Fisher exact test was used to compare all proportions. All reported P values are 2-sided. A value of $P < .05$ was considered statistically significant.

Results

Simulation Studies

The cell retrieval rate was found to decrease when Hank's solution without FBS was used, thus indicating the effectiveness of adding serum to the homogenizing buffer (Figure 2*A*). The cell retrieval rate was found to decrease when more than 30 g of feces were processed (Figure 2*B*). The cell retrieval rates were similar when incubation was conducted at room temperature and at 4°C (Figure 2*C*). Filtering of the stool suspension with the 48- or 96-μm filter resulted in significant clogging and thus hampered cell retrieval. However, a lot of fecal

residue remained after filtering with the 1000-μm filter, hindering the handling of the stool suspension thereafter. We therefore decided to use the 512-μm filter (Figure 2*D*). The dose of the magnetic beads applied was also examined. The cell retrieval rate increased in a dose-dependent manner up to 80 μL. In reality, a sufficient amount of genomic DNA derived from exfoliated colonocytes was obtained, even when 40 μL of magnetic beads were used (Figure 2*E*). Regarding the optimal incubation time of the magnetic beads for the complete binding of HT-29 cells to the beads, 30 minutes of incubation was found to be sufficient for the satisfactory binding of HT-29 cells to the beads (Figure 2*F*). For the retrieval of the cell-magnetic bead complexes on the magnet, a 10-minute reaction period was sufficient (Figure 2*G*).

The cell retrieval rates were 0.8% and 33.5% using the Percoll centrifugation method and the magnetic beads method, respectively, thus underscoring the advantage of the magnetic beads method (Figure 2*H*).

Cytology

Atypical cells were observed in colonocytes isolated from the feces of 32 of 116 patients with colorectal cancer, with a sensitivity rate of 28% (95% CI: 20–37; Table 2, Figure 3*A* and 3*B*). No atypical cells were observed in any of the 83 healthy volunteers, with a specificity rate of 100% (95% CI: 96–100). A significant difference ($P < .0001$) was found in the positivity rate between the patient group and the healthy volunteer group. The sensitivity rates for Dukes' A, B, and C or D colorectal cancers were 23% (7 of 30; 95% CI: 10–42), 32% (10 of 31; 95% CI: 17–51), and 27% (15 of 55; 95% CI: 16–41), respectively. No significant differences in the positivity rates were found among any of the stages. Furthermore, the sensitivity rates for cancers on the right side of the colon, including the cecum, ascending colon, and transverse colon, and for those on the left side of the colon, including the descending colon, sigmoid colon, and rectum, were 9% (3 of 35; 95% CI: 2–23) and 36% (29 of 81; 95% CI: 25–47), respectively. Therefore, the positivity rate was significantly higher for cancers on the left side of the colon ($P < .01$).

DNA Analysis

Overall analysis of stool samples. Sequence analysis showed distinct mutations in each of the analyzed genes in the tumor tissue and colonocytes isolated from feces (Figure 3*C–F*). Genetic alterations were observed in the colonocytes isolated from the feces of 82 of the 116 patients with colorectal cancer, yielding a sensitivity rate of 71% (95% CI: 62–79; Table 2). However, 10 of the 83 healthy volunteers were also positive for genetic alterations, producing a specificity value of 88% (95% CI: 79–94). A significant difference ($P < .0001$) was noted in the positivity rates of the patient group and the healthy volunteer group.

Genetic alterations were observed in 18 of the 30 patients with Dukes' A colorectal cancer, yielding a sensitivity rate of 60% (95% CI: 41–77). Furthermore, genetic alterations were observed among 26 of the 31 patients with Dukes' B colorectal cancer (84%; 95% CI: 66–95) and 38 of the 55 patients with Dukes' C or D colorectal cancer (69%; 95% CI: 55–81). No significant difference in sensitivity was found among any of the stages.

Genetic alterations were observed in colonocytes isolated from feces in 20 out of 35 patients with cancers originating on the right side of the colon (57%; 95% CI: 39–74) and in 62 out of 81 patients with cancers originating on the left side of the colon (77%; 95% CI: 66–85). No significant differences in the sensitivity rates were observed, although the sensitivity rate tended to be higher for cancers on the left side of the colon.

DNA analysis limited to colonocytes isolated from the feces of patients with colorectal cancer tissue involving genetic alterations. We assessed the performance of the present methodology for isolating cancer cells by examining the positivity rate of genetic alterations in colonocytes isolated from the feces of patients who showed alterations in their cancer tissues (Table 3). Among the 116 patients, a total of 93 (80%; 95% CI: 72–87) exhibited genetic alterations in the APC, K-*ras*, or p53 genes or BAT26 positivity in their cancer tissue: 51 patients exhibited APC mutations (44%, 95% CI: 35–53), 33 patients exhibited K-*ras* mutations (28%; 95% CI: 20–38), 62 patients exhibited p53 mutations (53%; 95% CI: 44–63), and 6 patients exhibited BAT26 positivity (5%; 95% CI: 2–11). Among the 93 patients with genetic alterations in their cancer tissues, the alterations were also successfully detected in colonocytes isolated from the feces of 80 patients (86%; 95% CI: 77–92). Among the 39 patients with Dukes' C or D advanced cancer who exhibited a genetic alteration in their cancer tissues, 36 patients exhibited genetic alterations in colonocytes isolated from their feces (92%; 95% CI: 79–98). Furthermore, genetic alterations were detected in colonocytes isolated from the feces of 18 of 24 patients with Dukes' A cancer (75%; 95% CI: 53–90) and 26 of 30 patients with Dukes' B cancer (87%; 95% CI: 69–96). No statistically significant difference was found among these 3 groups. In addition, genetic alterations could be detected in colonocytes isolated from the feces of 20 of 27 patients with cancers originating on the

Table 2. Incidences of Genetic Alterations of the APC, K-*ras*, p53, and MSI (BAT26) Genes as Well as Results From Cytology in all Patients and Healthy Volunteers

		Patient				Healthy volunteer	
		Tumor tissue		Isolated cell		Isolated cell	
	Marker	No.	Positivity (%) (95% CI)	No.	Sensitivity (%) (95% CI)	No.	Specificity (%) (95% CI)
Overall	Combined marker	93	80 (72–87)	82	71 (62–79)	10	88 (79–94)
Patients (n = 116), healthy volunteers (n = 83)	APC	51	44 (35–53)	47	41 (32–50)	1	99 (93–100)
	K-*ras*	33	28 (20–38)	33	28 (20–38)	1	99 (93–100)
	p53	62	53 (44–63)	45	39 (30–48)	6	93 (85–97)
	BAT26	6	5 (2–11)	4	3 (1–9)	3	96 (90–99)
	Cytology			32	28 (20–37)	0	100 (96–100)
Dukes' stage A (n = 30)	Combined marker	24	80 (61–92)	18	60 (41–77)		
	APC	14	47 (28–66)	11	37 (20–56)		
	K-*ras*	6	20 (77–39)	5	17 (6–35)		
	p53	6	20 (77–39)	9	30 (15–49)		
	BAT26	1	3 (1–17)	1	3 (1–17)		
	Cytology			7	23 (10–42)		
Dukes' stage B (n = 31)	Combined marker	30	97 (83–100)	26	84 (66–95)		
	APC	17	55 (36–73)	17	55 (36–73)		
	K-*ras*	10	32 (17–51)	9	29 (14–48)		
	p53	18	58 (39–75)	13	42 (25–61)		
	BAT26	2	6 (1–21)	1	3 (1–17)		
	Cytology			10	32 (17–51)		
Dukes' stages C and D (n = 55)	Combined marker	39	71 (57–82)	38	69 (55–81)		
	APC	20	36 (24–50)	19	35 (22–49)		
	K-*ras*	17	31 (19–45)	19	35 (22–49)		
	p53	27	49 (35–63)	23	42 (29–56)		
	BAT26	3	5 (1–15)	2	4 (0–13)		
	Cytology			15	27 (16–41)		
Right-sided colon cancer (n = 35)	Combined marker	27	77 (60–90)	20	57 (39–74)		
	APC	11	31 (17–49)	8	23 (10–40)		
	K-*ras*	16	46 (29–63)	12	34 (19–52)		
	p53	17	49 (31–66)	11	31 (17–49)		
	BAT26	2	6 (1–19)	1	3 (1–15)		
	Cytology			3	9 (2–23)		
Left-sided colon cancer (n = 81)	Combined marker	66	81 (71–89)	62	77 (66–85)		
	APC	40	49 (38–61)	39	48 (37–60)		
	K-*ras*	17	21 (13–31)	21	26 (17–37)		
	p53	45	56 (44–67)	34	42 (31–53)		
	BAT26	4	5 (1–12)	3	4 (1–10)		
	Cytology			29	36 (25–47)		

right side of their colon (74%; 95% CI: 54–89) and 60 of 66 patients with cancers originating on the left side of their colon (91%; 95% CI: 81–97). A statistically significant difference was found between the right- and left-side colon cancer patient groups ($P = .03$).

