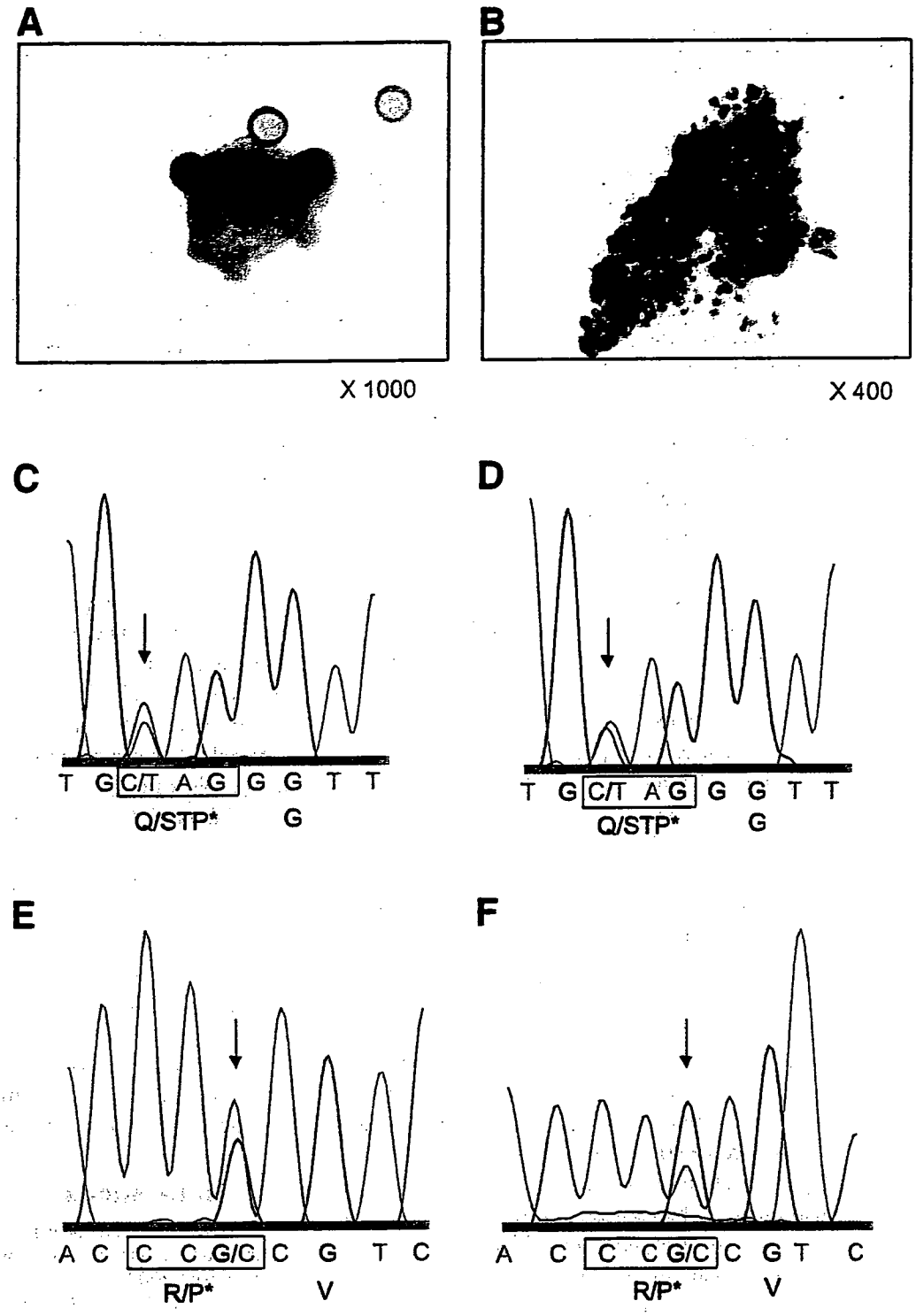

Figure 3. Cytology and DNA sequencing. Papanicolaou staining of colonocytes isolated from the feces of patients with colorectal cancer. (*A*) A patient with ascending colon cancer, Dukes' stage A. (*B*) A patient with rectal cancer, Dukes' stage C. Detection of mutations in tumor tissues and colonocytes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

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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

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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.

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.

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

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

levels. ADT was given before, during and after radiation therapy in 88.9, 88.1 and 79.8% of patients in PCS9901. Conformal therapy was performed in 43% and the median dose delivered was 68.4 Gy in PCS9901, which is increasing compared with 65 Gy in PCS9698. The PSA progression-free survival (<1.0 ng/ml) of patients receiving EBRT (66 Gy) and ADT at National Cancer Center Hospital (NCCH) was 65 and 57% at 5 and 10 years, respectively. The delivered dose was increased from 66 to 72 Gy about 2 years ago in NCCH. For patients in PCS9901, 24 and 16% had received dynamic and static 3D-CRT, respectively. Only 3% of them had received IMRT. RT has been recognized as curative treatment for PC in Japan.

