

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-SHAU 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 SHIRAIISHI)

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*-acetylglucosamine 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, cyclin 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 prednisone 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 PSA DT, 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 ≥ 0.2 ng/ml. Extra-prostatic 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 BRACHY THERAPY 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 anastomosis, Prostatecint 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 micro-metastasis 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 TAJI 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 over-expression of p53 and Ki-67. There definitely is harm from screening such as psychological stress, side effects and over-treatment 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.

Promoter hypermethylation of the potential tumor suppressor *DAL-1/4.1B* gene in renal clear cell carcinoma

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Renal clear cell carcinoma (RCCC) is a malignant tumor with poor prognosis caused by the high incidence of metastasis to distal organs. Although metastatic RCCC cells frequently show aberrant cytoskeletal organization, the underlying mechanism has not been elucidated. *DAL-1/4.1B* is an actin-binding protein implicated in the cytoskeleton-associated processes, while its inactivation is frequently observed in lung and breast cancers and meningiomas, suggesting that 4.1B is a potential tumor suppressor. We studied a possible involvement of 4.1B in RCCCs and evaluated it as a clinical indicator. 4.1B protein was detected in the proximal convoluted tubules of human kidney, the presumed cell of origin of RCCC. On the other hand, loss or marked reduction of its expression was observed in 10 of 19 (53%) renal cell carcinoma (RCC) cells and 12 of 19 (63%) surgically resected RCCC by reverse transcription-PCR. Bisulfite sequencing or bisulfite SSCP analyses revealed that the 4.1B promoter was methylated in 9 of 19 (47%) RCC cells and 25 of 55 (45%) surgically resected RCCC, and inversely correlated with 4.1B expression ($p < 0.0001$). Aberrant methylation appeared to be a relatively early event because more than 40% of the tumors with pT1a showed hypermethylation. Furthermore, 4.1B methylation correlated with a nuclear grade ($p = 0.017$) and a recurrence-free survival ($p = 0.0036$) and provided an independent prognostic factor ($p = 0.038$, relative risk 10.5). These results indicate that the promoter methylation of the 4.1B is one of the most frequent epigenetic alterations in RCCC and could predict the metastatic recurrence of the surgically resected RCCC.

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Key words: tumor suppressor gene; bi-sulfite sequencing; two-hit inactivation; recurrence-free survival rate; independent prognostic factor

Renal cell carcinoma (RCC) accounts for about 2% of human cancers worldwide, with an incidence of 189,000 and a mortality of 91,000 reported in the year of 2000.¹ Renal clear cell carcinoma (RCCC), which represents 75% of all RCC, exhibits frequent metastasis to distant organs without any clinical symptoms. Furthermore, 40–60% of RCCC tumors without metastasis at first presentation eventually develop metastasis as they progress.² Finally, metastatic RCCC becomes refractory to any therapeutic approaches, including chemo-, radio-, and hormonal therapies, resulting in a poor prognosis of patients, with a 5-year survival of less than 10%.³ Thus, understanding the molecular mechanisms of the development and progression of RCCC is a critical issue for controlling this refractory cancer.

Several genetic and epigenetic alterations have been reported in RCCC. The mutation of the *VHL* gene, associated with loss of heterozygosity (LOH) at the gene locus on chromosomal fragment 3p25–p26, was observed in ~50% of sporadic RCCC.⁴ Since the *VHL* encodes a component of an E3 ubiquitin ligase that promotes the degradation of hypoxia-inducible factors, loss of VHL function could be involved in angiogenesis, one of the most characteristic features of RCCC.⁵ Epigenetic inactivation of the *RASSF1A* gene is also reported frequently in RCCC.^{6–8} In addition, promoter methylation and/or aberrant expression of the *E-cadherin* and *beta-catenin* genes are also found at a high incidence in RCCC,

suggesting that disruption of cell adhesion and cytoskeleton organization is also involved in RCCC.^{9,10} On the other hand, mutation of the *H-*, *K-*, *N-ras* and inactivation of the *TP53* and *RB1* genes are relatively rare events,¹¹ while inactivation of the *p16/CDKN2A* gene is involved in a small subset of advanced RCCC.¹²

We have reported that the loss of function of the tumor suppressor in lung cancer 1 (TSLC1) protein, an immunoglobulin superfamily cell adhesion molecule, is implicated in a variety of human cancers in their advanced stages.^{13–17} In addition, we have demonstrated that TSLC1 directly binds to DAL-1/4.1B, an actin-binding protein, through its 4.1-binding motif. DAL-1 was originally isolated as an expressed fragment of the 4.1B gene, whose expression was down regulated in adenocarcinoma of the lung.¹⁸ Restoration of DAL-1 expression in non-small-cell lung cancer or breast cancer cell lines significantly suppressed cell growth *in vitro*.^{18,19} Moreover, loss of 4.1B expression was observed in human breast cancers and meningiomas, suggesting that the 4.1B gene is an additional target for inactivation in human cancers.^{1–21} Interestingly, 4.1B/DAL-1 interacts with spectrin, an actin-binding protein, and over expression results in altered cytoskeleton-associated properties, including cell adhesion and motility.²⁰

To analyze the role of TSLC1 and 4.1B in RCCC, we analyzed 55 surgically resected RCCC and 19 cell lines in the present study. While we could not detect loss of TSLC1 expression, we did find significant alterations in 4.1B gene expression in these tumors. Herein, we demonstrated that hypermethylation of the 4.1B gene was a frequent event and could provide an independent prognostic factor for metastatic recurrence after completely resected RCCC.

Material and methods

Cell lines

RCC cell lines, Caki-2, SW839, ACHN, 786-O, 769-P, A-704, A-498 and Hs891.T, were obtained from the American Type

Abbreviations: LOH, loss of heterozygosity; NDS, normal donkey serum; PCR, polymerase chain reaction; RCC, renal cell carcinoma; RCCC, renal clear cell carcinoma; RT-PCR, reverse transcription-polymerase chain reaction; SNP, single nucleotide polymorphism; SSCP, single-strand conformation polymorphism; TNM, tumor-node-metastasis.