Discussion

We have devised a simple, highly reliable methodology for isolating colorectal cancer cells from nonlaxative-induced, naturally evacuated feces from most patients with colorectal cancer. To date, several methods of isolating colorectal cancer cells from feces have been reported.[21,22,26,27]

Our new funnel-shaped filter system extensively improved the filtration efficiency of the stool suspension by enlarging the filtration area and selecting the optimal pore size; the system was capable of filtrating the entire stool suspension without filter clogging. These properties permit the omission of centrifugation and simplify the overall process because all steps can be performed at room temperature. Furthermore, the use of serum successfully increased the cell retrieval rate. We presume that this increase may be attributed to the suppression of protease activity or the inhibition of nonspecific reactions of the antibodies on the bead surface. Consequently, our new methodology also allows the extraction of high-quality DNA or RNA from exfoliated colonocytes. Very recently, Imperiale et al compared a panel of fecal DNA markers and Hemoccult II as screening tests for colorectal cancer. It is worth noting that, in their study, colonoscopy as a reference standard was used

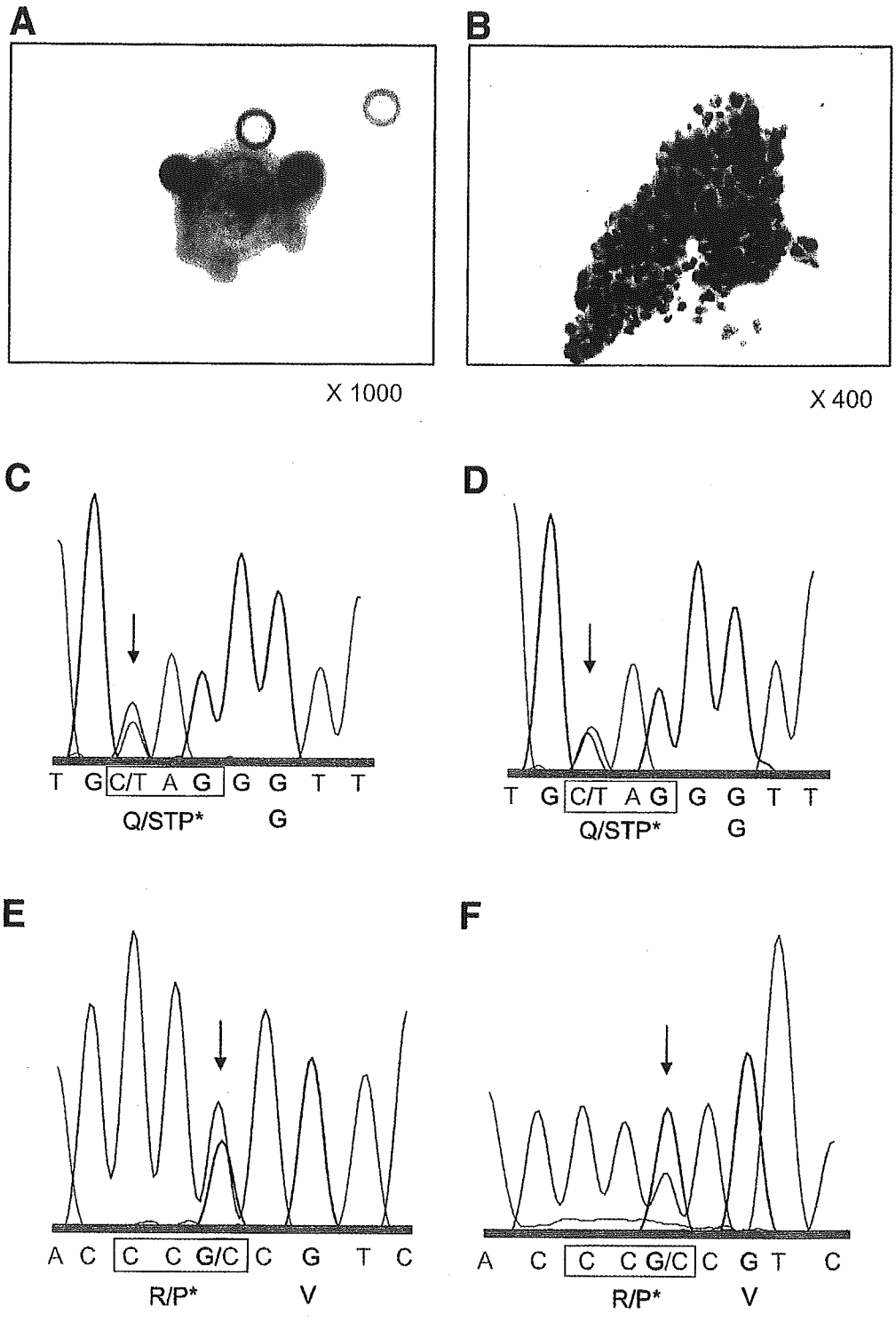

Figure 3. Cytology and DNA sequencing. Papanicolaou staining of colonocytes isolated from the feces of patients with colorectal cancer. (*A*) A patient with ascending colon cancer, Dukes' stage A. (*B*) A patient with rectal cancer, Dukes' stage C. Detection of mutations in tumor tissues and colonocytes isolated from the feces of patients with colorectal cancer. (*C*) A point mutation of the APC gene in a tumor tissue specimen obtained from a patient with rectal cancer, Dukes' stage B. (*D*) An identical mutation was detected in colonocytes isolated from the feces of the patient. (*E*) A point mutation of the p53 gene in a tumor tissue specimen obtained from a patient with ascending colon cancer, Dukes' stage A. (*F*) An identical mutation was detected in colonocytes isolated from the feces of the patient. *Wild/mutant.

in all subjects. They conducted those tests in a blinded fashion and showed that sensitivity of DNA analysis was 4-fold higher than that of Hemoccult test.[28] We believe that this report may prompt a study of fecal DNA test for colorectal cancer screening.

The idea to isolate cancer cells from feces originally derived from a study that described the abnormal expression of the CD44 gene in many tumors, including colon cancer and bladder cancer.[21,29,30] In the course of a series of studies, we predicted that normal mucous cells would die and be exfoliated during turnover and that the cancer cells would likely survive for a long time in the feces.

Although cytology is highly specific compared with direct sequence analysis, its sensitivity, especially for cancers on the right side of the colon is relatively low. From a technical aspect, our cytology method does not allow the

Table 3. Incidences of Genetic Alterations in Colonocytes Isolated From the Feces of Patients With Colorectal Cancer Tissue Involving Genetic Alterations of the APC, K-*ras*, p53, or MSI (BAT26) Gene

	Combined marker		APC		K-ras		p53		BAT 26	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
Overall	80/93	86% (77–92)	46/51	90% (79–97)	29/33	88% (72–97)	42/62	68% (55–79)	4/6	67% (22–96)
Dukes' stage A	18/24	75% (53–90)	11/14	79% (49–95)	5/6	83% (36–100)	5/6	83% (36–100)	1/1	100% (3–100)
Dukes' stage B	26/30	87% (69–96)	16/17	94% (71–100)	9/10	90% (56–100)	12/18	67% (41–87)	1/2	50% (1–99)
Dukes' stages C and D	36/39	92% (79–98)	19/20	95% (75–100)	15/17	88% (64–99)	21/27	78% (58–91)	2/3	67% (9–99)
Right-sided	20/27	74% (54–89)	8/11	73% (39–94)	12/16	75% (48–93)	11/17	65% (38–86)	1/2	50% (1–99)
Left-sided	60/66	91% (81–97)	38/40	95% (83–99)	17/17	00% (81–100)	31/45	69% (53–82)	3/4	75% (19–99)

NOTE. Number of positive cases in tumor tissue and colonocytes isolated from feces/number of positive cases in tumor tissue, with 95% confidence interval.

observation of cells unless there are 5×10^4 cells per slide. Technical improvements might increase the benefits of feces cytology. However, we believe that cytology is not suitable as a method for identifying cancer because of its low sensitivity, at least at present. From a practical point of view, we have conducted a study to determine the effect of the time and temperature after evacuation on the recovery rates of fecal colonocytes, and we have found that we can obtain almost the same number of colonocytes from stool materials 3 days after evacuation in comparison with 6 hours after evacuation if fecal material is kept at 4°C (data not shown). This observation may be important for the potential clinical application of this method.