The Role of High-Dose External Beam Radiotherapy in the Treatment of Prostate Cancer (By Michael Zelefsky)

Clinical trials during the last 5–10 years have demonstrated the need for increased radiation doses to achieve a maximal local tumor control for patients with clinically localized PC. Conventional RT with 65–70 Gy provided only 50 and 24% PSA control rate for T1–2 and T3 tumors, respectively. The 10 year PSA RFS rates for favorable risk patients treated to 75.6 Gy was 85% compared with 58% for 70.2 Gy and 47% for <70.2 Gy. For intermediate-risk patients, the 10 year PSA RFS for patients treated to 75.6 Gy was 54% compared with 45% for those with 70.2 Gy and 23% for dose levels <70.2 Gy. For unfavorable risk patients the 10 year PSA RFS for 75.6 Gy was 41% compared with 26% for 70.2 Gy and 10% for <70.2 Gy. A Cox regression analysis demonstrated that pretreatment PSA ($P < 0.001$), radiation dose, GS, clinical stage and neoadjuvant ADT were important independent predictors of PSA response. Post-treatment biopsy studies have also confirmed that higher doses >75.6 Gy have been associated with improved local control outcomes and improved metastasis-free survival. Higher radiation doses translate into improved tumor control which in turn reduces the risk of distant metastases and death from PC. With the advent of 3D-CRT and IMRT, the late side effects of therapy have been significantly reduced. These sophisticated treatment delivery systems have effectively reduced the volume of normal tissue carried to the higher radiation doses and have directly resulted in reduced frequencies of rectal bleeding and late urinary toxicities despite the application of dose levels as high as 86.4 Gy.

Wide Resection of the Prostate with Neoadjuvant Hormone Therapy (By Hiroyuki Fujimoto)

From randomized prospective studies, the efficacy of combining RP with neoadjuvant hormone therapy (NHT) for cT1-2 PC has not proven to be adequate in terms of biochemical or local control. For cT3-4 disease, RP alone is not favored because of high rate of positive margins and PSA failure. Urologists at the National Cancer Center have developed a new surgical method of wide resection of the prostate with 6–12 months of neoadjuvant ADT for patients with cT3-4 GS 7–9 tumors. From January 2000 to December 2003, 67 patients were enrolled for the non-nerve-sparing operation. Follow-up duration ranged from 210 to 1613 days (median 569 days). Wide resection was conducted without preservation of the bladder neck; the seminal vesicle, especially at their base, was covered by Denonvilliers' fascia. Pathological stage distribution was pT0 1 (2%), pT2 27 (42%), pT3a 17 (27%), pT3b 6 (9%) and pT4 13 (20%), respectively. The positive surgical margin rate was 10%. The projected 3 year PSA recurrence-free rate was 80% in all patients. Of note, pT0–pT3a patients had a 4 year PSA recurrence-free rate of 95%. No clinicopathological factors were found to be significant predictors for PSA recurrence. About 90% of patients were pad-free in 6 months. Preoperative risk analysis is necessary to avoid unsuccessful operation. Long-term follow-up is necessary.

Chairperson: Robert Myers

Improved Outcomes with Conformal Prostate Brachytherapy in the Treatment of Clinically Localized Prostate Cancer (By Michael Zelefsky)

Permanent seed brachytherapy has become an important treatment modality for PC. The advantage of seed implantation with I-125 or Pd-103 radioactive seeds is that the seeds can deliver a substantially higher radiation dose to the prostate and surrounding tissue compared with modern EBRT. The results of TRUS-based preplan brachytherapy at 10–15 year for favorable risk patients were excellent. However, for high-risk patients, preplan brachytherapy is associated with a poor outcome. Dr Grimm's data on brachytherapy alone showed that 126 patients with PC of GS < 7 were treated by the 'Seattle' method of prostate brachytherapy. Median PSA at presentation was 5.1 ng/ml. Median PSA-based follow-up time was 81.4 months. PSA progression-free survival based on the ASTRO failure definition is 85% at 10 years. Acute side effects do exist with brachytherapy, such as: urinary symptoms (31%), urethral stricture (11%), rectal bleeding (11%) and ED (35–40%). The limitations also include that preplan will not consistently reflect the anatomic conditions in the operation room. To overcome the distortion mismatch, intraoperative 3D-conformal treatment planning for prostate brachytherapy was developed at MSKCC. Procedure relies on real-time imaging and planning. Minimum dose delivered is 144 Gy and can be up to 288 Gy. Consequently, the improvement in conformality of the radiation dose distribution did lead to a reduction in toxicity, which then translates into a better biochemical outcome.

The Role of Salvage Radiotherapy after Prostatectomy (By Michael Zelefsky)

There has been an increasing interest in better defining the role of salvage RT for a rising PSA after RP. Only a select group of patients with disease confined to the prostate bed will benefit from RT. There are three ways of locating the possible source

of PSA relapse: diagnostic studies, PSA kinetics and prostatectomy pathology, albeit imprecise. The diagnostic studies may include MRI, bone scan, biopsy of the anastamosis, Prostascint study and PET. Immediate detectability of PSA after surgery suggests micro-metastatic disease. Delayed PSA recurrence suggests local residual disease. PSADT > 6 months suggests local disease. Presalvage RT PSA > 0.6 suggests distant failure. The pathology information is also helpful. Positive margin and positive extracapsular extension suggest local recurrence, whereas seminal vesical invasion and lymph node micrometastasis suggest distant failure. A multi-institutional study of salvage RT for failed RP (n = 537; *JAMA* 2004) showed that the long-term biochemical progression-free and metastasis-free rate at 8 years is around 30 and 55%, respectively. By multivariate analysis, high pretreatment PSA, high GS, negative surgical margin and short PSADT are the four independent risk factors predicting biochemical relapse after salvage RT. A nomogram is available for the prediction.

