TRANSRECTAL HIGH-INTENSITY FOCUSED ULTRASOUND IN THE TREATMENT OF LOCALIZED PROSTATE CANCER : A MULTICENTER STUDY

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We report a multicenter trial with transrectal high-intensity focused ultrasound (HIFU) in the treatment of localized prostate cancer. A total of 72 consecutive patients with stage T1c-2N0M0 prostate cancer were treated using the Sonablate 500^{TM} HIFU device (Focus Surgery, Indianapolis, USA). Biochemical recurrence was defined according to the criteria recommended by the American Society for Therapeutic Radiology and Oncology Consensus Panel. The median age and prostate specific antigen (PSA) level were 72 years and 8.10 ng/ml, respectively. The median follow-up period for all patients was 14.0 months. Biochemical disease-free survival rates in all patients at 1 and 2 years were 78% and 76%, respectively. Biochemical disease-free survival rates in patients with stage T1c, T2a and T2b groups at 2 years were 89, 67% and 40% ($p=0.0817$). Biochemical disease-free survival rates in patients with Gleason scores of 2-4, 5-7 and 8-10 at 2 years were 88, 72% and 80% ($p=0.6539$). Biochemical disease-free survival rates in patients with serum PSA of less than 10 ng/ml and 10-20 ng/ml were 75% and 78% ($p=0.6152$). No viable tumor cells were noted in 68% of patients by postoperative prostate needle biopsy. Prostatic volume was decreased from 24.2 ml to 14.0 ml at 6 months after HIFU ($p<0.01$). No statistically significant differences were noted in International Prostate Symptom Score, maximum urinary flow rate and quality of life analysis with Functional Assessment of Cancer Therapy. HIFU therapy appears to be minimally invasive, efficacious and safe for patients with localized prostate cancer with pretreatment PSA levels less than 20 ng/ml.

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Key words : Prostate cancer, High-intensity focused ultrasound, Minimally invasive surgery

INTRODUCTION

Prostate cancer is the most common malignancy in men and the second leading cause of death due to cancer in the United States[1]. Prostate cancer has been treated in various ways, depending on the severity of the condition, age of the patient, staging, Gleason score and serum prostate-specific antigen (PSA) level. Radical prostatectomy has long been regarded as appropriate therapy for patients with organ-confined prostate cancer. Despite excellent 5- to 10-year survival rates after radical prostatectomy for organ-confined disease, surgery is

associated with significant morbidity, including blood loss due to transfusion-related complications, erectile dysfunction in 30% to 70% of cases, and stress incontinence in up to 10% of patients[2,3]. In addition, surgical intervention is not typically considered for patients whose life expectancy is less than 10 years. Recently, a number of alternative less invasive treatments have been developed for patients with localized prostate cancer, either not appropriate for surgery or who do not want to risk the potential side effects of surgery. Three-dimensional conformal radiotherapy (3D-CRT), brachytherapy, intensity-modulated external beam radiotherapy, cryosurgical ablation of the prostate and laparoscopic radical prostatectomy have all been applied for the treatment of this group of patients[4-6]. However, in the event of treatment failure, these cannot be repeated and salvage radical prostatectomy is associated with a high morbidity rate[7].

High-intensity focused ultrasound (HIFU) delivers intense ultrasound energy with consequent heat destruction of tissue at a specific focal distance from the probe without damage to tissue in the path of the ultrasound beam[8]. HIFU non-invasively induces complete coagulative necrosis of a tumor without surgical exposure or insertion of instruments into the lesion. This advantage makes it one of the most attractive options for the localized treatment of tumors[9,10]. We report here a multicenter trial with 72 consecutive patients treated with HIFU for clinical stage T1c-2N0M0 localized prostate cancer.

PATIENTS AND METHODS

Inclusion and Exclusion Criteria

As a rule, the inclusion criteria for treatment were patients with biopsy proven and untreated stage T1c-2N0M0 localized prostate cancer[11]. Age, serum PSA levels, prostatic volume and WHO performance status should be less than 80 yrs, 20 ng/ml, treatable with a 4.0 focal length probe which means a prostatic volume less than 50 ml and 0-1. Patients with urethral stricture, anal stricture, bleeding tendency, renal dysfunction with serum Cr more than 2.0 mg/dl, hydronephrosis, larger than 5 mm calcifications in the prostate, uncontrolled diabetes mellitus, hypertension, angina, history of cardiac infarction or other malignant diseases were excluded from the study. None of the patients were receiving neoadjuvant hormonal and/or chemotherapy before HIFU. All patients were fully informed of the details of this treatment and gave written consent preoperatively.