Direct sequence analysis of colonocytes isolated from the feces of 83 healthy volunteers revealed mutations in 8 subjects (9%; 95% CI: 4–18), the breakdown of which was as follows: 1 APC1 mutation, 1 K-*ras* mutation, and 6 p53 mutations. Points of mutations identified of the p53, APC, and K-*ras* genes observed in the 83 healthy volunteers in this study were identical to that reported previously in tumors. These mutations of p53, APC, and K-*ras* in tumors are recorded in the database of OMIM. PCR errors were unlikely because multiple PCR reactions and sequence reactions were separately conducted. However, genetic alterations in precancerous lesions may have been present, although endoscopy findings macroscopically verified the absence of adenoma and carcinoma. The individuals in whom the present methodology revealed genetic alterations should be monitored to assess whether these findings were false-positive results or a predictor of tumorigenesis.

Oncogenes in feces are presumably derived from cancer cells exfoliated from the cancer tissue, and genetic alterations would not be detected in colonocytes isolated from feces if the original cancer tissue did not contain genetic alterations. In fact, among the 93 patients who exhibited genetic alterations in their cancer tissues, alterations were detected in colonocytes from the stools of 80 patients, producing a true sensitivity rate of 86% (80 of 93), although the present overall sensitivity was 71%. Furthermore, our methodology allows the isolation and retrieval of colorectal cancer cells from both early stage cancer and right-side colon cancer. Because the methodology allows processing at room temperature, we are currently constructing an automated, mechanized processing system on a commercial basis. A problem of our test was its relatively low specificity for a screening test as described previously. We consider that mutations observed in the healthy subjects might be attributable to the fact that they belonged to a high-risk group for colorectal cancer because these 83 volunteers were selected from among colonoscopy examinees recruited by the newly established National Cancer Center Research Center for Cancer Prevention and Screening, and the detection rate of cancers appeared to be considerably higher in the all examinees at the center than in the general population in Japan (unpublished observation). Therefore, we speculate that precancerous lesions with mutations of the genes tested might have been present in the colorectal epithelium of some of these healthy volunteers. We think that a prospective randomized study would be needed to determine the actual specificity of our method in a real screening population and to verify its clinical usefulness.

References

1. The Editorial Board of the Cancer Statistics in Japan. Cancer statistics in Japan—2003. Available at: http://www.fpcr.or.jp. Accessed 2003.
2. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. N Engl J Med 1993;328:1365–1371.
3. Hardcastle JD, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, James PD, Mangham CM. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. Lancet 1996;348:1472–1477.
4. Kronborg O, Fenger C, Olsen J, Jorgensen OD, Sondergaard O. Randomized study of screening for colorectal cancer with faecal-occult-blood test. Lancet 1996;348:1467–1471.
5. Towler B, Irwig L, Glasziou P, Kewenter J, Weller D, Silagy C. A systematic review of the effects of screening for colorectal cancer

using the faecal occult blood test, hemoccult. BMJ 1998;317:559–565.
6. Winawer S, Fletcher R, Rex D, Bond J, Burt R, Ferrucci J, Ganiats T, Levin T, Woolf S, Johnson D, Kirk L, Litin S, Simmang C. Colorectal cancer screening and surveillance: clinical guidelines and rationale—update based on new evidence. Gastroenterology 2003;124:544–560.
7. Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, Snover DC, Schuman LM. The effect of fecal occult-blood screening on the incidence of colorectal cancer. N Engl J Med 2000;343:1603–1607.
8. Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. Science 1992; 256:102–105.
9. Hasegawa Y, Takeda S, Ichii S, Koizumi K, Maruyama M, Fujii A, Ohta H, Nakajima T, Okuda M, Baba S, et al. Detection of K-*ras* mutations in DNAs isolated from feces of patients with colorectal tumors by mutant-allele-specific amplification (MASA). Oncogene 1995;10:1441–1445.
10. Smith-Ravin J, England J, Talbot IC, Bodmer W. Detection of c-Ki-ras mutations in faecal samples from sporadic colorectal cancer patients. Gut 1995;36:81–86.
11. Eguchi S, Kohara N, Komuta K, Kanematsu T. Mutations of the p53 gene in the stool of patients with resectable colorectal cancer. Cancer 1996;77:1707–1710.
12. Nollau P, Moser C, Weinland G, Wagener C. Detection of K-*ras* mutations in stools of patients with colorectal cancer by mutant-enriched PCR. Int J Cancer 1996;66:332–336.
13. Ratto C, Flamini G, Sofo L, Nucera P, Ippoliti M, Curigliano G, Ferretti G, Sgambato A, Merico M, Doglietto GB, Cittadini A, Crucitti F. Detection of oncogene mutation from neoplastic colonic cells exfoliated in feces. Dis Colon Rectum 1996;39:1238–1244.
14. Deuter R, Muller O. Detection of APC mutations in stool DNA of patients with colorectal cancer by HD-PCR. Hum Mutat 1998;11: 84–89.
15. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, Thibodeau SN, Shuber AP. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 2000;119:1219–1227.
16. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, Hibi K, Goodman SN, D'Allessio M, Paty P, Hamilton SR, Sidransky D, Barany F, Levin B, Shuber A, Kinzler KW, Vogelstein B, Jen J. Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst 2001;93:858–865.
17. Rengucci C, Maiolo P, Saragoni L, Zoli W, Amadori D, Calistri D. Multiple detection of genetic alterations in tumors and stool. Clin Cancer Res 2001;7:590–593.
18. Traverso G, Shuber A, Olsson L, Levin B, Johnson C, Hamilton SR, Boynton K, Kinzler KW, Vogelstein B. Detection of proximal colorectal cancers through analysis of faecal DNA. Lancet 2002; 359:403–404.
19. Traverso G, Shuber A, Levin B, Johnson C, Olsson L, Schoetz DJ Jr, Hamilton SR, Boynton K, Kinzler KW, Vogelstein B. Detection of APC mutations in fecal DNA from patients with colorectal tumors. N Engl J Med 2002;346:311–320.
20. Boynton KA, Summerhayes IC, Ahlquist DA, Shuber AP. DNA integrity as a potential marker for stool-based detection of colorectal cancer. Clin Chem 2003;49:1058–1065.
21. Yamao T, Matsumura Y, Shimada Y, Moriya Y, Sugihara K, Akasu T, Fujita S, Kakizoe T. Abnormal expression of CD44 variants in the exfoliated cells in the feces of patients with colorectal cancer. Gastroenterology 1998;114:1196–1205.
22. Davies RJ, Freeman A, Morris LS, Bingham S, Dilworth S, Scott I, Laskey RA, Miller R, Coleman N. Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. Lancet 2002;359:1917–1919.
23. Winter MJ, Nagtegaal ID, van Krieken JH, Litvinov SV. The epithelial cell adhesion molecule (Ep-CAM) as a morphoregulatory molecule is a tool in surgical pathology. Am J Pathol 2003;163: 2139–2148.
24. Balzar M, Prins FA, Bakker HAM, Fleuren GJ, Warnaar SO, Litvinov SV. The structural analysis of adhesions mediated by Ep-CAM. Exp Cell Res 1999;246:108–121.
25. Salem RR, Wolf BC, Sears HF, Lavin PT, Ravikumar TS, DeCoste D, D'Emilia JC, Herlyn M, Schlom J, Gottlieb LS. Expression of colorectal carcinoma-associated antigens in colonic polyps. J Surg Res 1993;55:249–255.
26. Iyengar V, Albaugh GP, Lohani A, Nair PP. Human stools as a source of viable colonic epithelial cells. FASEB J 1991;5:2856–2859.
27. Davidson LA, Lupton JR, Miskovsky E, Fields AP, Chapkin RS. Quantification of human intestinal gene expression profiles using exfoliated colonocytes: a pilot study. Biomarkers 2003;8:51–61.
28. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal cancer screening in an average-risk population. N Engl J Med 2004;351:2704–2714.
29. Matsumura Y, Tarin D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. Lancet 1992;340: 1053–1058.
30. Matsumura Y, Hanbury D, Smith J, Tarin D. Non-invasive detection of malignancy by identification of unusual CD44 gene activity in exfoliated cancer cells. BMJ 1994;308:619–624.

Received May 24, 2005. Accepted August 31, 2005.

Address requests for reprints to: Yasuhiro Matsumura, MD, PhD, National Cancer Center Research Institute East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. e-mail: yhmatsum@east.ncc.go.jp; fax: (81) 4-7134-6866.