Grant sponsors: the Ministry of Health, Labor, and Welfare, Japan; the Ministry of Education, Culture, Science, Sports, and Technology, Japan; the Program for the Promotion of Fundamental Studies in Health Sciences of Pharmaceutical and Medical Devices Agency (PMDA), Japan; the Foundation for the Promotion of Cancer Research of Japan; the National Institutes of Health; Grant number: NS41520.

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Received 22 March 2005; Accepted after revision 10 June 2005

DOI 10.1002/ijc.21450

Published online 8 September 2005 in Wiley InterScience (www.interscience.wiley.com).

Culture Collection (Rockville, MD); KMRC-1, KMRC-2, KMRC-3, VMRC-RCW, VMRC-RCZ and Caki-1 cells were from the Japanese Collection of Research Bio-resources (Tokyo, Japan); OS-RC-2, RCC10RGB, TUHR4TKB, TUHR10TKB and TUHR14TKB cells were from the Riken Cell Bank (Tsukuba, Japan). Cells were cultured according to the supplier's recommendations.

Surgical specimens

Fifty-five pairs of cancerous and adjacent noncancerous tissues of RCCC were surgically resected at the National Cancer Center Hospital or the Hospital of the University of Tokyo, after obtaining written informed consent from each patient. Pathological diagnosis was performed or confirmed at Pathology Division, National Cancer Center Research Institute, and the clinicopathological features were determined according to the 1997 Union Internationale Contre le Cancer.²² Analyses of human materials were carried out according to the institutional guidelines.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted using the RNeasy Mini Kit (QIAGEN, Valencia, CA). By using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA), 1 µg of total cellular RNA was reverse-transcribed, and an aliquot was amplified by polymerase chain reaction (PCR), using TITANIUM Taq DNA polymerase (BD Biosciences Clontech, Palo Alto, CA) to obtain a 572-bp fragment of DAL-1 cDNA and a 646-bp fragment of human β-actin cDNA in the same reaction. The primers used for PCR were 5'-GGTGGCGAGGGAGGTCACCTGACAAGGAACA G-3' and 5'-CGCTCCCACATTCATCTGGGTCATAGTCTCCG AG-3' for DAL-1 (1.0 µM, each) and 5'-GGTGGCGAGGGA GGTCACTGACAAGGAACAG-3' and 5'-CGCTCCCACATTC ATCTGGGTCATAGTCTCCGAG-3' for β-actin (0.2 µM, each).

Restoration of DAL-1 expression by 5-aza-2'-deoxycytidine

At day 0, 1×10^5 cells were seeded, treated with 5-aza-2'-deoxycytidine (10 µM; Sigma-Aldrich, St. Louis, MO) or PBS for 24 hr on days 2 and 5 and collected on day 8, as reported previously.²³

Loss of heterozygosity (LOH) analysis

Five DNA fragments containing single nucleotide polymorphisms (SNPs) on 18p11.3, namely IMS-JST067229, IMS-JST031621, IMS-JST082513, IMS-JST143134 and IMS-JST119847, were examined for LOH as described previously.²⁴

Bisulfite sequencing

Bisulfite sequencing was performed as described previously.²⁵ Briefly, genomic DNA was denatured with NaOH (0.3 M) and incubated with sodium bisulfite (3.1 M; Sigma) and hydroquinone (0.8 mM; Sigma), pH 5.0, at 55°C for 20 hr, followed by purification and treatment of DNA with NaOH (0.2 M) for 10 min at 37°C. Modified DNA (100 ng) was subjected to PCR to amplify a 92-bp DNA fragment, using a pair of primers (DAL-1 PR2F: 5'-CGGAGTTTCGGTGTTTTTTTGTAATAGG-3' and DAL-1 PR2R: 5'-GCGCCGCGACGTAAAACTAAAC-3'). The PCR products were subcloned to confirm the sequence of at least 4 clones for each sample.

Bisulfite single-strand conformation polymorphism (SSCP) analysis

For SSCP analysis, the 92-bp fragments were amplified by PCR using two primers, PR2F and PR2R, the latter of which was end-labeled with Texas Red. The PCR products were diluted 7 times with a loading buffer (90% deionized formamide, 0.01% New Fuchsin and 10 mM EDTA), heat-denatured for 3 min at 95°C, immediately cooled on ice for 3 min and then loaded onto the gel (0.5× MDE™ Gel Solution; BMA, Rockland, ME). Electrophoresis was carried out for 120 min at 20°C, using SF5200 (Hitachi Electronics Engineering, Tokyo, Japan) with cooling systems. The analysis was repeated 3 times using independent PCR products.

The criterion for hypermethylation was met when the ratio of the methylated fragments to the unmethylated fragments was more than 0.4.

Immunohistochemistry

Sections (5-µm thick) of formalin-fixed, paraffin-embedded specimens were obtained from the National Cancer Center Hospital. For antigen retrieval, the section was heated for 5 min at 120°C with 1 mM EDTA in an autoclave after de-paraffinization and dehydration. Nonspecific reactions were blocked with 5% normal donkey serum (NDS) in TBS. All sections were incubated with anti-DAL-1 antibody (diluted with 1% NDS in TBS 1:2,000) at 4°C overnight. This rabbit polyclonal antibody against 18 amino acids in the U2 domain of DAL-1 was generated by D. H. Gutmann (unpublished results). The sections were then incubated with a labeled polymer, horseradish peroxidase (DakoCytomation, Glostrup, Denmark), at room temperature for 1 hr, rinsed gently with TBS, covered with 3,3'-diaminobenzidine (DakoCytomation) and incubated for 3 min. All sections were counterstained with hematoxylin. 4.1B expression was determined as "membrane expression" when 4.1B signals were detected along the cell membrane in more than 80% of the cells and as an "aberrant expression" or "no expression" when the majority of the 4.1B signals were observed diffusely in the cytoplasm or were undetected.

Statistical analysis

The Kruskal-Wallis test and Mann-Whitney *U*-test were used to examine the correlation with clinicopathological characteristics. Recurrence-free survival was analyzed by the Kaplan-Meier method and the Log-rank test. Multivariate analysis was carried out using the Cox proportional hazard model. The software Stat View 5.0 (SAS institute, Cary, NC) was used for the analysis. Differences with *p* values of less than 0.05 were considered significant.