HIFU Eqipment

For this study, we used the Sonablate 500^{TM} (Focus Surgery, Indianapolis, IN, USA) HIFU machine. This treatment module includes the ultrasound power generator, transrectal probes, the probe positioning system, and a continuous cooling system (Fig. 1). The

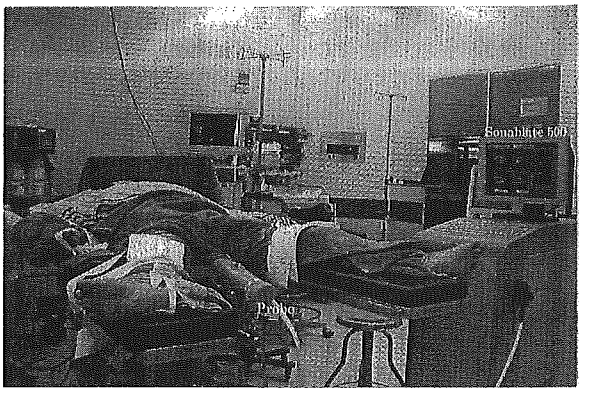

Fig. 1. The Sonablate-500^{TM} type device consists of an operator's console, imaging monitor, transrectal probe and an automatic continuous cooling system.

transrectal HIFU probes use proprietary transducer technology with low-energy ultrasound (4 MHz) for imaging of the prostate and for the delivery of high-energy ablative pulses (site intensity, 1,300-2,200 W/cm^2). The single piezoelectric crystal alternates between high-energy power for ablative (3 sec) and low-energy for ultrasound imaging (6 sec)[10].

Prior to beginning the treatment, the operator uses longitudinal and transverse sonograms to obtain an image of the prostate and selects the prostate tissue volume to be ablated by a set of cursors on these images. The probe houses a computer-controlled positioning system that directs each ablative pulse to the targeted region of the prostate. Each discrete high-energy focused ultrasonic pulse ablates a volume of $3 \times 3 \times 10$ mm^3 of tissue[10]. The total acoustic power is initially set at 24 W and 37 W for 3.0 and 4.0 cm focal length probes, respectively. The individual focal lesion produces almost instantaneous coagulative necrosis of the tissue due to a temperature rise of 80° to 98°C in the focal zone[8]. Under computer control, the ultrasound beam is steered mechanically to produce consecutive lesions in a manner such that all focal lesions overlap

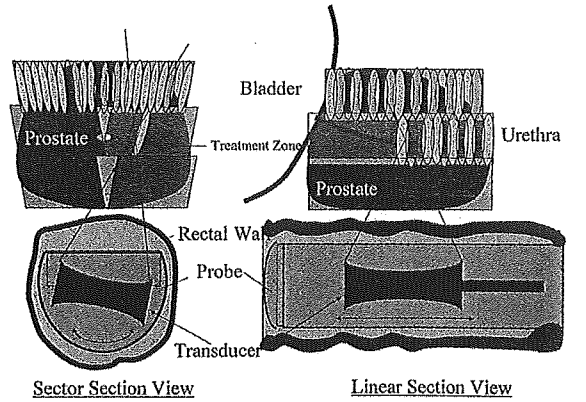

Fig. 2. The computer-controlled transducer ablates the entire prostate tissue. Focal lesions are overlapped in linear rows (left) at each of the lateral sector positions (right) to create a volume lesion.

laterally and longitudinally to ensure necrosis of the entire targeted prostate volume (Fig. 2). An automatic cooling device is used during treatment to maintain a constant baseline temperature of less than 18°C in the transrectal probe that helps to prevent thermal injury of the rectal mucosa.

HIFU Procedure

All patients were anesthetized by general, epidural, spinal or intravenous anesthesia, and were placed in a supine and open leg position. A condom was placed over the probe and degassed water was used to inflate the condom that was covered with ultrasound gel for close coupling of the ultrasound probe to the rectal wall, and the probe was inserted manually into the rectum. The probe was fixed in position by an articulating arm attached to the operating table. After selection of the treatment region of the prostate from the verumontanum to the bladder neck, the treatment was started. Transrectal probes with focal lengths of 3.0 and 4.0 cm were used according to the size of the prostate as determined by transrectal ultrasound (TRUS), with larger glands requiring longer focal lengths. The treatment continued layer by layer (10 mm thickness) from the apex to the base (Fig. 2). Usually, three successive target areas (anterior, mid-part and base) were defined to treat the whole prostate. After treatment was completed, a transurethral balloon catheter was inserted into the bladder[10].