Supported in part by a grant for research on advanced medical technology from the Ministry of Health, Welfare, and Labor and a research and development project of the Industrial Science and Technology Program supported by the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Reprinted from
Jpn J Clin Oncol 2005;35(11)680–689
doi:10.1093/jjco/hyi180

Meeting Report

The 18th International Symposium: Controversies in Prostate Cancer Diagnosis and Treatment

Yeong-Shiau Pu[1], Hiroyuki Fujimoto[2], Tadao Kakizoe[3] and Robert Myers[4]

[1]Department of Urology, National Taiwan University Hospital, Taipei, Taiwan, [2]Urology Division, National Cancer Center Hospital, [3]National Cancer Center, Tokyo, Japan and [4]Department of Urology, Mayo Clinic College of Medicine, MN, USA

Jpn J Clin Oncol 2005;35(11)680–689
doi:10.1093/jjco/hyi180

Meeting Report

The 18th International Symposium: Controversies in Prostate Cancer Diagnosis and Treatment

Yeong-Shiau Pu[1], Hiroyuki Fujimoto[2], Tadao Kakizoe[3] and Robert Myers[4]

[1]Department of Urology, National Taiwan University Hospital, Taipei, Taiwan, [2]Urology Division, National Cancer Center Hospital, [3]National Cancer Center, Tokyo, Japan and [4]Department of Urology, Mayo Clinic College of Medicine, MN, USA

Received August 16, 2005; accepted September 7, 2005; published online November 7, 2005

INTRODUCTION

The 18th International Symposium of the Foundation for the Promotion of Cancer Research, 'Controversies in Prostate Cancer: Diagnosis and Treatment' was held in Tokyo on 24–26 January 2005. The symposium was organized by Drs Tadao Kakizoe, Robert Myers, Hiroyuki Fujimoto and Yoichi Arai with Dr Takashi Sugimura as the advisor.

WELCOME AND INTRODUCTION

Dr Kakizoe chaired the session and expressed his sincere concerns about the ongoing big snow storm in the United States. Professor T. Sugimura opened the Eighteenth International Symposium. Since 1988, 578 speakers from 24 nations around the world have been invited to discuss various cancers comprehensively, usually one cancer at a time. This was the second time where prostate cancer (PC) was discussed. Dr Sugimura pointed out that prostate-specific antigen (PSA) has made a huge progress possible since late 1970s. Dr Sugimura then used himself as an example to explain the notion of cancer survivor. The Japanese Emperor is also a cancer survivor who had PC which has been surgically removed by Chairman Dr Kakizoe.

For reprints and all correspondence: Yeong-Shiau Pu, Department of Urology, National Taiwan University Hospital, Taipei, Taiwan. E-mail: yspu@ha.mc.ntu.edu.tw; ys.pu@msa.hinet.net

Abbreviations: ADT, androgen-deprivation therapy; AR, androgen receptor; BPH, benign prostatic hyperplasia; BCR, biochemical recurrence; DRE, digital rectal examination; 3D-CRT, 3-dimension conformal radiotherapy; EORTC, European Organization for Research and Treatment of Cancer; ED, erectile dysfunction; EBRT, external beam radiotherapy; GS, Gleason's score; HRPC, hormone-refractory prostate cancer; IMRT, intensity modulated radiotherapy; LHRHa, luteinizing hormone-releasing hormone agonist; MSKCC, Memorial Sloan-Kettering Cancer Center; NVB, neurovascular bundle; PZ, peripheral zone; PC, prostate cancer; PSA, prostate-specific antigen; PSADT, PSA-doubling time; PSAV, PSA velocity; QOL, quality-of-life; RP, radical prostatectomy; RT, radiotherapy; TZ, transition zone; TRUS, transrectal ultrasonography

Survivors from PC may be sensitive to follow-up PSA reports, which represents a new issue of care.

OPENING REMARKS: PROSTATE CANCER—A CHALLENGE FOR THE 21ST CENTURY

Dr Robert Myers gave the opening remarks. He indicated that PC is a challenge for the early 21st century. There are several questions that need to be answered about PC, which includes: cancer significant or insignificant, screen or not to screen, chemoprevention, who should be treated, what is the optimal treatment and how, response to PSA rise after treatment, timing for androgen-deprivation, and the best approach for androgen-independent prostate cancer (AIPC)? Current American Cancer Society guidelines 2005 (www.cancer.org) suggests that doctors should offer PSA and digital rectal examination (DRE) at age 50 to men without serious medical problems expected to live at least 10 years. American Academy of Family Physicians (www.aafp.org), however, concludes that there is insufficient evidence on which to make recommendation for or against routine screening for PC using PSA or DRE. Similarly, US Services Preventive Task Force (www.ahrq.gov) also holds the opinion that PSA screening can detect early-stage PC but mixed and inconclusive evidence that early detection improves health outcomes. Dr T.A. Stamey even published a highly debatable article (*BJUI* 2004) entitled 'The era of serum PSA for biopsy of the prostate is now over in the USA'. Then what is beyond PSA? Dr Ornstein et al. and Dr Fradet et al. published a serum proteomic profiling and an uPM3 gene-based urine test, respectively, both of which seemed to increase the accuracy of PC detection. Dr Nelson et al. established in 2004 a 70-gene model to predict PC aggressiveness by genomic approach. The challenge in treatment was outlined nicely in the report of Prostate Cancer Foundation to the Nation 2004. Three major issues are the absence of reliable markers, how to predict treatment response and a low enrollment for clinical trials.

SESSION 1: EPIDEMIOLOGY AND PATHOLOGY

CHAIRPERSON: DAVID BOSTWICK

PROSTATE CANCER: CONTROLLING THE EPIDEMIC (BY PETER BOYLE)

PC caused about 11 million incident cases or 6.1% of men worldwide in 2002. The second leading male cancer is PC, although the death rate is much lower than the incidence rate. In Europe, there were an estimated 2.8 million incident cases of cancer and 1.7 million cancer deaths in 2004. In men, there was an estimated 1.5 million incident cases of cancer of all forms (except skin cancer) diagnosed. In the United States, PC was the commonest form of incident cancer in men in 1996–2000. If we look at the mortality rate, however, lung cancer was the commonest form of cancer death in the same period and the PC death rate was much lower than its incidence rate. By person-years of life lost due to cancer, lung cancer caused most lives lost in 2000 in the United States, followed by breast, colorectal and pancreas cancer. PC, in fact, ranked in the middle. If compared with person-years of life lost per death, PC caused the least amount of lives lost compared with other cancers. PC has a steep rise of incidence with increasing age. There are no other cancers that have such a steep association between incidence rate and age. The underlying concept of PC control is to specify a series of actions in three domains, which will bring about a reduction in PC mortality: primary prevention, secondary prevention or screening, and tertiary prevention. Three randomized screening trials to see if screening reduces mortality have been done or are underway. The Quebec study should be omitted because of unsatisfactory methodology and unreliable data obtained. Two other trials with one in the United States and the other in Europe are still awaited. The major question today is 'will PSA screening reduce PC mortality'. The cohort-specific mortality rate for PC has remained virtually unchanged in generations of men born in last century in nearly every country of the world. More importantly, the adverse effects of radical prostatectomy (RP) on quality-of-life (QOL) could change a modest mortality reduction obtained by PSA screening into negative QOL-adjusted years.

ACTIVE MONITORING USING PSA-DOUBLING TIME FOR STAGE T1C PROSTATE CANCER WITH FAVORABLE BIOPSY FEATURES (BY YOSHIYUKI KAKEHI)

It is difficult to differentiate biologically aggressive cancers from indolent ones at diagnosis. Dr Kakehi and his associates performed a prospective cohort study of active monitoring of disease progression using PSA-doubling time (PSADT) in newly diagnosed T1c patients (PSA $\leq$ 20) who chose to have a delayed treatment. All of them had favorable pathological features. After registration, patients were monitored with PSA every 6 months and re-biopsied at the 13th month. Patients who showed a PSADT of <2 years at any check-point or out of the criteria by re-biopsy are recommended to start treatment. Eighty percent of the 197 patients accepted the active monitoring protocol. Among them, 53% had a PSADT longer than 10 years or even a decreasing PSA and 19%, however, were rapid PSA risers. Active monitoring protocol for stage T1c PC with selective delayed intervention using PSADT offers individualized strategy according to the biological behavior.