Results

Loss of 4.1B expression in RCC

We initially examined the expression of the 4.1B gene in normal kidney and 19 RCC cell lines by RT-PCR. As shown in Figure 1a, a significant amount of 4.1B mRNA was detected in normal kidney. On the other hand, 10 of 19 (53%) RCC cell lines lacked 4.1B mRNA expression. Next, we analyzed the expression of 4.1B mRNA in 19 surgically resected RCCC as well as several noncancerous renal tissues from the same patients. Semi-quantitative analysis by RT-PCR revealed that 4.1B mRNA was absent or markedly reduced in 12 of 19 (63%) of these primary RCCC (Fig. 1b). These results suggest that the 4.1B gene may be a target for inactivation in renal carcinogenesis.

Promoter hypermethylation of the 4.1B gene in RCCC

The 4.1B gene harbors a typical DNA sequence matching the criteria of a CpG island in its upstream region, exon 1, and the beginning of intron 1. To elucidate the molecular mechanisms underlying the loss of 4.1B expression, we examined the methylation status of the 4.1B promoter in RCC cells. By using bisulfite sequencing, we had previously determined that hypermethylation of the 14 CpG sites within the 92-bp fragment around the 4.1B promoter strongly correlates with loss of expression in non-small-cell lung cancer cell lines.²⁴ Bisulfite sequencing of the same fragment revealed that these CpG sites were highly methylated in TUHR10TKB and A704 cells lacking 4.1B expression, whereas they were not methylated in KMRC1 cell expressing a significant amount of 4.1B transcript (Figs. 2a and 2b). A similar analysis showed that hypermethylation was observed in 9 of 19 (47%) RCC cell lines, where hypermethylation strongly correlated with loss of 4.1B expression (*p* = 0.0004, Fig. 1a). To examine the methylation status of the promoter quantitatively, we analyzed the promoter fragments by SSCP after PCR amplification of the bisul-

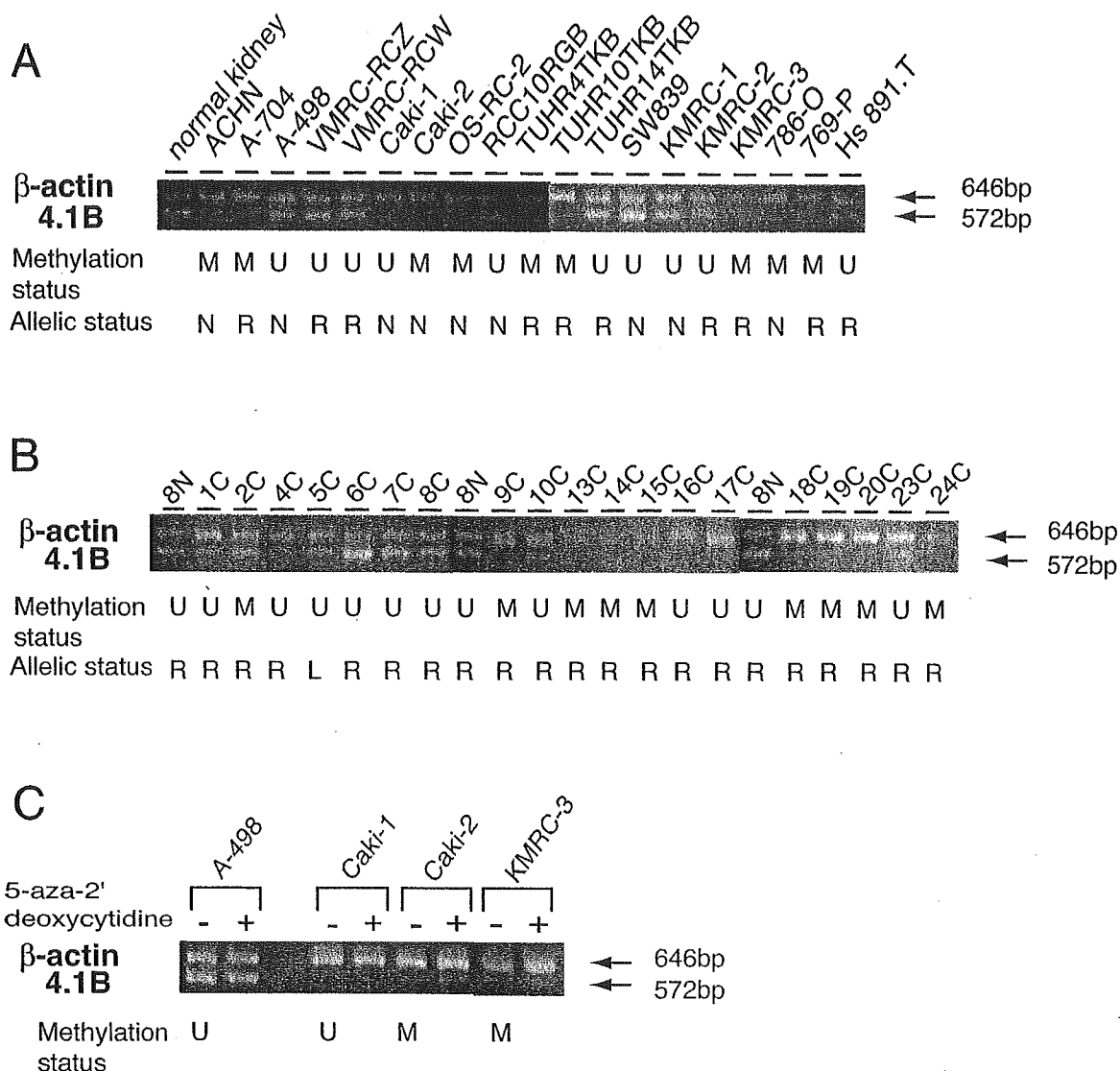


FIGURE 1 – Expression of the *4.1B* gene in RCC. (a) and (b): RT-PCR analysis of *4.1B* and β -actin in RCC cell lines (a) and surgically resected RCC (b). C and N in (b) indicate cDNA from a cancerous and noncancerous portion of the kidney, respectively. The results of methylation status determined in Figure 2 and allelic status are included as a reference. M and U indicate the hypermethylated and unmethylated promoter of the *4.1B*, respectively. R and L indicate retention and loss of heterozygosity, respectively. N in (a) indicates not informative. (c): RT-PCR analysis of *4.1B* and β -actin in RCC cells treated with 5-aza-2'deoxyctidine (+) or PBS (-).