WHY IS PROSTATE CANCER INCREASING IN ASIAN COUNTRIES INCLUDING JAPAN? (BY TAIJI TSUKAMOTO)

Dr Tsukamoto first stated that 30 and 90 men die of PC every day in Japan and the United States, respectively, and that 60 and 600 men are diagnosed as PC every day in Japan and the United States, respectively. The age-adjusted mortality rates per 100 000 of PC in Japanese men have gradually increased with 0.5 in 1950, 3.8 in 1975, 6.0 in 1990 and 8.4 in 2001. The increase has skyrocketed since 1990. This increase pattern of the disease is similar to that found in the United States where the incidence suddenly started to increase in 1985, reached the maximum in 1993 and decreased thereafter. Prolongation of lifespan, early detection with PSA, changes in lifestyle and genetic predisposition may be responsible for elevated incidence and mortality rates. PSA examination in clinics detected 5–8% of patients with PC among those with lower urinary tract symptoms. The PC incidence for Japanese living in Hawaii is higher than Japanese living in Japan but still lower than Americans. It is reported that mutation pattern in surgical specimens is different between Caucasian and Japanese men. It has been shown that Japanese men have a smaller prostate volume than American, Scotland and Dutch men across all age groups between 40 and 80 years of age.

CLOSING

CHAIRPERSON: ROBERT MYERS

SUMMARY OF SYMPOSIUM AND CLOSING REMARKS (BY TADAO KAKIZOE)

Dr Kakizoe gave the closing remarks by summarizing the lectures that have been given in the past two-and-a-half days. We learned that PC is the most common cancer of males in several developed countries. PC is also increasing sharply in some Asian countries including Japan, Korea and Taiwan. PC is full of heterogeneity of phenotypes and genotypes. PSADT is a good marker for watchful waiting; latent cancer, screened cancer and clinical cancer show same overexpression of p53 and Ki-67. There definitely is harm from screening such as psychological stress, side effects and overtreatment and the benefit of screening is to be shown. Serum PSA is correlated with PC volume. PSA < 2.5 is advised as a new cut-point for Americans. We need to develop new markers and imaging procedures. In chemoprevention of PC, we doubted that 'prevented' tumors may not be biologically and clinically important. NVB preservation may be associated not only with sexual function but also with better continence recovery. Significant progress has been made in the molecular mechanism of prostate tumorigenesis, development of gene therapy and immunotherapy in Japan. We also had lectures that covered the optimal timing of ADT and the recent progress in chemotherapy for PC. There are multiple choices of therapies for PSA recurrence after RP. We also know the expected results of salvage RT and ADT for these patients. Various forms of RT have become major tools for the treatment of these patients. There are multiple nomograms available to be used to predict treatment outcome. A successful and fruitful symposium was concluded.

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A Flexible Endoscopic Surgical System: First Report on a Conceptual Design of the System Validated by Experiments

Toshiaki Kobayashi[1], Sean Lemoine[1,2], Akihiko Sugawara[1,2], Takaaki Tsuchida[1], Takuji Gotoda[1], Ichiro Oda[1], Hirohisa Ueda[3] and Tadao Kakizoe[1]

[1]National Cancer Center, [2]Japan Association for the Advancement of Medical Equipment and [3]Pentax Corporation, Tokyo, Japan

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Background: Surgery is a standard diagnostic and therapeutic procedure. However, its technical difficulty and invasiveness pose problems that are yet to be solved even by current surgical robots. Flexible endoscopes can access regions deep inside the body with less invasiveness than surgical approaches. Conceptually, this ability can be a solution to some of the surgical problems.
Methods: A flexible (surgical) endoscopic surgical system was developed consisting of an outer and two inner endoscopes introduced through two larger working channels of the outer endoscope. The concept of the system as a surgical instrument was assessed by animal experiments.
Results: Gastric mucosa of the swine could be successfully resected using the flexible endoscopic surgical system, thereby showing us the prospect and directions for further development of the system.
Conclusion: The concept of a flexible endoscopic surgical system is considered to offer some solutions for problems in surgery.

Key words: surgical robot – endoscopic surgery – surgery – robotics – endoscope

INTRODUCTION

We recently reported a new concept for endoscopic mucosal resection of gastric cancer with the use of a magnetic anchor. The anchor consisted of microforceps and a magnetic weight in order to grasp, stabilize and pull up the gastric mucosa (1). During the experiments, we thought that the procedure would be easier if one more endoscope was present to hold and stabilize the mucosa instead of the magnetic anchor.

Concerning flexible endoscopes, there are some ultrathin endoscopes that can be inserted into the working channels of standard endoscopes, such as gastrointestinal endoscopes. If the outer endoscope is able to contain larger and multiple working channels, several thin endoscopes could be inserted through the outer endoscope. This would allow for the resecting procedures. Such a system could also be applied to the fields where current surgical robots are targeting.

One of the problems with current surgical robots is inaccessibility to regions located deep inside the body, particularly regions reached through narrow and winding routes, such as the digestive tracts and blood vessels. However, some early gastric cancers can be resected endoscopically with much less invasiveness than surgery. These surgeries cannot be performed by current surgical robot systems because those regions were not originally considered places for the systems to operate.

An experimental flexible endoscopic surgical system was developed to cope with these problems of accessibility, consisting of a flexible outer endoscope with two working channels through which two inner flexible endoscopes could be inserted. These inner endoscopes were designed to have similar functions as flexible gastrointestinal endoscopes allowing for performance of standard endoscopic procedures even when introduced through the outer endoscope.

The uses of the flexible endoscopic system as a surgical instrument, as well as its functionality, were confirmed during gastric mucosal resection of the swine. This is in contrast to the current limitations for surgical robotics in terms of lesion access.

MATERIALS AND METHODS

FLEXIBLE SURGICAL ENDOSCOPE

As shown in Fig. 1, the flexible surgical endoscope consists of an outer flexible endoscope and two inner flexible endoscopes inserted into the working channel of the outer endoscope. The specifications of these endoscopes are listed in Table 1.