Clinical Follow-up and Definition of Outcome

Patient status and treatment-related complications were followed up by all available means, including periodic patient visits and self-administered questionnaires dealing with urinary continence and erectile function using Functional Assessment of Cancer Therapy (FACT) questionnaire. Urinary symptoms and urinary flow rate analysis were performed using International Prostate Symptom Score (I-PSS) index and urowflowmetry[12,13]. Serum PSA was assayed every 1 to 6 months during follow-up. A postoperative prostate needle biopsy under TRUS was performed on all patients at 6 months. The American Society for Therapeutic Radiology and Oncology (ASTRO) consensus definition, i.e., three consecutive increases in post treatment PSA after a nadir has been achieved, was used to define biochemical failure[14]. The time to biochemical failure was defined as midway between the post treatment PSA nadir and the first of three consecutive PSA increases. None of the patients received androgen deprivation after HIFU or other anticancer therapy before documentation of a biochemical recurrence. HIFU related complications were defined by Japanese version of National Cancer Institute-Common Toxicity Criteria version 2.0[15].

Statistical Analyses

All statistical analyses were performed by the Department Statistics in Indiana University. The chi-square test was used to assess the correlation between preoperative and postoperative parameters. The distributions of biochemical disease-free survival times were calculated according to the Kaplan-Meier curves and the logrank test was used to compare curves for groups. All p values less than 0.05 reflected statistically significant differences.

RESULTS

A total of 75 patients were entered in the trial. The prostate was treated in 1 (75) or 2 (14) HIFU sessions in a total of 89 procedures (1.2 sessions/patient). One patient with stage T1b, 1 patient with a serum PSA of 20.60 ng/ml and 1 patient on whom treatment was stopped during the procedure because of appearance with large microbubbles in the prostate were excluded. The median age, serum PSA level and prostatic volume of the 72 patients analysed were 72 yrs (range 45 to 79), 8.10 ng/ml (range 2.10 to 19.80) and 22.1 ml (range 8.5 to 52.8), respectively. The TNM stage was T1c in 40 patients, T2a in 18 patients and T2b in 14 patients. All patients had a histological diagnosis of prostatic adenocarcinoma according to the Gleason grading system. The Gleason score was 2 to 4 in 9 patients, 5 to 7 in 55 patients, 8 to 10 in 6 patients and unknown in 2 patients (Table 1).

The median time of HIFU treatment and hospitalization was 169 min (range 65 to 485 min) and 5.0 days (range 2 to 55), respectively. The gland size decreased from an initial volume of 24.2 ml to a final median volume of 14.0 ml ($p < 0.01$) in 45 patients. Totally, 49 out of 72 (68%) had negative follow-up biopsies at 6 months after HIFU. Biochemical disease-free survival rates were analyzed in 60 patients. Twelve patients were excluded from the analysis for unsatisfactory followup. The median follow-up period for all patients was 14.0 months (range 2 to 24). Biochemical disease-free survival rates in all patients at 1

Table 1. Characteristics in 72 patients with localized prostate cancer

Median age (range)	72 (45–79)
Median PSA (range)	8.10 ng/ml (2.10–19.80)
Prostate volume (range)	22.1 (8.5–52.8)
Pretreatment PSA (%):	
10 or less	44 (61)
10.1–20	28 (39)
Clinical stage (%):	
T1c	40 (56)
T2a	18 (25)
T2b	14 (19)
Gleason score (%):	
2–4	9 (13)
5–7	55 (76)
8–10	6 (8)
Unknown	2 (3)
Median mos followup (range)	14.0 (2–24)

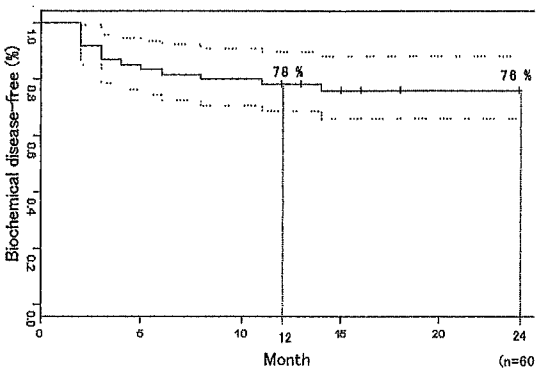

Fig. 3. Kaplan-Meier biochemical disease-free survival curves in all patients.

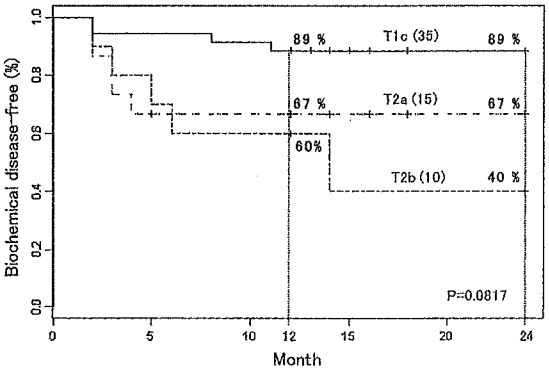

Fig. 4. Kaplan-Meier biochemical disease-free survival curves according to clinical stage.