fite-treated DNA. As shown in Figures 2a and 2c, clones with known sequences in terms of CpG methylation showed distinct mobility in SSCP analysis, where clone I with no methylation and clone VI with complete methylation showed the slowest and the fastest mobility, respectively. Bisulfite SSCP of RCC cells revealed that TUHR10TKB and A704 cells showed a pattern of hypermethylation, while KMRC1 cell showed a pattern of no methylation, in agreement with the results obtained using bisulfite sequencing (Figs. 2a and 2d). Next, we examined the methylation

status of the *4.1B* in surgically resected RCC. As shown in Figure 2e, DNA from tumors 4C, 5C and 6C showed no methylation, while that from 13C, 14C and 15C showed hypermethylation. DNA from noncancerous renal tissues 4N and 13N showed no methylation. A similar analysis revealed that 25 of 55 (45%) surgically resected RCC showed hypermethylation. *4.1B* promoter methylation strongly correlated with loss of *4.1B* expression in a subset of surgically resected RCC examined ($p = 0.0063$, Fig. 1b, Table I).

FIGURE 2 – Methylation analysis of the *4.1B* promoter. (a): Schematic representation of the methylation status of the *4.1B* promoter. A hatched box and an open box indicate a CpG island and exon 1 of the *4.1B*. Vertical bars indicate CpG sites numbered 1–40. Black and white circles represent methylated and unmethylated CpG, respectively. Rows 1–4 indicate the results of independent clones. (b): Bisulfite sequencing of the *4.1B* promoter in 3 RCC cells. Sequence traces in each sample correspond to the genomic sequence (-65 bp to -23 bp from the transcription initiation site) shown in the top line. CpG sites, numbered 19–22, are underlined. Asterisks indicate the nucleotides corresponding to methylated cytosine residues at the CpG sites. (c)–(e): Bisulfite SSCP analyses of the cloned DNA fragments of known sequences (c), RCC cells (d), and surgically resected RCC and corresponding noncancerous kidney (e). C and N in (e) indicate DNA from a cancerous and noncancerous portion of the kidney, respectively. Presence or absence of *4.1B* expression determined in Figure 1 is shown as (+) or (-), respectively (d) (e).

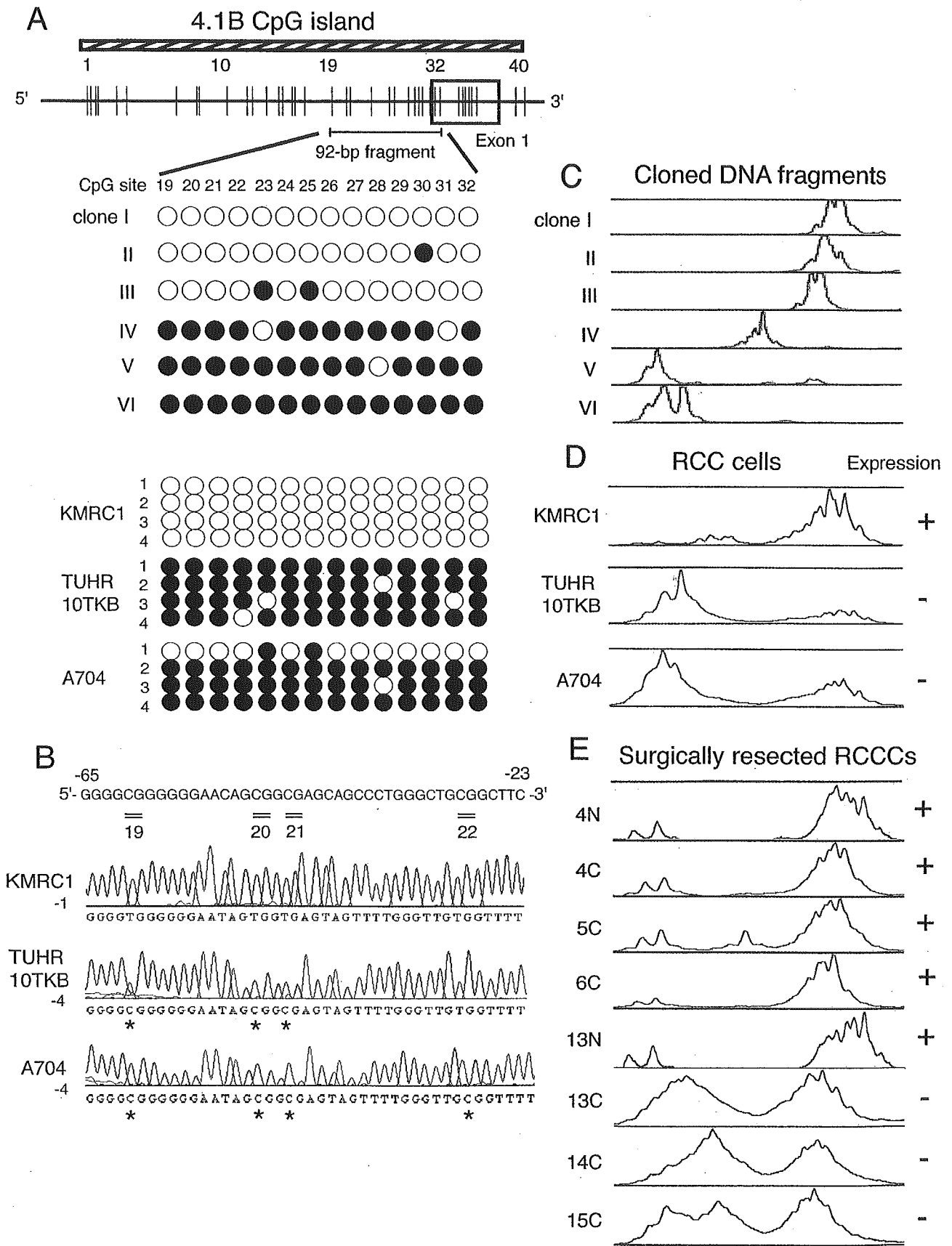


FIGURE 2.