CHAIRPERSON: YEONG-SHIAU PU

LATENT PROSTATE CANCER: CURRENT CONCEPTS (BY DAVID BOSTWICK)

There are four ways of finding latent cancer: screening biopsy, transurethral resection of the prostate, cystoprostatectomy and autopsy. According to cystoprostatectomy series reported worldwide, the prevalence of latent cancer ranged from 9 to 61% with most being between 37 and 46%. Several reports indicated that the prevalence of latent cancer is about the same across countries or races but is progressively increased with aging. About 80% of men by age 80 develop PC. Dr Bostwick raised a working hypothesis of origins of PC that inflammation plus unique environment with time causes increased oxidative stress which with time causes genetic instability. Genetic instability then causes high-grade prostate intraepithelial neoplasia (PIN) after years and finally causes cancer. It has been shown that there were no differences between latent, screened and clinical cancers in terms of Gleason's score (GS), Ki-67 index, DNA ploidy and p53 overexpression. There are no consistent difference between latent and clinical cancer after controlling for patient age, cancer location and cancer volume; cancer volume and cancer doubling time account for many differences between latent and clinical cancer. To better detect cancer, Dr Bostwick advocate a novel PCA3 (uPM3) urine test which is a nucleic acid amplification assay that provides an innovative method for a more accurate detection of PC.

LATENT CANCER, NODULAR HYPERPLASIA AND DIFFUSE ENLARGEMENT OF THE PROSTATE: MORPHOMETRIC AND HISTOPATHOLOGICAL ANALYSES (BY TAIZO SHIRAISHI)

To clarify the pathology of the development of these disorders Dr Shiraishi et al. compared histopathological findings of the prostate from different age groups. Whole-mount sections of prostates obtained from males at autopsy without clinical diagnosis of PC and benign prostatic hyperplasia (BPH) were used to assess the relationship between age and prostate weight, prostate histological composition in the transition zone (TZ) and peripheral zone (PZ), and comparison of latent cancer prevalence by age groups. They found that a rapid increase in prostate weight from birth to the 20s was followed by a slow rise thereafter. Significant volume increases were observed in all three components of glandular epithelium, glandular lumen and stroma in the TZ from the 40s to 70s. The epithelial and stromal volumes, however, tended to decrease in the PZ in an age-dependent manner. Tumor and hyperplasia have a long natural history, usually starting in the fourth decade of life. There was an age-dependent prostatic enlargement, especially due to the TZ zone. Large prostates can be classified into three

types according to PZ/TZ ratio. TZ latent cancer is more common in enlarged prostates.

Familial Prostate Cancer (By Kazuhiro Suzuki)

Positive family history is a strong risk factor for PC, and men with positive family history with PC are recommended to take cancer screening at the age of 40. HPC and FPC accounts for 3–5% and 15–20% in the United States, respectively. In Japan, Dr Suzuki and co-workers found that HPC and FPC accounts for ~1 and 3%, respectively. Clinical characteristics of HPC/FPC did not differ from sporadic cancers except for early onset. However, family history of PC increased the positive predictive value of patients with gray-zone PSA values. HPC served as a good model to analyse the genetic susceptibility to PC. Since the susceptibility locus at 1p24–25 named as HPC1 has been reported, several loci were reported in association with HPC development. In 2001, the first candidate susceptibility gene HPC2/ELAC2 was reported. Since then, several genes including RNASEL and MSR1 were identified as susceptibility genes. Their study showed that HPC2/ELAC2 and RNASEL were involved in the development of HPC/FPC in the Japanese population. Genome-wide linkage analysis demonstrated suggestive linkage near D8S550 on 8p23 and D1S2667 on 1p36.

SESSION 2: PREVENTION AND DIAGNOSIS

Chairperson: Peter Boyle

Pro's and Con's of Screening for Prostate Cancer in 2005 (By Fritz Schröder)

The benefit of a cancer screening is based on clinically relevant decrease of cancer mortality with acceptable costs. PC mortality in the United States between 1979 and 2000 decreased for 19.2%. However, the only way of showing or disproving the value of population screening for PC is through a valid randomized trial of screening. Such trials are ongoing in Europe and in the US. Results, depending on power and mortality differences can be expected after 2006. The European Randomized Study of Screening for Prostate Cancer (ERSPC) had recruited more than 200 000 men aged between 50 and 74 from 1993 through 2004. The trial performed screening every 4 years and will follow-up subjects for 10 years. Different assumptions of mortality reduction ranged from 20 to 50% which impacts the power of the trial and also the end of follow-up year. There has been no significant difference in mortality between groups yet. Dr Draisma and co-workers developed simulation models (MISCAN) based on results of the Rotterdam section of the ERSPC, which enrolled 42 376 men and in which 1498 cases of PC were identified. The models were used to predict mean lead times and overdetection rates. Mean lead times and rates of overdetection depended on age at screening. For a single screening test at age 55, the estimated mean lead time was 12.3 years and the overdetection rate was 27%; at age 75, the estimates were 6.0 years and 56%, respectively. For a screening program with a 4 year interval from age 55 to 67, the estimates were 11.2 years and 48%, respectively. This screening program raised the lifetime risk of PC from 6.4 to 10.6%, an increase of 65%. In annual screening from age 55 to 67, the estimated overdetection rate was 50% and the lifetime cancer risk was increased by 80%. It appears that these data support a screening interval of >1 year.

Current Controversies in Prostate Cancer Screening (By William Catalona)

About 17% of US men are diagnosed with PC during their lifetime and ~16% of these will die of it. Therefore, 3% die of PC and, thus, are eligible for screening benefits. PSA tests in the United States were introduced in around 1991 in a broad sense. By 1995 the mortality rates leveled off and have been decreasing from 3 to 4% per year, more rapidly than for any other cancer. When Dr Catalona started a PSA screening study in 1989, he used a 4 ng/ml PSA cutoff. When FDA approved in 1994 that PSA in conjunction with DRE can be used as a tool for early detection of PC, 4 ng/ml cutoff was chosen. However, PSA cutoff of 4 misses many clinically-significant PCs. If we do not adjust for the verification bias by statistical methods, the ideal cutoff would be 2.6. If we do, the figure would be 1.4. Thus, for a man with a healthy prostate, which means no BPH, prostatitis, or PC, his PSA should be <1 ng/ml. However, older men (≥60 years) do have BPH or prostatitis, the ideal cutoff would then be 4.1 and 2.1 for unadjusted and adjusted calculations, respectively. So based on the Prostate Cancer Prevention Trial (PCPT), a significant proportion of men with a PSA <4 are found to have PC. Of note, even at a low PSA range (<4 ng/ml), PSA does correlate with the likelihood of having PC and high-grade PC. Dr Catalona pointed out that although PSA is not a good marker for curable PC in very large or small tumors, it is a good marker for both PC and curable PC in intermediate-sized tumors. Cancer volume also correlates well with tumor recurrence rate. PSA velocity (PSAV) is among all the most significant PSA measurements that we can make. PSAV is associated with the risk for cancer, biochemical progression and cancer-specific mortality.

Screening, Biopsy and Life Expectancy (By Hidetoshi Yamanaka)

Life expectancy may be one of the most important issues in the development of optimal screening systems, which can detect clinically significant (i.e. life-threatening) and also curable tumors. The risk of cancer-related death is being higher as the age at diagnosis being younger. The use of age-specific reference ranges of PSA (ASRR PSA) may be able to detect small cancer in younger men without increasing the number of unnecessary biopsies in older men, and may also be cost advantage for screening. Dr Yamanaka showed that among 6744 men participated, 556 men had at least one abnormal finding on PSA levels (>4 ng/ml), DRE or transrectal ultrasonography (TRUS). Of the 556 patients, 331 were biopsied, and 119 were diagnosed with PC. The diagnostic efficiency of

the ASRR PSA was optimal with cutoffs of 3.0, 3.5, 4.0 and 7.0 ng/ml for men with 60–64, 65–69, 70–79 and >80 years, respectively. The sensitivity of the ASRR PSA was higher than the traditional 4.0 cutoff without much compromise in specificity. Dr Yamanaka and co-workers initiated another prospective study in 2000. Between 2000 and 2003, 28 930 men aged 50–69 years old had their PSA levels measured in the population-based screening study. The cutoff for biopsy indication was set at 3.0 and 3.5 ng/ml in the age range of 50–64 and 65–69, respectively. A total of 719 men (2.5%) and 1307 men (4.7%) were in the PSA of ASRR to 4.0 and >4.0 ng/ml, respectively. Of the 719 men with PSA between ASRR to 4.0, 131 (18%) were biopsied, and the positive biopsy rate was 19% (25/131), which was slightly lower than the 31% in the PSA range from 4.1 to 10 ng/ml. ASRR PSA may be useful to detect early-stage PC in younger men with only 2.5% increase in the number of men with abnormal PSA, compared with the traditional PSA cutoff of 4.0 ng/ml for men aged 50–69. Dr Yamanaka also proposed that the number of biopsy cores should be set according to the life-expectancy and prostate volume in younger men.