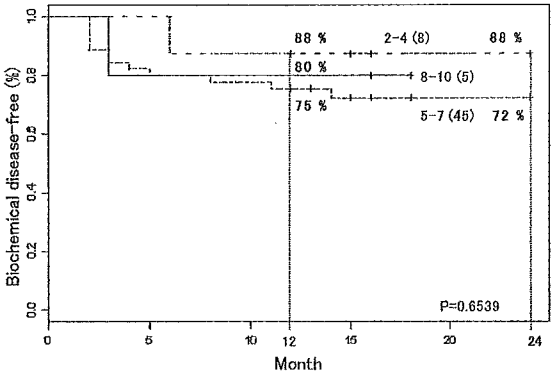

Fig. 5. Kaplan-Meier biochemical disease-free survival curves according to Gleason score.

and 2 years were 78% and 76%, respectively (Fig. 3). Biochemical disease-free survival rates in patients with stage T1c, T2a and T2b groups at 2 years were 89%, 67% and 40% ($p = 0.0817$, Fig. 4). Biochemical disease-free survival rates in patients with Gleason 2–4, 5–7 and 8–10 groups at 2 years were 88, 72% and 80% ($p = 0.6539$, Fig. 5). The biochemical disease-free survival rate in patients whose serum PSA less than 10 ng/ml and 10 – 20 ng/ml were 75% and 78% ($p = 0.6152$).

Prostatic volume was decreased from 24.2 ml to 14.0 ml at 6 months after HIFU ($p < 0.01$, Fig. 6). No statistically significant difference was noted in I-PSS, Q-max and FACT quality of life analysis (Fig. 7, 8 and 9).

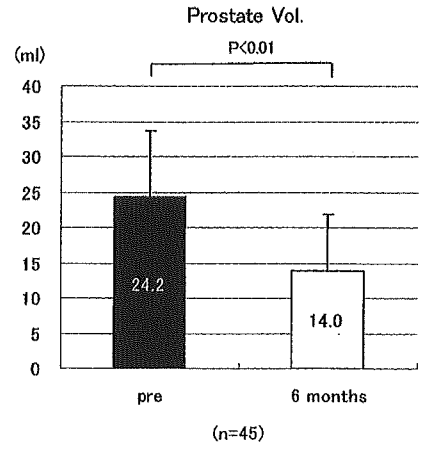

Fig. 6. Changes of prostatic volume.

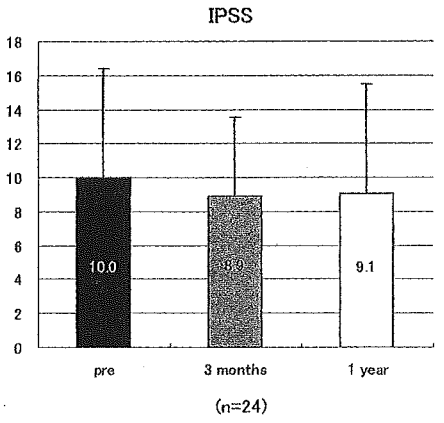

Fig. 7. Changes of International Prostatic Symptom Score.

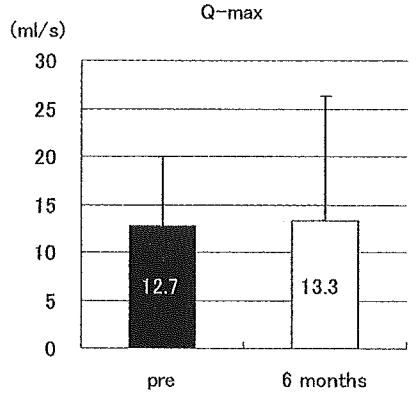

Fig. 8. Changes of maximum flow rate.

Thirteen out of 72 patients developed a urethral stricture, 6 and 4 patients developed epididymitis and prostatitis. Postoperative erectile dysfunction was noted in 12 out of 31 (39%) patients who were potent preoperatively. Nephrotic syndrome, transient urinary incontinence, transit stooly incontinence, balanoposthitis or retrograde ejaculation was observed in 1 patient each (Table 2).