TABLE I - METHYLATION AND EXPRESSION STATUS OF 4.1B AND CLINICOPATHOLOGICAL CHARACTERISTICS IN RCCC

	Number of cases	4.1B Promoter		p-value
		Hypermethylation (%)	No methylation (%)	
4.1B expression				
RT-PCR				
Analyzed	19	9 (47)	10 (53)	
Positive	7	1 (14)	6 (86)	
Reduced	2	0 (0)	2 (100)	
Negative	10	8 (80)	2 (20)	0.006 ¹
Immunohistochemistry				
Analyzed	20	10 (50)	10 (50)	
Membrane	9	1 (11)	8 (89)	
Aberrant	5	3 (60)	2 (40)	
Negative	6	6 (100)	0 (0)	0.004 ²
Clinicopathological Characteristics				
Analyzed	55	25 (45)	30 (55)	
Age (years)				
60 and older	32	15 (47)	17 (53)	
Under 60	23	10 (43)	13 (57)	NS ¹
Gender				
Male	37	17 (46)	20 (54)	
Female	18	8 (44)	10 (56)	NS ¹
Pathological stage				
I	36	15 (42)	21 (58)	
II	8	4 (50)	4 (50)	
III	8	4 (50)	4 (50)	
IV	3	2 (67)	1 (33)	NS ¹
TNM classification				
pT1a	17	8 (47)	9 (53)	
pT1b	21	8 (38)	13 (62)	
pT2	8	4 (50)	4 (50)	
pT3a	2	1 (50)	1 (50)	
pT3b	5	3 (60)	2 (40)	
pT3c	2	1 (50)	1 (50)	NS ¹
pT4	0	0 (0)	0 (0)	
pN0	54	25 (46)	29 (54)	
pN1,pN2	1	0 (0)	1 (100)	NS ¹
pM0	53	23 (43)	30 (57)	
pM1	2	2 (100)	0 (0)	NS ¹
Nuclear grade				
G1	22	5 (23)	17 (77)	
G2	27	17 (63)	10 (37)	
G3	6	3 (50)	3 (50)	0.017 ¹

NS, not significant.

¹Mann-Whitney U-test. ²Kruskal-Wallis test.

We then examined the role of promoter methylation in gene silencing of the *4.1B* gene by treating RCC cells with the demethylating agent 5-aza-2'-deoxycytidine. Semi-quantitative RT-PCR analysis revealed that the expression of *4.1B* mRNA following 5-aza-2'-deoxycytidine treatment was only observed in the Caki-2 and KMRC-3 cell lines harboring the hypermethylated *4.1B* promoter, but not in the Caki-1 cell line lacking *4.1B* promoter methylation. These results suggest that *4.1B* promoter methylation is causally related to loss of *4.1B* expression (Fig. 1c).

LOH analysis of the *4.1B* gene

We next analyzed the allelic status of the chromosomal fragment, 18p11.3, around the *4.1B* locus in RCC cells, using 5 highly polymorphic SNP markers. Ten of 19 RCC cell lines showed retention of heterozygosity in at least 1 locus per tumor. Five of these RCC cell lines (A704, TUHR4TKB, TUHR10TKB, KMRC3 and 769-P) harbored a hypermethylated *4.1B* promoter and lacked *4.1B* expression. These findings suggest that the *4.1B* gene is inactivated by bi-allelic methylation in some RCC cell lines. In contrast, 9 RCC cell lines did not show heterozygosity at any loci examined, strongly suggesting that one allele of the *4.1B* gene was deleted. Four of these RCC cell lines (ACHN, Caki-2, OS-RC-2, and 786-O) showed promoter hypermethylation with loss of *4.1B* expression, suggesting that the *4.1B* gene was inactivated by 2 hits

involving both promoter methylation and LOH. Last, LOH was only observed in 4 of 54 (7.4%) informative cases in surgically resected RCCC, suggesting that bi-allelic methylation may represent the major mechanism to suppress *4.1B* expression in primary RCCC.

Aberrant expression of *4.1B* protein in surgically resected RCCC

We then examined *4.1B* protein expression in human normal kidney as well as primary RCCC, using a polyclonal antibody against U2 domain of human *4.1B*.¹³ As shown in Figure 3a, *4.1B* protein was expressed in the baso-lateral membrane of the proximal convoluted tubules, from which RCCC arises. *4.1B* protein expression was also found in the basement membrane of the glomeruli, but not in the distal convoluted tubules, Henle's loops or collecting ducts in normal human kidney. An immunohistochemical study of 20 surgically resected RCCC revealed that 9 tumors (45%) demonstrated significant expression of *4.1B* protein along the cell membrane, 8 of which (89%) carried the unmethylated *4.1B* promoter (Fig. 3b). On the other hand, 6 tumors (30%), all of which (100%) harbored the hypermethylated *4.1B* promoter, showed absence of *4.1B* protein expression (Fig. 3c). In this regard, loss of *4.1B* protein expression significantly correlated with *4.1B* promoter hypermethylation ($p = 0.0040$, Table I). In addition, 5 tumors (25%) showed an aberrant pattern of *4.1B* expression, in which weak signals of *4.1B* protein were detected