CHAIRPERSON: WILLIAM CATALONA

CANCER-ASSOCIATED CARBOHYDRATE ALTERATION OF PSA (BY CHIKARA OHYAMA)

Carbohydrates on tumor cell surface play important roles in cancer invasion and metastasis. Cancer-associated carbohydrate alteration in serum PSA has never been demonstrated. PSA is a glycoprotein containing ~8% of carbohydrate composed of an *N*-glycan. The structure of carbohydrate on PSA is thought to be a biantennary N-linked oligosaccharide of the *N*-acetyllactosamine type. In order to apply the cancer-associated carbohydrate alteration to the improvement of PSA assay, Dr Ohyama and co-workers first performed an intensive structural analysis of PSA purified from human seminal fluid. The predominant core structure of *N*-glycan of seminal fluid PSA was a complex type biantennary oligosaccharide and was consistent with the structure reported previously. *Lens culinaris* (LcH), *Aleuria aurantia* (AAL), *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA) lectins were tested for their binding affinity to the carbohydrates on PSA. They also analysed serum PSA from randomly selected patients with PC and BPH. Among the lectins examined, the MAA-bound fraction of PSA increased in LNCaP supernatant compared with seminal fluid and BPH tissue. Free PSA from PC patients had increased binding to MAA compared with that from BPH patients. Distinct binding of free PSA to MAA lectin between PC and BPH could be a potential measure for improved specificity of PSA assay.

MOLECULAR MARKERS OF PROSTATE CANCER (BY OSAMU OGAWA)

Development of PC is affected by both genetic and environmental factors. In case–control studies on a Japanese population, Dr Ogawa and co-workers found that PC risk was significantly associated with particular polymorphisms on CYP17, SRD5A2, vitamin D receptor, cycline D1 and PSA genes. Dr Ogawa further did a study that correlated 13 polymorphic markers with the cause-specific survival of 122 advanced PC. Among them, CYP19 and IGF-1 long-allele genotypes were found to be significantly associated with reduced survival. In a study using tissue microarray (TMA) technology, specimens were obtained from 52 patients undergoing RP, Ki-67, p53 and androgen receptor (AR) antigen expression were examined. They found that TMA GS ($P = 0.038$), TMA primary Gleason grade ($P = 0.013$), Ki-67 labeling index ($P < 0.001$), p53 ($P = 0.045$) and AR antigen expression ($P = 0.046$) were significant variables for predicting biochemical relapse. Moreover, TMA primary Gleason grade and Ki-67 were independently associated with treatment failure. They demonstrated nicely that more accurate prediction of prognosis can be made by combining traditional clinicopathological parameters and molecular expressions determined by tissue TMA.

IMAGING MODALITIES FOR STAGING PROSTATE CANCER (BY HISATAKA KOBAYASHI)

CT, MRI, ultrasound and nuclear medicine are currently the major modalities for evaluating PC. They, however, have not provided satisfactory information about the local-regional extent of invasion. MRI is a powerful modality for evaluating PC with its high spatial resolution and excellent soft tissue contrast. Currently, three MRI methods: T2-weighted imaging, MR spectroscopic imaging and dynamic MRI with Gd-DTPA contrast are valuable for diagnosing loco-regional spread of primary tumors. Combination of these MR modalities gives more precise assessment of the prostate. Fluoro-deoxyglucose (FDG) does not work well for the diagnosis or local staging of PC. Nuclear medicine techniques with FDG or monoclonal antibodies have proven to be valuable for the detection of positive lymph nodes. The In-111-labeled PSMA (murine) monoclonal antibody (Prostascint) specifically detects metastasis-positive lymph nodes. With MRI using nanotechnology, Dr Kobayashi and co-workers have recently developed a method to visualize the lymphatic flow from cancer tissue to sentinel lymph nodes.

CHAIRPERSON: WILLIAM CATALONA

CHEMOPREVENTION OF PROSTATE CANCER: ASSESSING BENEFIT AND RISK IN THE PROSTATE CANCER PREVENTION TRIAL (CONTENTS BY ERIC KLEIN; PRESENTED BY WILLIAM CATALONA)

The PCPT that compares the rate of cancer reduction between finasteride 5 mg/day and placebo in 18 000 men (normal DRE and PSA <3.0 ng/ml) demonstrated a 25% reduction in the 7 year period prevalence of PC in the finasteride group. In brief, there was a 6.4% reduction (RR = 0.75) in the prevalence of PC and a 1.3% increase (RR = 1.27) in high-grade

disease. Tumors of GS ≤6, =7 and ≥8 markedly decreased, unchanged and slightly increased, respectively. More and more insignificant cancers are now being identified. The burden of cure, however, is heavy, which includes anxiety over initial diagnosis, discomfort of biopsy and staging procedures, uncertainty of cure, inconvenience of therapy, treatment-related morbidity, and cost of management of incontinence, bowel dysfunction, and erectile dysfunction (ED). A mathematical model of risk and benefit for finasteride was developed. The benefit/risk ratio of finasteride use was estimated by calculating the ratio of absolute risk reduction in the finasteride arm to the absolute risk of excess high-grade cancers. Using this model, for all cancers detected in the PCPT, the baseline benefit/risk ratio increased from 4.6/1 to 5.1/1, 6.2/1 and 9.2/1 for assumptions of 10, 25 and 50% histologic artifact, respectively. The baseline ratio increased from 4.6/1 to 8.2/1 for the assumption of a 25% overdetection bias, and to 9.1/1, 10.9/1 and 16.3/1 for combined assumptions of a 25% overdetection bias and 10, 25 and 50% histologic artifact, respectively.

EQUOL: ONE ISOFLAVONE FOR CHEMOPREVENTION OF PROSTATE CANCER (BY HIDEYUKI AKAZA)

Many reports have shown that soybean isoflavones may have a significant role in preventing PC. Dr Akaza's group has previously published a case–control study that some Japanese are able to metabolize daidzein, one of soybean isoflavones, to equol (equol producers), and that the incidence of PC is higher in non-producers. They recently conducted a similar case–control study involving Japanese living in Japan, Koreans living in Korea and Japanese immigrants to the United States. There were no differences in daidzein or genistein between each group. The percentage of equol producers differs significantly between cases and controls being 29 and 46% in Japan ($P = 0.004$) and 30 and 59% in Korea ($P = 0.001$), respectively. The percent equol producers for Japanese immigrants to the United States were markedly lower than that for Japanese and Koreans. Dietary factors may play important roles. The daily amount of green tea consumption may affect the production of equol in Japanese men.

SESSION 3: THERAPY OF LOCALIZED PROSTATE CANCER

CHAIRPERSON: PETER SCARDINO

HYPOFRACTIONATED CARBON-ION RADIOTHERAPY FOR PROSTATE CANCER (BY HIROHIKO TSUJII)

The characteristics of carbon-ion radiotherapy (C-ion RT) include superior dose distribution and high biological effect. The therapeutic merit of heavy-ion radiation is that the density of ionization increases with depth of penetration, which generates a higher biological effect. The dose fractionation of 66.0 GyE in 20 fractions through three ports over 5 weeks has been used as a recommended regimen. The PTV is 10 mm wider than the CTV but is only 5 mm next to the rectum to exclude the anterior rectal wall as much as possible. A total of 248 patients were treated with this regimen with the follow-up >6 months. An average pretreatment PSA value in these patients was 37.7 ng/ml and the median was 19. Incidence of radiation morbidities in the rectum and the genitourinary system were considered acceptable. Only four patients (1.6%) developed Grade 2 rectal bleeding, which eventually accounted for 0.4%. Seventeen patients (6.9%) developed Grade 2 urinary morbidities but most of them eventually improved to the incidence of 3.6% without specific treatment. These morbidity profiles compared favorably with contemporary series. All 248 patients have been free from local recurrence. The 5 year overall and cause-specific survival rates were 89.5 and 92.1%, respectively. The 5 year biochemical relapse-free survival (RFS) was 81.6%.

RADICAL RETROPUBIC PROSTATECTOMY (BY ROBERT MYERS)

In the only randomized prospective study from Scandinavian Prostatic Cancer Group showed that RP compared with watchful waiting significantly reduced disease-specific mortality, but there was no significant difference in terms of overall survival. At Mayo clinic, a significant stage migration has been seen from 1987 to 2003 with T3 disease dropping from 25 to 3% and T1c disease increasing from 2 to 56%. According to Dr Binder, the Da Vinci Surgical System costs very much including about 1.25 million of the robot, 110 000 yearly service contract, and 1600–3900 instruments (8–30 uses) with all in Euros. Dr Myers also pointed out the advantage of tactile sensation with open surgery and long learning curve with the laparoscopic surgery. Dr Myers then presented his results on RP on 307 patients within the last year. The positive margin rate was 13.6 and 11.7% for the total series and the ones with bilateral nerve bundle preservation, respectively. The overall pad-free rate was 93%. According to an unpublished retrospective study, the satisfactory intercourse rate with or without assistance in his patients was 84%.