For analysis of HIFU treatment using Sonablate 500^{TM}, ultrasound imaging for identifying prostate and quality levels were categorized more than good in patients with 92%. A transrectal probe was easily

Table 2. Complications

Complication	Grade 1	Grade 2	Grade 3	Grade 4	Total
Urethral stricture	0	0	13	0	13
Erectile dysfunction (31 potent patients)	0	0	12	0	12
Epididymitis	2	2	2	0	6
Prostatitis	2	0	2	0	4
Nephrotic syndrome	0	0	1	0	1
Balanoposthitis	1	0	0	0	1
Uninary incontinence (grade 1)	1	0	0	0	1
Stooly incontinence	1	0	0	0	1
Retograde ejaculation	1	0	0	0	1

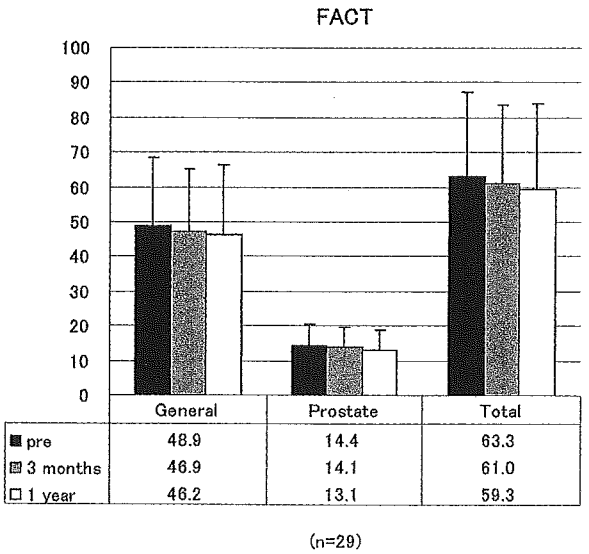

Fig. 9. Quality of life change by FACT general and prostate.

inserted into the rectum in 97% of the patients. Totally, 96% of the HIFU treatment was categorized as an easy procedure.

DISCUSSION

In 1995, Madersbacher et al. reported the effectiveness of HIFU in 10 cases of localized prostate cancer[8]. Histologically, HIFU-treated lesions of the prostate demonstrated a coagulation necrosis with sharp boundaries. In 1996, Gelet et al. reported preliminary experiences with HIFU using the Ablatherm device (EDAP-Technomed, Lyon, France) for treating localized prostate cancer[16]. Beerlage et al. reported the results of HIFU treatments in 111 patients with clinical stage T1-3N0M0 prostate cancer and a PSA level less than 25 ng/ml. The treatment for the first 49 patients was performed selectively (i.e. unilateral or bilateral treatment in one or two sessions depending on findings from TRUS and biopsies) and the whole prostate was treated in the remaining 62 patients. A complete response (defined as a PSA level < 4.0 ng/ml and a negative biopsy) was achieved in 60% of the whole prostate treated patients with and in 25% of selectively treated patients[17].

In 2001, Gelet et al. reported their long-term follow-up data in which a complete response was obtained in 66% of patients with no residual cancer (regardless of PSA levels) or no increases in PSA levels in three consecutive examinations with a PSA velocity < 0.75 ng/ml/year for patients with negative biopsies[18]. More recently, Chaussy and Thuroff summarized clinical outcomes by the ASTRO definition as 84.2% stability rate in the HIFU group and 80% rate in the combination with transurethral resection of the prostate (TURP) and HIFU group in 1 year[19]. In summarizing our clinical outcome using the ASTRO definition, the biochemically disease-free survival rate was 76% at 2 years follow-up. Patients with stage T1c, T2a and T2b showed resectively 89, 67% and 40% biochemical disease-free survival rates at 2 years follow-up ($p=0.0817$). The clinical outcome in our series of patients with preoperative PSA less than 20 ng/ml were comparable to the outcome of patients treated with radical prostatectomy[2,3].

In our series, postoperative urethral strictures at near verumontanum in the prostatic urethra occurred in 21% of the patients. Recently, TURP or bladder neck incision immediately before or after HIFU was found to reduce the treatment-related morbidity such as postoperative prolonged urinary retention, urinary catheterization time and urinary infection[20,21]. Neoadjuvant hormonal therapy also might be useful to reduce the volume of the prostate which can reduce the time of treatment and rate of morbidity. However, the upper limit of the gland volume is 50 ml even after reducing the size of the prostate with neoadjuvant androgen deprivation or TURP in our series. Generally, radicalism of prostate cancer and preservation of sexual function are always controversial because postoperative impotence depends on preservation of neuro-vascular bundles that sometimes includes tumor invasion. In our study, 39% of the patients exhibited erectile dysfunction after the HIFU therapy. One out of 12 patients who desired treatment for postoperative erectile dysfunction recovered with sildenafil citrate. We considered this rate to be lower than that compared to radical prostatectomy[2,3]. Further experience is required to confirm this important conclusion.