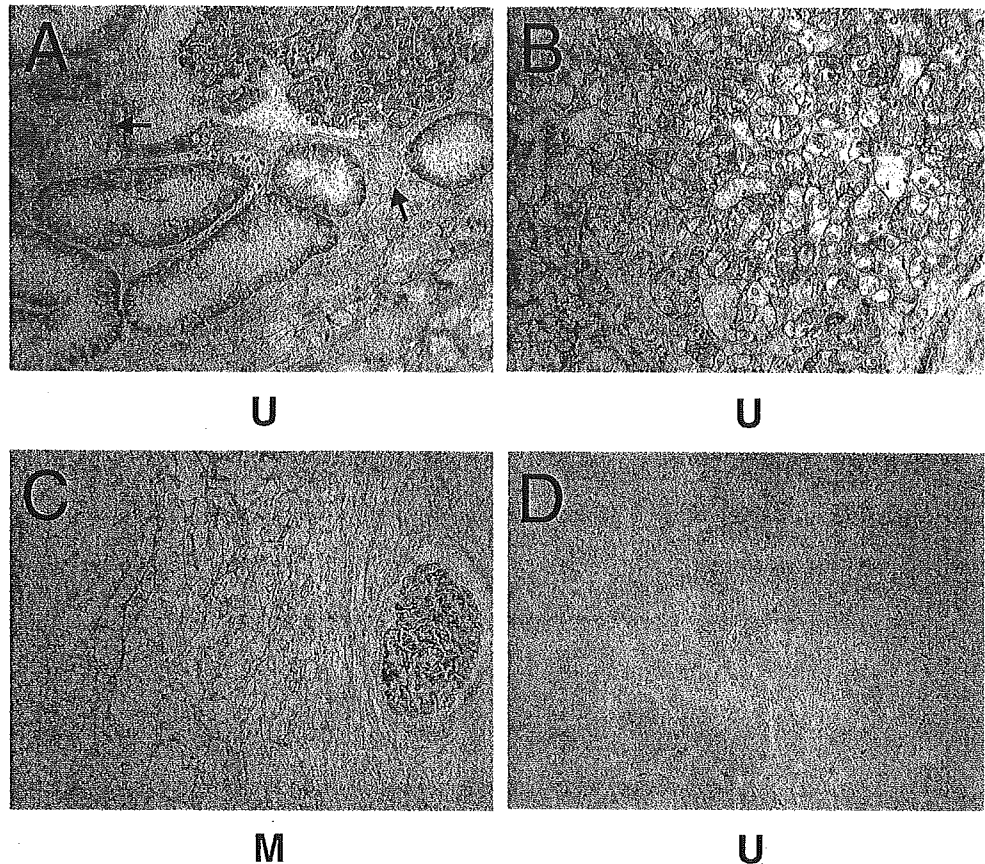


FIGURE 3 – Immunohistochemical analysis of 4.1B protein in human normal kidney (a) and surgically resected RCCC (b)–(d). (a) Expression of 4.1B is detected along the basolateral membrane of the proximal convoluted tubules and in the basement membrane of the glomeruli, but not in the distal convoluted tubules (arrows). (b): RCCC7C. 4.1B is detected along the cell membrane (membrane expression). (c) RCCC19C. 4.1B expression is absent (no expression). The basement membrane of the glomeruli (right) serves as a positive control. (d) RCCC5C. 4.1B is present diffusely in the cytoplasm (aberrant expression). M and U indicate tumors with hypermethylated and unmethylated *4.1B* promoter, respectively. Original magnifications, $\times 400$.

diffusely in the cytoplasm, but not at the cell membrane (Fig. 3d). Including these tumors with aberrant protein localization, 4.1B expression was abrogated in a total of 11 of 20 surgically resected RCCC (55%).

Clinicopathological features of RCCC with hypermethylation of the 4.1B gene

To understand the clinicopathological significance of the promoter methylation of the *4.1B* gene in surgically resected RCCC, we examined the pathological stage, tumor-node-metastasis (TNM) classification and nuclear grade of the tumors as well as the age and gender of the 55 patients. As shown in Table I, *4.1B* hypermethylation was observed in 15 of 36 (42%) tumors representing stage I and in 8 of 17 (47%) tumors with pT1a, whereas the incidence of hypermethylation did not increase significantly in tumors in more advanced stages. These results suggest that *4.1B* hypermethylation occurs in a subset of tumors as a relatively early event in multi-stage renal carcinogenesis. Correlation of the *4.1B* hypermethylation with lymph node metastasis (pN) or distant metastasis (pM) could not be determined because the great majority of tumors examined were pN0 and pM0 at the time of resection. Interestingly, *4.1B* hypermethylation was preferentially observed in tumors with higher nuclear grade ($p = 0.017$). On the other hand, the age and gender of the patients were not correlated with *4.1B* hypermethylation.

Hypermethylation of the 4.1B gene correlates with the recurrence-free survival of the RCCC patients

Finally, we examined the significance of *4.1B* methylation as a prognostic factor of metastatic recurrence for RCCC patients. Of 55 patients examined for *4.1B* methylation, 53 patients who received complete surgical resection of RCCC were examined for their prognosis, whereas the other two patients were excluded

from the analyses because they harbored metastasis at the time of resection. Kaplan-Meier analysis revealed that the recurrence-free survival of patients with tumors of *4.1B* methylation was significantly shorter than that observed in patients with the unmethylated *4.1B* promoter ($p = 0.0036$, Fig. 4). Furthermore, the multivariate analysis by the Cox hazard model indicated that *4.1B* methylation was an independent prognostic factor, as shown in Table II ($p = 0.038$; relative risk, 10.5).

Discussion

The present study demonstrates that the epigenetic inactivation of the *4.1B* gene is involved in primary RCCC and represents an independent prognostic factor for RCCC patients. Analysis of the expression, methylation and allelic status of the *4.1B* gene revealed that hypermethylation and loss of expression were strongly correlated with each other in both the cell lines and surgically resected RCCC ($p < 0.0001$), as observed in other tumor suppressor genes. The 92-bp fragment including 14 CpG sites that we examined in this study contained a putative transcription start site of *4.1B* gene and a Sp1-binding sequence, which suggests that some methyl-CpG binding proteins might suppress the transcription through interaction with this regulatory motif. While LOH at the *4.1B* locus on 18p11.3 was not frequently observed in surgically resected RCCC, we demonstrated a two-hit inactivation of the *4.1B* in a subset of cell lines by the promoter hypermethylation associated with LOH as well as through bi-allelic hypermethylation. These findings suggest that *4.1B* may act as a potential tumor suppressor in human RCCC. It is worth noting that loss of *4.1B* expression was also observed in Caki-1 cells and several tumors without *4.1B* methylation (Figs. 1a and 1b). In this regard, treatment of Caki-1 cells with 5-aza-2'-deoxycytidine did not restore *4.1B* expression (Fig. 1c). These results suggest that some mechanisms other than promoter methylation, such as histone deacetyla-