FUNCTIONAL OUTCOME OF RADICAL PROSTATECTOMY (BY YOICHI ARAI)

Nerve-sparing RP is beneficial for the preservation of sexual potency. Whether urinary continence is also improved by the nerve-sparing procedure remains controversial. Dr Arai and co-workers examined the effect of neurovascular bundle (NVB) preservation during RP on short-term post-operative urinary continence. Eighty-five patients undergoing RP were prospectively enrolled. Electrophysiological testing was performed to confirm NVB preservation. Macroanatomical assessment was incorrect in 20% of the bundles compared with the electrophysiological assessment. The degree of NVB preservations (both NVB preserved or resected or one side preserved) were reclassified in 33% of the patients. Bilateral nerve-sparing group had significantly better post-operative urinary control than the unilateral nerve-sparing group and the non-nerve-sparing group. However, there was

no significant difference between groups in urinary control by macroanatomical classification. Similarly, the bilateral nerve-sparing group showed a significant better recovery of erectile function than the unilateral nerve-sparing and non-nerve-sparing group.

SESSION 4: CURRENT FAR EAST STATUS OF PROSTATE CANCER

CHAIRPERSON: FRITZ SCHRÖDER

STATUS OF PROSTATE CANCER IN KOREA (BY CHONGWOOK LEE)

The incidence rate of PC per 100 000 Koreans adjusted for the world population was reported to be only 2.98 in 1989. However, since the 1990s, the incidence of PC has dramatically increased in Korea. From 1995 to 2002, PC showed the highest rate of increase (2.11-fold increase) among all cancers in Korean males. In 1996, PC became one of the top 10 incident cancers in men in Korea and rose to the sixth in 2002 when the age-standardized incidence rate was 7.71 per 100 000. According to Korean Central Cancer Registry, PC accounts for 3% of male incident cancers in 2003. The mortality rate also rose rapidly in the past 10 years. The 5 year PC survival in 2003 was higher in the United States (over 90%) than Korea and Japan (~50–60%). At their institution of Seoul National University Hospital, over 70% of PCs diagnosed were of Stage D during the late 1980s, whereas Stage D cancers decreased to <50% during the new millennium. Meanwhile, the increases in average life span of Koreans and the westernization of life style, including diet pattern, may have contributed to the increase of PC. As the differences in PC incidence between Koreans residing in the United States and Korea have been observed, environmental changes may also be a significant factor.

STATUS OF PROSTATE CANCER IN TAIWAN (BY YEONG-SHIAU PU)

PC was the sixth leading male cancer (incidence rate 15.8 per 100 000 men) in 2000 and resulted in 742 deaths (mortality rate 6.45 per 100 000) in 2003. The incidence would rise up to 30 per 100 000 in 2004, over 7 times of that in 1990. The age-adjusted incidence and mortality rates in Taiwan are among the highest in Asian countries, which is higher than Japan and Korea but lower than Philippines. Widespread use of PSA and aging may be responsible for the rapid rise of PC in Taiwan in the past decade. However, westernized dietary habit is still controversial. It has been shown that among all risk factors, population aging was the strongest factor contributing to the increase of mortality rate in an age-period-cohort analysis in Taiwan. A case–control study on the risk factors of PC in a patient population comprised mainly of veterans (63%) in Taiwan showed that PC patients tended to have engaged in more physical activity (OR 2.2), have a lower body mass index (OR 2.0) and be less likely to consume vegetables cooked with pork lard (OR 0.47). In the past, up to 80% of PCs were locally advanced or metastatic at diagnosis. Nowadays, a stage migration from late to early stages was seen in Taiwan. In 1999, a pathological review of 49 cystoprostatectomy specimens revealed latent PC in 33% and high-grade PIN in 49% of the prostates removed. The age-adjusted abnormal PSA (≥4.0 ng/ml) rate was ~5%, very similar to that of a Japanese population. The cancer detection rate by screening in a health check-up setting was only 0.3%, which is significantly lower than those of Western countries. The PSA positive predictive value for a referral population was ~15% for subjects having a PSA between 4 and 10 ng/ml.

SESSION 5: MOLECULAR BIOLOGY AND NOVEL THERAPIES

CHAIRPERSON: EDWARD MESSING

FUNCTION OF ANDROGEN RECEPTOR IN PROSTATE CANCER DEVELOPMENT (BY SHIGEAKI KATO)

Androgen exerts a wide range of biological effects. Most of the biological actions of androgen are considered to exert through nuclear vitamin receptor-mediated gene expression. AR knockout (ARKO) male mice generated by the conventional method are expected to suffer from testicular feminization mutant (Tfm) abnormalities with infertility. Therefore, it is impossible to generate ARKO mouse lines by natural mutations. Dr Kato generated the floxed AR mice, and then crossed with female AR(−/+) heterozygote expressing Cre to generate ARKO mice line. The AR(−/Y) KO males grew healthy with typical features of Tfm abnormalities, and genital organs were atrophic with a marked decrease of serum testosterone levels, but with normal estrogen level. Hot spot mutation (T877A) in human AR ligand binding domain (LBD) is often found in hormone-refractory prostate cancer (HRPC). It is to be studied whether such an AR mutation leads to dominant proliferation. Dr Kato applied the floxed AR mice to 'knock' the human AR T877A mutant LBD cDNA into the corresponding mouse gene locus to express endogenous mouse–human hybrid AR mutant. The mice looked normal in external genital organs and reproduction. However, the prostate size in the AR (T877A/Y) mice observed at age of 17 weeks was clearly increased. No antagonistic action of hydroxyflutamide against prostate development was observed. These findings suggest that hypersensitivity of AR mutants to antagonists and endogenous steroid hormones may potentiate hormone dependency in PC development.

PERSONALIZED PEPTIDE VACCINATION FOR PROSTATE CANCER (BY KYOGO ITOH)

Antitumor vaccines have emerged as a promising therapeutic approach. Dr Itoh et al. recently devised a new peptide-based vaccination. In addition, they recently reported a benefit of the combination of the peptide vaccination and low-dose estramustine phosphate in patients with metastatic HRPC who had received the previous vaccination. Forty-nine patients

with HLA-A24+ or -A2+ HRPC were enrolled in the Phase I/II study. Those who showed a progressive disease in the vaccination alone treatment period were offered a combined treatment with vaccination and low-dose estramustine phosphate (280 mg/day). All patients developed Grade 1 or 2 local redness and swelling at the injection site. Best clinical response of the 49 cases with the vaccination alone was 5 partial responses, 5 stable diseases and 39 progressive diseases. Median time to progression was 2.5 months. Furthermore, the majority of patients treated with the combination therapy showed a decrease of PSA. Among the 14 patients receiving the combined treatment, 7 (50%) achieved partial responses. The median survival time with the combined therapy was 25 months. QOL were not deteriorated during the treatment. They did another study on 33 HRPC patients treated with the combined therapy and 33 matched HRPC control patients. All patients failed the estramustine phosphate-based therapy. Cause-specific survival in the 33 HRPC patients treated with the combined therapy was longer than that of the control group (log-rank $P = 0.002$). Peptide vaccination was an independent factor of an improved survival by multivariate analysis.

IN SITU GENE THERAPY FOR PROSTATE CANCER (BY HIROMI KUMON)

More than 500 gene therapy protocols have been tested against cancer in the world by January 2005. Intraprostatic adenoviral vector transduction of the herpes simplex virus-thymidine kinase (HSV-tk) gene followed by the systemic administration of ganciclovir (GCV) is a form of cytoreductive gene therapy that has been examined extensively in preclinical studies and Phase I/II trials at Baylor College of Medicine (BCM). The safety and potential efficacy of HSV-tk + GCV *in situ* gene therapy were confirmed in 36 patients with biochemical recurrence (BCR) of localized PC after definitive radiation therapy. Dr Kumon et al. in collaboration with BCM conducted a Phase I/II study using the identical protocol. As of the time of presentation, seven patients have been treated with three at the first dose level of 1×10^9 and four at the second dose level of 1×10^{10} PFU. No adverse events were observed, although transient vector shedding into urine and mild antibody response to adenovirus were detected. PSA responses were detected in 71% (5/7) of the patients. In one patient treated at the first dose level, PSA fell <4 ng/ml for over 1 year. Recently, the patient received the second treatment at the higher level of 1×10^{10} 2 years after the initial treatment, resulting in a repeated PSA response. In order to augment specific immune response, new strategies including immune gene therapy and combination therapy should be devised. IL-12 is a potent cytokine having antitumor activities involving IFN-gamma release, expansion and activation of NK and T cells, and differentiation of CD4+ cells into Th1 cells. *In situ* Adv-IL12 gene therapy was initiated on 17 May 2004 at BCM. A Phase I/II protocol was also approved by the IRB at Okayama University Hospital. In addition, novel therapeutic targets including RTVP-1 and REIC/DKK-3 for *in situ* gene therapy for PC have been investigated extensively at both institutions. Extensive preclinical studies are underway at OUM.