For reprints and all correspondence: Toshiaki Kobayashi, Cancer Screening Technology Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan. E-mail: tkobayas@ncc.go.jp

The outer endoscope also has a 2.8 mm working channel and a charge coupled device (CCD) enabling the endoscope to operate in a similar fashion as standard gastrointestinal endoscopes. The endoscopic images are observed on cathode ray tube (CRT) monitors in the same manner as video-endoscopes.

Each of the inner endoscopes has a 2.0 mm working channel allowing accessories such as forceps and an electrocautery tip to be introduced and used. Unlike the outer endoscope, the inner endoscopes have optic fiber bundles for image visualization, instead of a CCD. These endoscopic images are also observed on CRT monitors. However, a video-adaptor, i.e. a small CCD video camera, must be connected onto each eye piece of the inner endoscopes in order to view the image on the monitors.

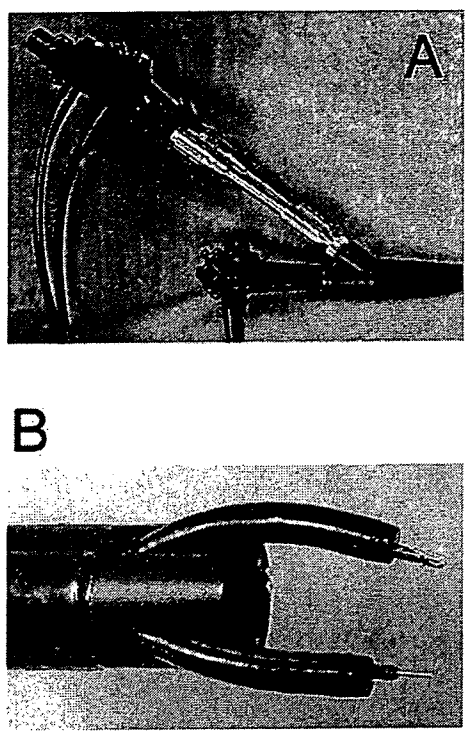

Figure 1. The flexible endoscopic surgical system. (A) The inner endoscope is inserted through a telescopic connecting device, which connects to the opening for the working channel of the outer endoscope near its control section. (B) At the tip of the outer endoscope two inner endoscopes protrude laterally, obtaining a certain distance between the two endoscopes.

Table 1. Specifications of the flexible endoscopic surgical system

	Outer endoscope	Inner endoscope
Total length (mm)	975	1395
Working length (mm)	665	1050
Insertion portion diameter (mm)	20	4.9
Tip bending (degree) (up/down, right/left)	210/120, 120/120	210/120, 120/120
Field of view (degree)	140	120
Depth of field (mm)	4–100	3–50
Channel diameter (mm)	7, 7, 2.8	2

These combined endoscopes are manipulated manually by three physicians together with the help of several assistants. The system, as a whole, operates similar to surgical robotic systems.

Physicians

Two series of experiments were conducted. The first series was performed by a senior endoscopist and three resident physicians in order to assess the system with consideration to its endoscopic nature. The senior endoscopist was trained within the specialty of internal medicine, whereas one of the resident physicians was in training for internal medicine and the other two were for surgery.

The purpose of the subsequent series was to assess the concept of the flexible surgical endoscope from the viewpoint of surgeons. Consequently, the procedure was performed by two senior endoscopists, one having more than 15 year experience as a surgeon and the other having some surgical training, in addition to two residents who were in training for surgery.

These two series were performed on separate occasions, with none of the physicians performing in both series.

Test Subject

Three female swine, under intravenous anesthesia, were laid on an examination table in the left lateral position. Within the first experiment, a 35.6 kg and a 34.1 kg swine were used. In the following experiment, a 41.8 kg swine was used. During these experiments, the law for the humane treatment and management of animals was observed.

Procedure

The procedure was similar to standard endoscopic mucosal resection with the exception of one more endoscope for stabilization of the gastric mucosa.

First, an incision was made in the mucosa surrounding the region of stomach intended for resection (2,3). The outer endoscope was inserted through the esophagus into the gastric cavity. Subsequently, using the telescopic connecting devices (Fig. 1), the inner endoscopes were inserted into the working channels of the outer endoscope and introduced into the gastric cavity.

The outer endoscope was placed near the region in which the first incision was made. Thereafter, the resecting procedure was performed using an electrosurgical knife through one of the working channels of the inner endoscopes, whereas the other contained forceps. Within the procedure, the operator decided which side of the working channels would use the electrosurgical knife.

These procedures were observed on three CRT monitors, each of which was connected to its endoscopic counter part.

The resecting procedures were performed on the anterior wall of the gastric angle, the anterior wall of the middle gastric body and the greater curvature of the middle gastric body in the

first series for the assessment of endoscopic features. Within the following series, the resecting procedures were performed on two regions adjacent to the greater curvature of the lower gastric body.

RESULTS

Concerning insertion of the outer endoscope through the esophagus into the gastric cavity, some difficulties were encountered owing to the large diameter of the outer endoscope and the relatively small size of the swine in both experimental series. However, the outer endoscope was introduced into the gastric cavity.

As for insertion of the inner endoscopes through the working channels of the outer endoscope, there were no difficulties experienced, even when the outer endoscope was bent due to insertion through the esophagus. Access to regions of the gastric wall was limited to the greater curvature due to the rigidity of the outer endoscope.

Maneuverability of the flexible endoscopic surgery system was satisfactory regarding the experiments were the first experiences for the physicians involved, despite some problems to solve.

The images from the outer endoscope were similar to those of standard gastrointestinal video-endoscopes due to the CCD system used in the outer endoscope. However, the images from the inner endoscopes were inferior to those of the outer endoscope. This inferiority was attributed to the limited number of optical fibers within the inner endoscope and deterioration of the image caused by conversion from optical images to electrical images through the use of a video-adapter. Consequently, during most of the procedure, endoscopic images were mainly observed using the monitor for the outer endoscope.