D'Amico et al. compared the outcome of a cohort

treated with 3D-CRT versus a matched cohort treated with brachytherapy plus external radiation therapy. The 5-year estimate of PSA failure-free survival rate after 3D-CRT alone was 45% and 67% when both radiation treatments were combined[22]. More recently, Kupelian et al. compared the biochemical disease-free survival rate after permanent seed brachytherapy, external beam radiation therapy (EBRT), combined seeds and EBRT, or radical prostatectomy for clinical stage T1–2 localized prostate cancer[23]. The 5-year biochemical disease-free survival rate for radical prostatectomy, EBRT <72 Gy, EBRT ≥72 Gy, permanent seed brachytherapy and combined seeds and EBRT were 81, 51, 81, 83% and 77%, respectively. Although not directly comparable, the results after treatment with HIFU appear to be similar to those after radiotherapy, even when both brachytherapy and EBRT are combined.

For many reasons, transrectal HIFU appears to be highly attractive as a minimally invasive treatment for localized prostate cancer. HIFU treatment requires no incision or puncture, with no bleeding, can be performed on an outpatient basis and is repeatable even when patients with local recurrence have already been treated with radiation therapy[24]. In addition, radiation therapy including brachytherapy and even surgery can be performed after HIFU.

Transrectal HIFU has considerable potential as a noninvasive treatment modality for patients with localized prostate cancer especially whose PSA less than 20 ng/ml.

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

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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.

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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.

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.

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.

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′-TGCACATCTCATGGGGGTTATAG-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′-