SESSION 6: THERAPY OF N+ AND ADVANCED PROSTATE CANCER

CHAIRPERSON: CHONGWOOK LEE

HORMONAL THERAPY FOR PROSTATE CANCER: TIMING AND CONTROVERSIES (BY EDWARD MESSING)

Based on the VA cooperative studies in the early 1970s, in which 'early' androgen-deprivation therapy (ADT) delayed progression to metastatic disease but did not prolong survival, withholding ADT until there were symptomatic metastases (or at least documented bone metastases) became the standard of care for using this treatment. Recently, there have been several randomized trials indicating that for patients with aggressive local disease, early ADT, either alone or in combination with RT or RP has demonstrated significantly improved overall survival compared with deferred ADT. Morbidities may come from three aspects: treatment, cancer and PSA anxiety. As for RT, large mature randomized studies have shown a survival benefit in high-risk patients in the early ADT arm than in the deferred arm. Dr Messing summarized that local control is far better with early ADT group and the survival advantage is modest, primarily for very high-risk patients. For definitive ADT, the large MRC study showed that early treatment probably prolongs survival and reduces serious morbidity in those with T3, T4 and M+ disease. Dr Messing pointed out that in the trial EST 3886 where all patients underwent RP and were found to have micrometastatic disease in pelvic nodes (*NEJM* 1999), the overall survival, cancer-specific survival and progression-free survival were all better in the early ADT group than in the deferred group. In another randomized study European Organization for Research and Treatment of Cancer (EORTC) 30846 by Dr Schröder et al., there was only an insignificant trend favoring the early ADT group. Patients in this study appeared to have more advanced disease than those in EST 3886. In the large randomized trial enrolling 3000 men with non-metastatic PC, immediate orchiectomy or luteinizing hormone-releasing hormone agonist (LHRHa) compares favorably with delayed ADT in terms of cancer-specific survival but not non-cancer-specific survival. Dr Messing concluded that early ADT prolongs survival for poor risk or localized/regional PC. However, no clear data indicating early ADT confers a survival benefit for low and even moderate risk disease.

CHEMOTHERAPY FOR ADVANCED PROSTATE CANCER (BY DAVID SOLIT)

PC has long been considered as chemoresistant as shown by a meta-analysis of 26 studies done between 1987 and 1999 on 1683 patients, which showed only 8% response rate. Patients who have a PSA decline of over 50% after chemotherapy have

a better survival than those who do not. Both the two mitoxantrone trials showed benefits in palliative effects but not survival compared with steroid alone. However, these results cannot be extrapolated to other clinical states, specifically to asymptomatic patients. Several Phase II studies showed that single-agent docetaxel had PSA response rates ~40–50% and objective response rates ~24–40%. SWOG 9916 is a multicenter, randomized Phase III study comparing taxotere plus estramustine (D + E) versus mitoxantrone plus prednisone (M + P). The results showed that patients on D + E had a median overall survival of 18 months compared favorably with the M + P group where the median overall survival was 16 months ($P = 0.01$). Another randomized study TAX327 that enrolled over 1000 patients was to compare docetaxel q3 weeks plus prednisone versus docetaxel q week plus prednisone versus M + P. The results showed a survival benefit with the group of docetaxel q3 week plus prednisolone over the group of M + P (median survival 18.9 versus 16.4 months, $P = 0.009$). Most patients treated with these docetaxel protocols were well tolerated. To build on hormone and chemotherapy, we may need more novel and active agents. Dr Solit specified as an example a novel cytotoxic agent ixabepilone, which targets a binding site in tubulin shared with taxanes but overcomes various mechanisms mediating resistance to taxanes. The agent is under clinical investigation in Memorial Sloan-Kettering Cancer Center (MSKCC), Dana Farber Cancer Center and UCSF.

SESSION 7: THERAPY OF LOCALLY ADVANCED PROSTATE CANCER

CHAIRPERSON: DAVID SOLIT

RISING PSA AFTER RADICAL PROSTATECTOMY: RESULTS OF RADIATION AND OF ANDROGEN DEPRIVATION THERAPY (BY PETER SCARDINO)

After RP 25–40% of patients eventually experience BCR. Without further treatment the median time from BCR to metastases is 7–8 years. With ADT at metastases and other palliative measures, the median time from metastases to death is 5–6 years. Today, most patients with BCR are treated with RT or ADT before metastases appear. Dr Scardino and co-workers designed a nomogram (*JNCI* 1998) with which one can predict the 60-month recurrence-free probability after RP using preoperative PSA, biopsy Gleason's grade and clinical stage. Undetected local recurrence may give rise to late distant metastases, as has been shown after primary RT. Salvage RT is the only potentially curative therapy for men with failing RP. A multi-institutional study using salvage RT for failing RP showed that the 4 year progression-free probability is 45%. About 30% of them had a long-term disease-free state. The most important factor that predicts response to salvage RT is the PSA level at time of RT (PSA $\leq$ 2 ng/ml). Up to 50% of selected patients with a rising PSA after RP have locally recurrent PC. In the absence of positive lymph nodes at RP, two-thirds respond to salvage RT and one-third remain free of disease 5 years later. These patients typically respond to ADT for over a decade. Individual prognosis depends on PSADT, Gleason grade and pathologic stage, and is predictable from a nomogram. Once the PSA rises again (BCR castrate) metastases rapidly appear (median 9 months) and patients succumb to their cancer (median 26 months).

TREATMENT OF PATIENTS WITH PSA RECURRENCE AFTER RADICAL PROSTATECTOMY (BY SEIJI NAITO)

The standard therapy for patients with PSA recurrence after RP has not been established yet. Dr Naito and co-workers investigated the clinical outcome of RP by a multi-institutional randomized controlled trial to evaluate the significance of salvage RT and endocrine therapy for PSA recurrence after RP. They accrued 1192 patients who underwent RP during 1996–2002 with neither neoadjuvant nor adjuvant therapy from 36 institutes affiliated with the Japan Clinical Oncology Group (JCOG). All patients had a post-operative PSA < 0.2 ng/ml. PSA recurrence was defined as PSA $\geq$ 0.2 ng/ml. Extraprostatic extension (i.e. more than pT3) was observed in 33% of patients. During the median follow-up of 3.8 years, 25.3% developed a PSA recurrence. Preoperative PSA, pT stage and pathology GS were independent prognostic factors predicting PSA progression. In the protocol JCOG 0401, patients who have a PSA recurrence after RP are randomized into treatment group of either RT +/− ADT (experimental arm) or ADT alone (standard arm). In both arms, the treatment is started at PSA between 0.4 and 1.0 ng/ml. Patients in the standard arm are treated with bicalutamide and LHRHa if bicalutamide fails. In the experimental arm, a total dose of 64.8 Gy/36 Fr (50 days) external beam radiotherapy (EBRT) is delivered to the prostatic bed. In case of RT failure, bicalutamide will be started followed by LHRHa in case bicalutamide fails. The primary end point is time to treatment failure (TTF) of bicalutamide. The study was activated on 17 May 2004 and will clarify whether salvage RT has an advantage over ADT alone for PSA recurrence after RP.

CHAIRPERSON: HISATAKA KOBAYASHI

EXTERNAL BEAM RADIOTHERAPY FOR PROSTATE CANCER (BY MINAKO SUMI)

Innovative treatment technologies of RT such as 3-dimension conformal radiotherapy (3D-CRT), intensity modulated radiotherapy (IMRT), image-guided RT (IGRT) and brachytherapy are being rapidly incorporated into practice in Japan. The Patterns of Care Study (PCS) evaluated the standard of practice for PC according to institutional stratification in Japan. Studies of practice patterns for patients treated in 1996–98 (PCS9698) and 1999–2001 (PCS9901) have been performed. The specific trends found in the study were the prevalence of higher radiation doses and the use of 3D-CRT for the treatment of clinically localized PC. In comparison with the United States, patients treated with RT in Japan were found to have more advanced and poorly differentiated diseases with higher PSA