Some differences in use of the inner endoscopes for the resecting procedures between the first series and the second series were noticed. In the first series, the physicians appeared to have difficulties in some of the procedures such as accessing the mucosa, stabilization of the mucosal flap and resection procedures. These procedures were considered standard techniques for actual surgery, which means surgical experiences are required even to maneuver the flexible endoscopic surgical system.

Within the second series conducted by endoscopists with surgical experience, the resecting procedures were satisfactory, despite the fact this was their first experience using the system (Fig. 2). Through cooperation between the operator and assistants using verbal commands, manipulation of the inner endoscopes and the outer endoscope could be achieved. The functions of the inner endoscopes could be modified by changing the instruments inserted into the working channels. The flexible nature of the inner endoscopes allowed additional functions such as stabilization of the gastric wall by the longitudinal flank of the endoscope, as shown in Fig. 2C.

Within all the experiments, resecting procedures were completed without any complications such as perforation of the gastric wall. Consequently, five mucosal pieces, with sizes of

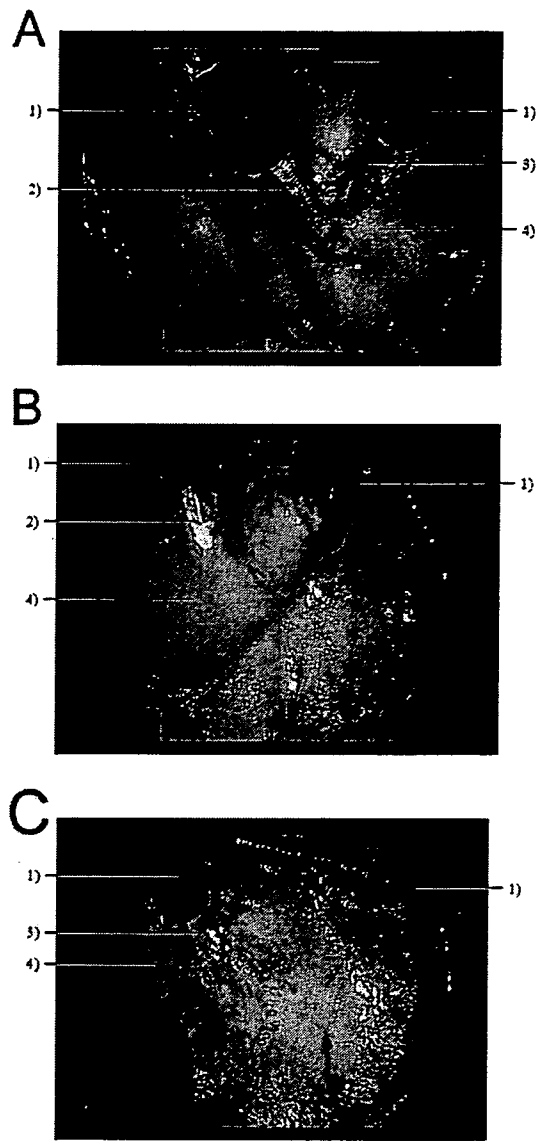

Figure 2. Images of the resecting procedures. (1) Inner endoscope, (2) forceps, (3) electrosurgical knife and (4) mucosal flap. (A) The right inner endoscope, with an electrosurgical knife introduced through its working channel, was maneuvered by the operator. The left inner endoscope, with forceps, was maneuvered by an assistant. (B) The tip of the right inner endoscope is holding up the mucosal flap in order to assist the forceps of the left inner endoscope to grasp the mucosal flap. (C) The right inner endoscope is pulling up the mucosal flap using forceps concealed in this image. In addition, using the flexibility of the endoscope, the gastric wall is stabilized by the longitudinal flank of the inner endoscope.

2.8×1.6 cm^2, 2.8×2.7 cm^2 and 2.6×2.0 cm^2 in the first series, and 3.2×2.7 cm^2 and 4.0×3.4 cm^2 in the second series were each resected in a single piece.

DISCUSSION

Surgical procedures are good options for diagnosis and treatment providing several advantages over non-surgical

approaches, especially in cases of malignant diseases. Although surgery is well accepted as a standard procedure in medicine there are still some problems left unsettled.

The technical difficulty of surgery is a common problem particularly for trainees, but even for experienced surgeons who have some technical limitations. Surgical procedures are difficult for regions deep in the body because the visual field for surgeons is limited, the number of surgical instruments which can be introduced is limited and the movements of these instruments are limited. One of the exemplary regions of this problem is the pelvic cavity, which includes surgery of rectal and prostate cancers.

Invasiveness is an inherent drawback to surgery, discouraging patients to undergo surgical treatment even when it is appropriate. It is true that surgery should be avoided when there are other less invasive alternatives.

Surgical robots such as the da Vinci system and the Zeus system are highly advanced medical instruments allowing for fine movements when appropriately manipulated by surgical experts. These systems are expected to solve some surgical problems such as invasiveness and the difficulty (4–8). Thus far, the systems have been able to solve some of the problems associated with surgery.

As for the invasiveness of surgery, endoscopic surgeries such as laparoscopy can be performed with robotic systems, utilizing smaller incisions than those of other standard open surgical approaches. The precise movements of surgeons are facilitated by robotic systems. However, laparoscopic procedures can be performed even without the robotic systems with the same amount of invasiveness.