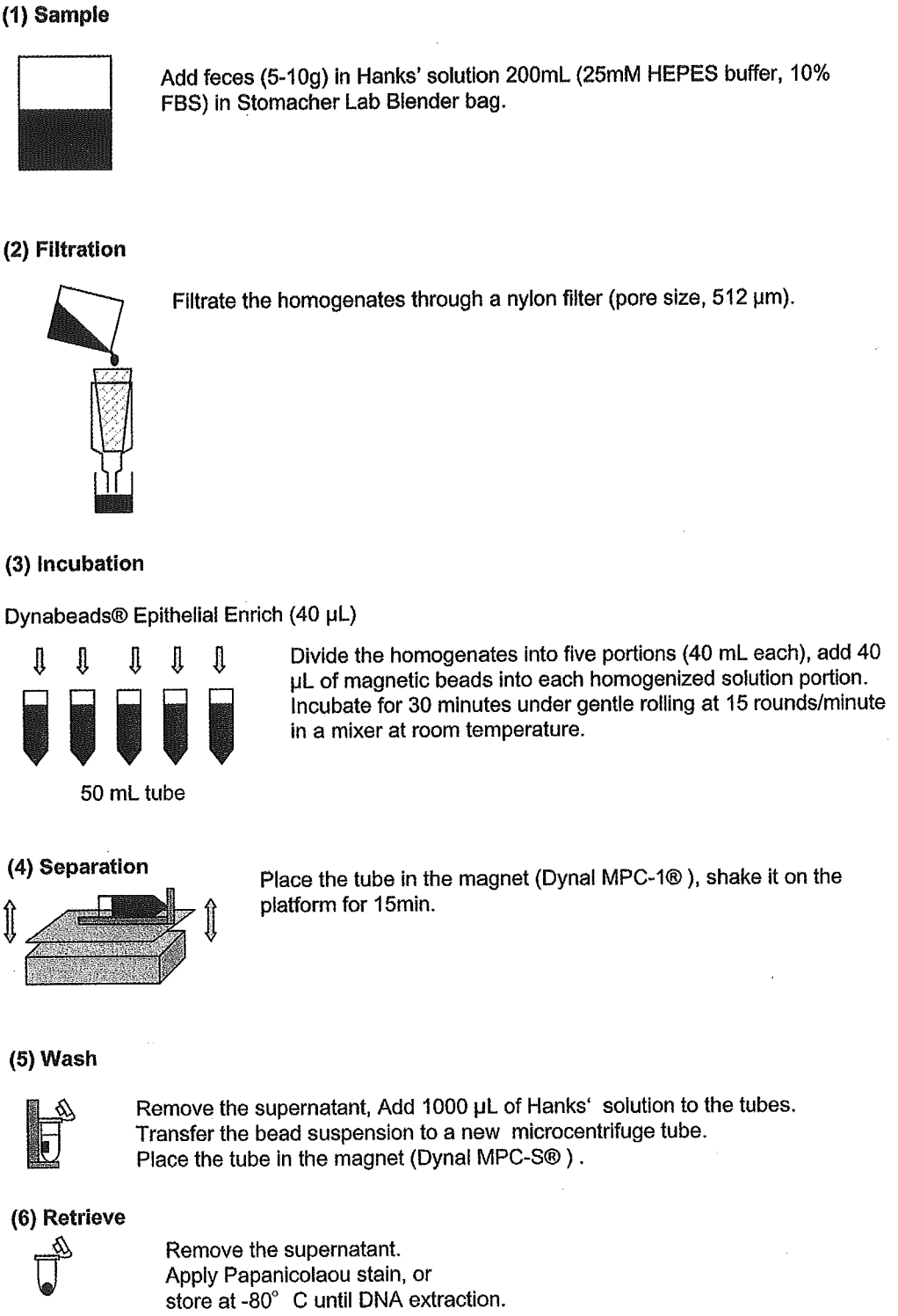

Figure 1. Schematic of procedure for isolating colonocytes from feces.

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-

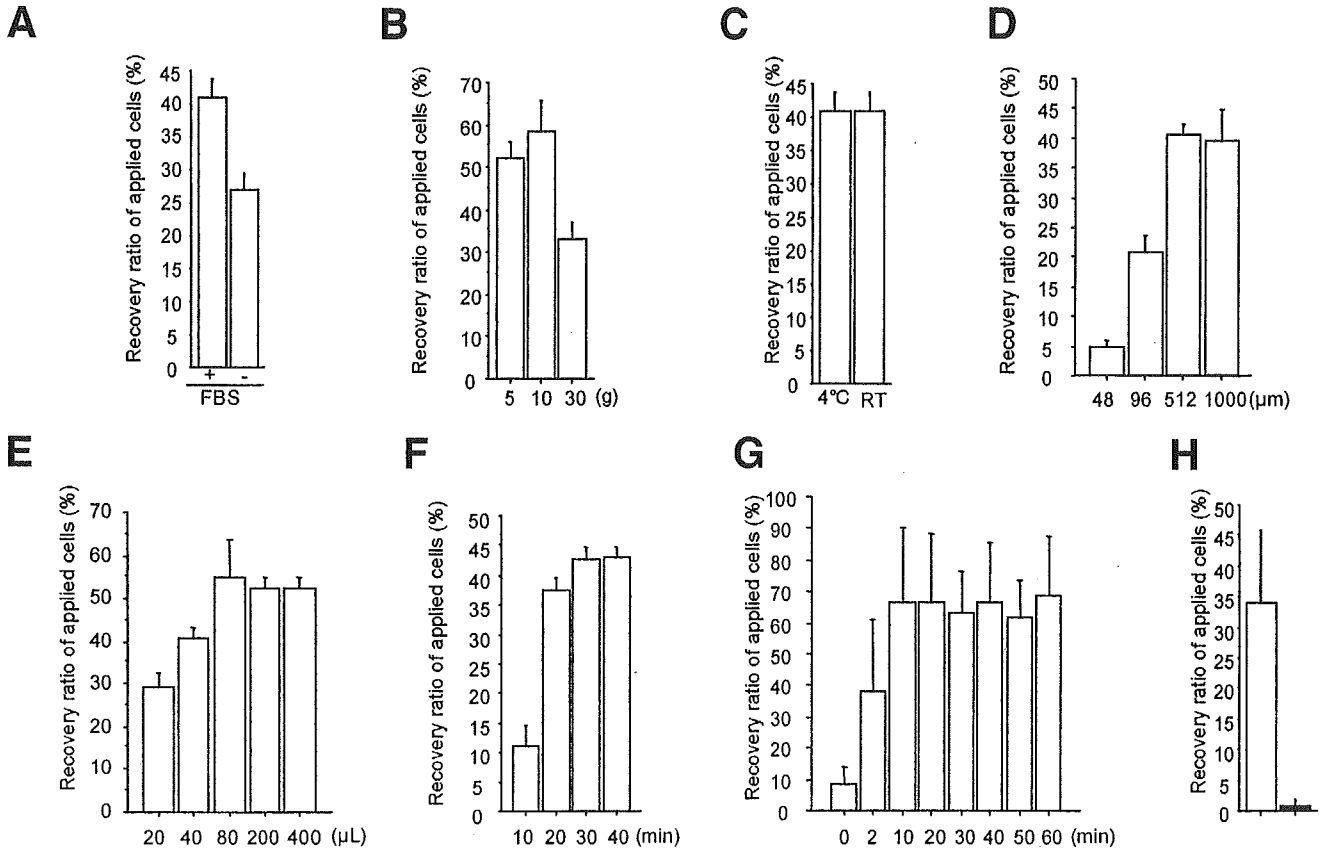

Figure 2. Simulation study to establish the optimal conditions for retrieving HT-29 colorectal cancer cells from feces and to compare the cell retrieval rates for the magnetic beads methods and the Percoll centrifugation method. Feces from healthy volunteers were divided into several portions, each of 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 Patients (n = 116), healthy volunteers (n = 83)	Combined marker	93	80 (72–87)	82	71 (62–79)	10	88 (79–94)
	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