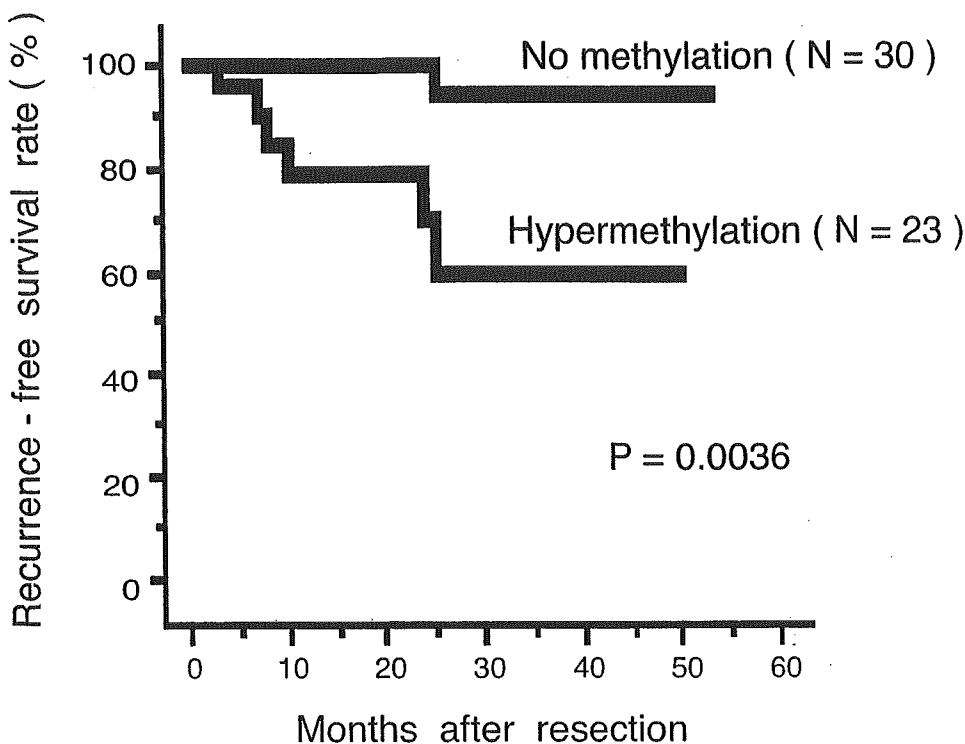


FIGURE 4 – Recurrence-free survival of the patients who received complete resection of RCCC with hypermethylated and unmethylated *4.1B* promoters. Intervals between the primary surgical resection and the metastatic recurrence at the lung, bone, liver, or pancreas are plotted in the Kaplan–Meier analysis. Log-rank *P* is included. *N* indicate number of cases.

TABLE II – PROGNOSTIC VALUE OF *4.1B* METHYLATION STATUS, PATHOLOGICAL STAGE AND NUCLEAR GRADE FOR RECURRENCE-FREE SURVIVAL IN RCCC

Variable	Kaplan–Meier analysis		Multivariate proportional hazard analysis		
	<i>p</i> -value	Relative risk	95% confidence interval	<i>p</i> -value	
<i>4.1B</i> methylation status ¹ (U vs. M)	0.0036	10.5	1.1–97.4	0.038	
Pathological stage (I, II vs. III, IV)	0.039	4.0	0.83–19.6	0.083	
Nuclear grade (1 vs. 2, 3)	0.059	1.8	0.18–18.1	0.62	

¹U, no methylation; M, hypermethylation.

tion and deficiency of transcription factors, might be involved in the regulation of *4.1B* expression in additional populations of RCCC.

Immunohistochemical studies using anti-*4.1B* antibody provided information about *4.1B* expression, but also *4.1B* subcellular localization in primary RCCC. In this study, we found a group of tumors with *4.1B* mislocalization, in addition to RCCC tumors lacking *4.1B* expression due to promoter hypermethylation. In the tumors with abnormal *4.1B* subcellular localization, *4.1B* protein was expressed diffusely within the cytoplasm, but not along the cell membrane. Some membrane proteins anchoring DAL-1 to the cell membrane might be inactivated in these cases. This mislocalization might impair the ability of *4.1B* to function as a potential tumor suppressor. In this regard, Robb *et al.* have recently shown that growth suppression of meningioma cells by *4.1B*/DAL-1 requires proper membrane localization.²⁶ This aberrant pattern of subcellular distribution in RCCC tumors would be associated with impaired *4.1B* function.

By using bisulfite-SSCP, a sensitive and highly quantitative method to detect the methylation status, we found *4.1B* promoter hypermethylation in 25 of 55 (45%) surgically resected RCCC. It has been speculated that the DNA methylation changes are rather rare events in RCCC in comparison with other major malignancies.^{27,28} In fact, previous studies have reported that the incidences of hypermethylation in representative tumor suppressor genes, including the *VHL*, *p16/CDKN2A*, *p14/ARF* and *APC* genes, are less than 16% in RCCC.^{8,28} However, the extensive analyses have demonstrated that the promoters of the *Timp-3* and *RASSF1A* genes are methylated in 60% and 23–91% of primary RCCC, respectively,

suggesting that several critical genes are inactivated frequently by methylation in RCCC as are in many other tumors.^{6–8} The incidence of promoter methylation of the *4.1B* (45%) that we have observed in this study is comparable to that of the *Timp-3* and *RASSF1A* genes. Therefore, loss of *4.1B* function appears to be strongly selected for the malignant growth of RCCC cells.