Current robotic systems may also pose problems (4–8), such as the limited number of surgeons who can manipulate the system, which is usually one. Additional training for the specific manipulating methods of the systems is another problem, as well as introduction costs. Consequently, it is currently not clear what the benefits of these robotic systems are, especially when assessed from the patient side. Moreover, problems which even surgical experts suffer from have not been solved.

Flexible endoscopes have been developed to cope with the problems of accessing regions through narrow tracts such as the esophagus and the tracheobronchial tree. Even in these regions flexible endoscopes can perform surgical procedures similar to standard surgery. Therefore, endoscopes are naturally considered functional even in other cavities such as the abdomen and pelvic cavities.

It would be easier and more functional to perform an operation using several endoscopic instruments introduced through the end of one endoscope, rather than conducting resection using only one endoscopic instrument introduced into one endoscope, as done in standard endoscopic procedures. The simplest model for this concept is the flexible endoscopic surgical system we developed and examined within these trials.

We assumed that there would be several problems with the flexible endoscopic surgical system when used clinically as it is merely a conceptual model to confirm its feasibility of use. However, despite those problems, the system was able to perform surgical resection. In addition, the problems encountered within the first experiment were inherent in all technical procedures.

Of interest, these problems showed us that, when indicated for resecting procedures, the flexible endoscopic surgical system is easier to manipulate by surgeons and not by endoscopists despite its endoscopic appearances.

The images of the inner endoscopes were not satisfactory because a CCD was not used in these endoscopes. Consequently, resecting procedures were monitored by images from the outer endoscope which contained the CCD. In this situation, the operator had to control the inner endoscope via observations on the monitor of the outer endoscope. This is different from standard endoscopic procedures in which images are observed on the monitor of the endoscope which the operator is controlling.

In general, it is not easy for trainees to understand appropriate surgical procedures, i.e. where to cut and where to stabilize. Verbal communication during operation is important to facilitate appropriate assistance, which was not adequately utilized in the first series. These issues are to be learned through years of experience and cannot be achieved instantly.

As mentioned above, the difference between the two experiments may reveal that for these flexible endoscopes, surgical experience is an important factor, when the system is indicated for surgical procedures. The limitation of the inner endoscopes, not having CCD may have emphasized this issue. Consequently, the next system is to consist of two inner endoscopes with a CCD for each. This would allow the operators to control the inner endoscopes in such a manner as used for standard gastrointestinal endoscopic procedures.

Furthermore, we think that there should be two styles of design for future flexible endoscopic surgical systems; one with increased surgical maneuverability designed particularly for the techniques of surgeons, the other preserving flexible endoscopic maneuverability for endoscopists. Although it has not been decided yet which design is more appropriate for a future surgical system, endoscopists may be able to become accustomed to the flexible endoscopic surgical system with surgical maneuverability when the system is popularized.

In addition to the merits mentioned above, flexible endoscopic materials can theoretically be made compatible with X-ray systems such as fluoroscopes and computed tomography (CT) systems, exemplified by such procedures as X-ray guided bronchoscopy. In the future, the materials used for flexible endoscopic constructions are expected to acquire compatibility with the magnetic fields of magnetic resonance imaging (MRI) systems.

As mentioned before, limitations in visualization pose surgical problems even for experienced surgeons. This may only partially be solved by the subjective ability of surgeons to presume the identity of invisible objects using their tactile sense and their intuition. Actually, the compatibility with imaging systems was one of the important requirements for the design of the flexible endoscopic surgical system,

allowing visibility of anatomical information invisible to the surgeon's eyes.

In order to make one more step towards the future for less invasive and more effective medical treatments, we believe that future surgical systems should acquire the accessibility to narrow regions located deep inside the body together with the compatibility of imaging systems such as CT and MRI. Thus, from the flexible nature and structural characteristics of a non-jointed, smooth outer sheath, we selected the flexible endoscope as the conceptual basis of development for our system. It is the combination of these and the aforementioned aspects that allows for minimization in invasiveness, through the use of pre-existing natural structures and tracts for lesion access to deep regions, and with the presence of multiple interchangeable inner-scopes, an increase in distal tip functionality at the surgical site. Although there are several factors still to discuss and develop, the concept of the flexible endoscopic surgical system is considered an appropriate development for a future surgical robotic system with this current system being a successful step towards that furture.

Acknowledgments

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DNA HYPOMETHYLATION ON PERICENTROMERIC SATELLITE REGIONS SIGNIFICANTLY CORRELATES WITH LOSS OF HETEROZYGOSITY ON CHROMOSOME 9 IN UROTHELIAL CARCINOMAS

TOHRU NAKAGAWA, YAE KANAI,* SAORI USHIJIMA, TADAICHI KITAMURA, TADAO KAKIZOE AND SETSUO HIROHASHI

From the Pathology Division, National Cancer Center Research Institute (TN, YK, SU, SH), Department of Urology, Faculty of Medicine, Tokyo University (TN, TKi) and National Cancer Center (TKa), Tokyo, Japan

ABSTRACT

Purpose: DNA methylation has important roles in genomic stability. Accordingly DNA hypomethylation on pericentromeric satellite regions may induce chromosomal instability through heterochromatin decondensation and chromosomal recombination enhancement. We elucidated the significance of aberrant DNA methylation on pericentromeric satellite regions during urothelial carcinogenesis.

Materials and Methods: We examined DNA methylation status on satellites 2 and 3 by Southern blotting and determined the allelic status of chromosome 9 using 6 microsatellite markers (D9S775, D9S925, D9S304, D9S303, D9S283 and D9S747) in 27 transitional cell carcinomas of the bladder, ureter or renal pelvis and corresponding noncancerous tissues.