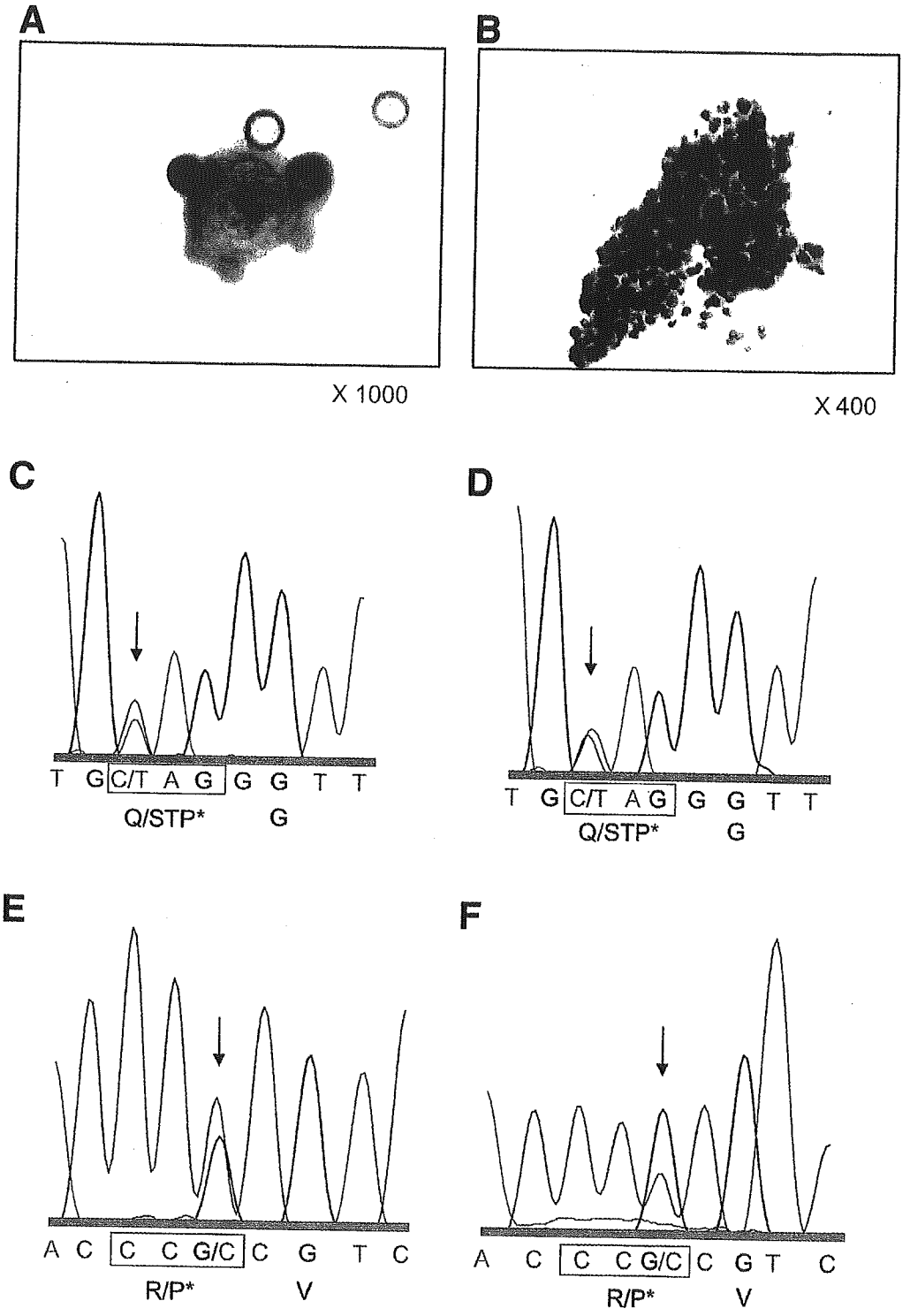

Figure 3. Cytology and DNA sequencing. Papanicolaou staining of colonocytes isolated from the feces of patients with colorectal cancer. (*A*) A patient with ascending colon cancer, Dukes' stage A. (*B*) A patient with rectal cancer, Dukes' stage C. Detection of mutations in tumor tissues and colonocytes 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.

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Meeting Report

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

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

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

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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

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.