It is interesting that the incidence of *4.1B* methylation is more than 40% in tumors with pT1a but does not increase as the T classification advances. The T classification of RCC is determined by the tumor size and the degree of invasion into the renal capsule or vein. In this regard, our findings suggest that *4.1B* promoter hypermethylation is involved in a subset of tumors in a relatively early stage, and is not significantly associated with the tumor size or the degree of invasion at the time of surgical resection. Another interesting result is the significant correlation of *4.1B* promoter hypermethylation with the nuclear grade, which is an indicator of nuclear abnormality of cancer cells (*p* = 0.017). It is worth noting that *4.1B* interacts with 14-3-3, a crucial modifier of the G2 checkpoint, by sequestering Cdc2-cyclin B1 complex in the cytoplasm.^{29,30} While Robb *et al.* recently suggest that 14-3-3 might not represent the critical *4.1B* effector protein,³¹ there is emerging data to support a role for *4.1B* in the regulation of apoptosis.^{19,26}

One of the most serious clinical problems of RCCC is a frequent metastatic recurrence that occurs even after the tumors are completely resected in their early stages. *4.1B* is an actin-binding protein involved in actin cytoskeleton organization and actin-mediated processes, including cell motility and adhesion.^{19,20} It is possible, therefore, to hypothesize that loss of *4.1B* function might be involved in metastasis of RCCC cells to distant organs. Our


findings that 4.1B promoter methylation is an independent prognostic factor of metastatic recurrence for RCCC patients would support this hypothesis. Furthermore, the observation that the recurrence-free survival of patients with tumors of 4.1B promoter hypermethylation was significantly shorter than that in patients without 4.1B promoter hypermethylation ($p = 0.0036$) suggests that 4.1B expression might represent a surrogate marker for this metastatic feature. It should be noted that 2 patients with metastasis at the time of resection, who were excluded from this analysis, also showed 4.1B promoter hypermethylation in the primary RCCC. In conclusion, our results provide the first demonstration that 4.1B promoter hypermethylation was involved in the development and/or progression of RCCC and may represent an independent and novel prognostic factor of the metastatic recurrence for RCCC patients.

Acknowledgements

We are grateful to Dr. E. Surace for his technical assistance. This work is supported in part by Grant-in-Aid for the Third-Term Comprehensive Control Research for Cancer from the Ministry of Health, Labor, and Welfare of Japan; a Grant-in-Aid for Special Projects for Cancer Research from the Ministry of Education, Culture, Science, Sports, and Technology of Japan; a Grant from the Program for the Promotion of Fundamental Studies in Health Sciences of Pharmaceutical and Medical Devices Agency (PMDA) of Japan to Y. M. and a Grant from the National Institutes of Health (NS41520) to D.H.G. D. Y., S. K., and M. S.-Y. are recipients of Research Resident Fellowships from the Foundation for the Promotion of Cancer Research of Japan. M. M. is a recipient of Research Fellowships from PMDA.

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 ライフサイエンス出版

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【禁 無断転載・複製】

尿道カテーテルについて

ある種の排尿障害の改善の目的で、また術後の尿流確保の目的などで、一時的に尿道内にチューブを挿入し留置することは、泌尿器科領域の基本的な診療手技の一つだ。

今から30年以上前、私が泌尿器科の臨床に加わるようになった頃は、ネラトンカテーテルというゴム製のカテーテルを経尿道的に膀胱に留置するのが普通だった。そのままではカテーテルが身体から脱落するので、ペニス包帯、略してペニ包という手技でカテーテルをペニスに固定していた。すなわち、ガーゼの端を2本、ヒモ状に切って細くより合せ、これでネラトンカテーテルのペニス先端部から出た部分を、交叉させたヒモで十字状にしぼる。別にガーゼ1枚をバイアスに折りたたんで3cmほどのガーゼのベルトを作り、先ほどのネラトンカテーテルをしばったひもをペニスの冠状溝周囲にこのガーゼで固定する。器用に実施された場合、でき上がりも美しく、固定も何日も長持ちした。

こんな時代に、ある日、米国からフォーリーカテーテルなるものが紹介された。バルーンの部分の材質の薄さ、耐久性、これを膨らませたり、しばませたりする、水の通路の細さ、いったん注入した水が抜けないための注入口の逆流防止の機構、いずれも美しい！と思うほど完成度の高い医療器具だった。以来、ペニ包はあつという間に姿を消し、男女ともにフォーリーカテーテルが使われるようになった。材質もシリコンとなり、口径も多様となり、内腔がつぶれないようバネの入ったもの、3チャンネルのものなど、さまざまな工夫がなされ、今日に至っている。術後の尿道を介した尿流の確保に必然の医療機器として、尿閉の患者さんその他、患者さんはもちろん私どもも大いにその便利さの恩恵に浴してきた。

平成17(2005)年5月、私は偶然に発見された左腎痛に対して小開腹による腎部分切除術を受けた。経過は順調で1週間で退院し、2週間目にはジュネーブに5日間の出張をするなど、いたって元気にしている。しかし、振り返って術後の1週間の生活を考えると、硬膜外麻酔チューブか

ら持続注入される稀釈モルフィンのおかげで創の痛みはほとんどなく、第1日目からどンドン歩行できた。最も苦痛だったのは、5日間留置されたフォーリーカテーテルの違和感だった。体動時に思いがけず引っぱられたり、絶えず覚える尿道の違和感、ときどき襲ってくる強い尿意などは想定外だった。だから5日目にフォーリーカテーテルも硬膜外チューブも、すべてのチューブ、カテーテル類が抜去されたときの爽快感は大変なものだった。朝、夕、病棟内を飛ぶように10周した。

私はフォーリーカテーテルを究極の医療器具の一つと考え、それを必要とされる患者さんに使用するの当然と考えていた。しかし実際に自ら体験してみると、上述したように、その違和感は大変に強かった。これを今まで想像することなく、よく説明することなく、患者さんに使用していたことの想像力の欠如、自らの不明を恥じた。

フォーリーカテーテルを必要とする医療状況は今後も変わらないだろう。だとしたら、術後の一定期間、尿を体外に誘導し、正確に尿量が測定でき、かつ自ら排尿しないでもよい、とする目的のためには、さらなる技術的工夫がありうるのではないだろうか？ 口径の工夫、材質の工夫、膀胱からの脱落防止機構の工夫……。

今、世の中にはトランスレーショナル・リサーチという言葉が大はやりである。しかし、上に述べたようなリバー・トランスレーショナル・リサーチもありうるのではないか？ いや、あるべきと思う。当り前と思われている技術、たとえば腹部手術後に術後一定期間留置される経鼻胃管は、多くの患者さんが苦痛を訴える。百年間、進歩のない技術はおかしいと思う。

私たちは、想像力を豊かにして常識を疑い、地味な研究にも取り組むべきだと思う。分子生物学や、最先端の機器を使った研究のみが、臨床研究ではないはずである。心したいものである。

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Reprinted from

Jpn J Clin Oncol 2005;35(11)667-671

doi:10.1093/jjcolhyi177

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

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