Results: DNA hypomethylation on satellites 2 and 3 was detected in 2 (7%) and no (0%) noncancerous tissues, and in 11 (41%) and 12 (44%) urothelial carcinomas, respectively. DNA hypomethylation in urothelial carcinomas significantly correlated with histological grade ($p = 0.0012$ and 0.0043), invasion depth ($p = 0.0055$ and 0.0228) and morphological structure (papillary vs nodular, $p = 0.0161$ and 0.0297) for satellites 2 and 3, respectively. Loss of heterozygosity on at least 1 locus of chromosome 9 was detected in 14 urothelial carcinomas (52%). DNA hypomethylation on satellites 2 ($p = 0.0098$) and 3 ($p = 0.0034$) significantly correlated with loss of heterozygosity on chromosome 9.

Conclusions: DNA hypomethylation on pericentromeric satellite regions may participate in the development and progression of urothelial carcinomas by inducing loss of heterozygosity on chromosome 9.

KEY WORDS: urothelium; carcinoma, transitional cell; DNA methylation; chromosomal instability; loss of heterozygosity

DNA methylation has important roles in transcriptional regulation, chromatin remodeling and genomic stability.[1] Satellites 2 and 3, which are related families containing a frequent 5 bp repeat, are abundant in pericentromeric heterochromatin regions on chromosomes 1, 9 and 16, and heavily methylated in normal somatic cells.[2] DNA hypomethylation on such pericentromeric satellite regions may induce chromosomal instability through heterochromatin decondensation and chromosomal recombination enhancement.[3,4] DNA hypomethylation on satellites 2 and 3 has been reported to cause chromosomal instability, such as the formation of multiradiate chromosomes composed of chromosomes 1, 9 and 16, in ICF (immunodeficiency-chromosomal instability-facial anomalies) syndrome.[2]

In human cancers overall DNA hypomethylation accompanied by region specific hypermethylation is generally observed.[1] Aberrant DNA methylation may be involved in carcinogenesis by at least three possible mechanisms: induction of genomic instability as a result of decreased methylation level,[5–7] increased gene mutagenicity caused by deamination of 5-methylcytosine to thymine and repression of gene transcription through CpG island methylation in specific gene regulatory regions, including tumor suppressor genes.[1] For example, frequent chromosomal 1q copy gain with a pericentromeric break point has been reported in hepatocellular carcinomas showing DNA hypomethylation on satellite 2.[8]

The role of DNA hypomethylation in urothelial carcinomas is not fully understood, although aberrant hypermethylation on CpG islands around the promoter region and decreased expression of tumor suppressor genes, such as the *p16* and *E-cadherin* genes, have been reported.[9,10] In addition, loss of heterozygosity (LOH) on chromosome 9 is the most common genetic abnormality in urothelial carcinomas.[11] Consequently we focused on the clinicopathological significance of DNA hypomethylation on pericentromeric satellite regions in urothelial carcinomas and examined whether this hypomethylation is the underlying mechanism for LOH on chromosome 9 during human urothelial carcinogenesis.

MATERIALS AND METHODS

Patients and tissue samples. Paired specimens of primary urothelial carcinoma and corresponding noncancerous tissue were obtained from surgically resected specimens from 27 patients (U1 to U27) treated at National Cancer Center Hospital, Tokyo, Japan. The patients were 22 men and 5 women with a mean age ± SD of 67.6 ± 10.5 years (range 50 to 85).

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* Correspondence: Pathology Division, National Cancer Center Research Institute, 5–1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan (FAX: 81–3-3248–2463; e-mail: ykanai@ncc.go.jp).

The primary tumor sites were the bladder, ureter and renal pelvis in 13, 5 and 9 patients, respectively. Based on histological examination 5 (19%), 10 (37%) and 12 (44%) tumors were classified as G1, G2 and G3–4 transitional cell carcinoma, while 11 (41%) and 16 (59%) were superficial (pTa and pT1) and invasive (pT2 to pT4), respectively.[12] Morphologically 21 tumors (78%) were papillary carcinoma and 6 (22%) were nodular carcinoma. Noncancerous specimens were obtained from the urothelium distant from the carcinoma.[13] In cases of widely spreading carcinomas in situ, as diagnosed histopathologically in preoperative biopsy specimens, the muscle layer of the bladder or the renal parenchyma was collected as noncancerous specimens since macroscopic examination cannot necessarily discriminate noncancerous urothelium from carcinoma in situ.

Southern blotting for pericentromeric satellite regions. High molecular weight DNA was isolated from fresh tissue samples by phenol-chloroform extraction and dialysis. DNA methylation status was assessed by digesting DNA with *Msp* I and *Hpa* II, which cut at the sequence CCGG. *Hpa* II does not cut when the internal cytosine is methylated. High molecular weight DNA (5 μg) was digested for 24 hours with 10 U *Msp* I or *Hpa* II/μg DNA. DNA fragments were separated by electrophoresis, transferred to nitrocellulose membranes and hybridized with ^{32}P labeled DNA probes. Previously described oligonucleotides were used as probes for satellites 2 and 3.[14]

Analysis of LOH on chromosome 9. Genomic DNA was amplified by polymerase chain reaction (PCR) using oligonucleotide primers for 6 microsatellite loci on chromosome 9, namely D9S775, D9S925, D9S304, D9S303, D9S283 and D9S747. Primer sequences were D9S775 (9p23) 5′-AAAGTAGCCATCCGTGTGT-3′ and 5′-GCTTTCTTTGATGGTTTACAG-3′, D9S925 (9p21–22) 5′-GTCTGGGTTCTCCAAAGAAA-3′ and 5′-TGTGAGCCAAGGCCTTATAG-3′, D9S304 (9p21) 5′-GTGCACCTCTACACCCAGAC-3′ and 5′-TGTGCCCACACACATCTATC-3′, D9S303 (9q21) 5′-CAACAAAGCAAGATCCCTTC-3′ and 5′-TAGGTACTTGGAAACTCTTGGC-3′, D9S283 (9q22) 5′-TGCTGGATTTCAGGTAGGG-3′ and 5′-ATGGTTATGCGGGTGTATTTCTC-3′, and D9S747 (9q32) 5′-GCCATTATTGACTCTGGAAAAGAC-3′ and 5′-CAGGCTCTCAAAATATGAACAAAAT-3′. The 5′ ends of forward primers were labeled with 6-carboxyfluorescein and PCR amplifications were performed with 20 ng genomic DNA. Subsequently PCR products were fractionated by electrophoresis (ABI 3100 sequencer, Applied Biosystems, Foster City, California) according to the manufacturer protocol. Data were analyzed with the GeneScan, version 3.7 computer program (Applied Biosystems). When 2 amplified bands per locus were detected in the noncancerous tissue specimen, the case was considered informative for LOH analysis. LOH was recorded when signal intensity for a tumor allele was decreased by more than 50% relative to the matched normal allele in informative cases, as described previously.[15–17] Replication error was identified by the presence of band shifts or the presence of novel bands in PCR products.

Statistics. Correlations between any 2 of DNA methylation status, allelic status and clinicopathological parameters were analyzed by the chi-square test with $p < 0.05$ considered significant.

RESULTS

DNA methylation status on pericentromeric satellite regions and its correlation with clinicopathological parameters. Figure 1 shows examples of Southern blotting. In 25 (93%) and all 27 (100%) noncancerous tissue specimens examined significantly larger DNA fragments were detected in *Hpa* II digests compared with *Msp* I digests at satellites 2 and 3, respectively, indicating that these regions were heavily methylated. In 11 (41%) and 12 (44%) urothelial carcinomas smaller fragments were detected in *Hpa* II digest compared

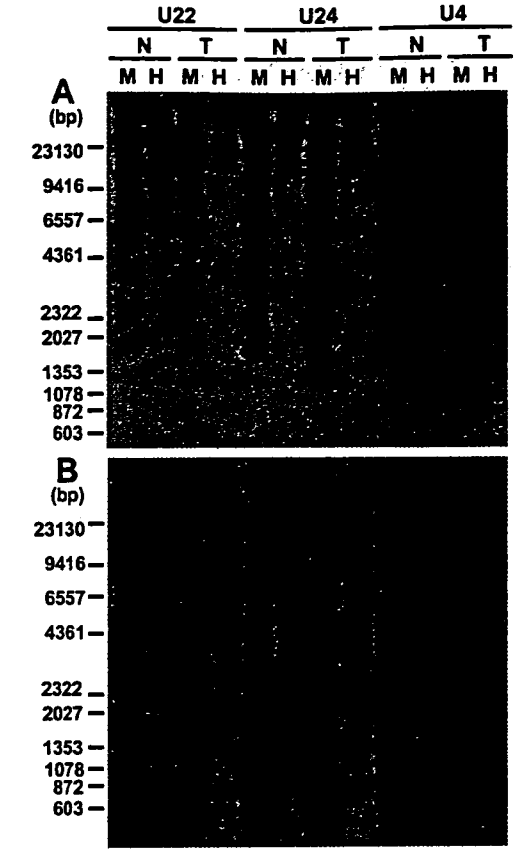

FIG. 1. Examples of Southern blotting for satellites 2 (*A*) and 3 (*B*) in cases of urothelial carcinoma. Methylation status was assessed by digesting DNA with *Msp* I (*Lane M*) and *Hpa* II (*Lane H*). DNA fragments were separated by electrophoresis, transferred to nitrocellulose membranes and hybridized with ^{32}P labeled DNA probes. Larger bands were detected in lane H compared with lane M in all noncancerous tissue (*N*), and in U22T and U24T, indicating that satellite 2 region was heavily methylated (*A*). In U4T lane H showed same hybridization pattern as lane M, indicating that this region was hypomethylated. (*B*) In all noncancerous tissues, and U22T and U24T satellite 3 region was heavily methylated, whereas this region was hypomethylated in U4T. *T*, cancerous tissue.

with corresponding normal tissues or *Hpa* II digest showed almost the same hybridization pattern as the *Msp* I digest of the same sample and the corresponding normal tissue, indicating that these regions were hypomethylated. In almost all carcinoma samples in which DNA hypomethylation was detected hypomethylation occurred on satellites 2 and 3.

DNA hypomethylation on pericentromeric satellite regions significantly correlated with histological grade (chi-square test $p = 0.0012$ and 0.0043), invasion depth (chi-square test $p = 0.0055$ and 0.0228) and morphological structure (papillary vs nodular chi-square test $p = 0.0161$ and 0.0297) for satellites 2 and 3, respectively (table 1), but not with age or gender (data not shown).

Allelic status of chromosome 9 and its correlation with clinicopathological parameters. Figure 2 shows examples of electropherograms of PCR products. Figure 3 shows the results of LOH analysis. Table 2 lists the incidence of LOH on each locus. LOH for at least 1 marker was found in 14 of the 27 informative cases (52%) (table 2).

The presence of LOH on at least 1 locus on chromosome 9 significantly correlated with histological grade (chi-square test $p = 0.0313$, table 3). LOH on at least 1 locus was detected in all 6 nodular carcinomas and its incidence (100%) was significantly higher than in papillary carcinomas (chi-square test $p = 0.0074$, table 3).

Correlation between DNA methylation status on pericentromeric satellite regions and allelic status of chromosome 9. DNA