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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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 nonsmall-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

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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.

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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′-GGTGCGGAGGGAGGTCACTGACAAGGAACAG-3′ and 5′-CGCTCCCACATTCATCTGGGTCATAGTCTCCGAG-3′ for DAL-1 (1.0 μM, each) and 5′-GGTGCGGAGGGAGGTCACTGACAAGGAACAG-3′ and 5′-CGCTCCCACATTCATCTGGGTCATAGTCTCCGAG-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′-CGGAGTTTCGGTGTTTTTTGTAATAGG-3′ and DAL-1 PR2R: 5′-GCGCCGCGACGTAAAAACTAAAC-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 Figure1*a*, 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. 1*b*). 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 nonsmall-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. 2*a* and 2*b*). 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. 1*a*). To examine the methylation status of the promoter quantitatively, we analyzed the promoter fragments by SSCP after PCR amplification of the bisul-

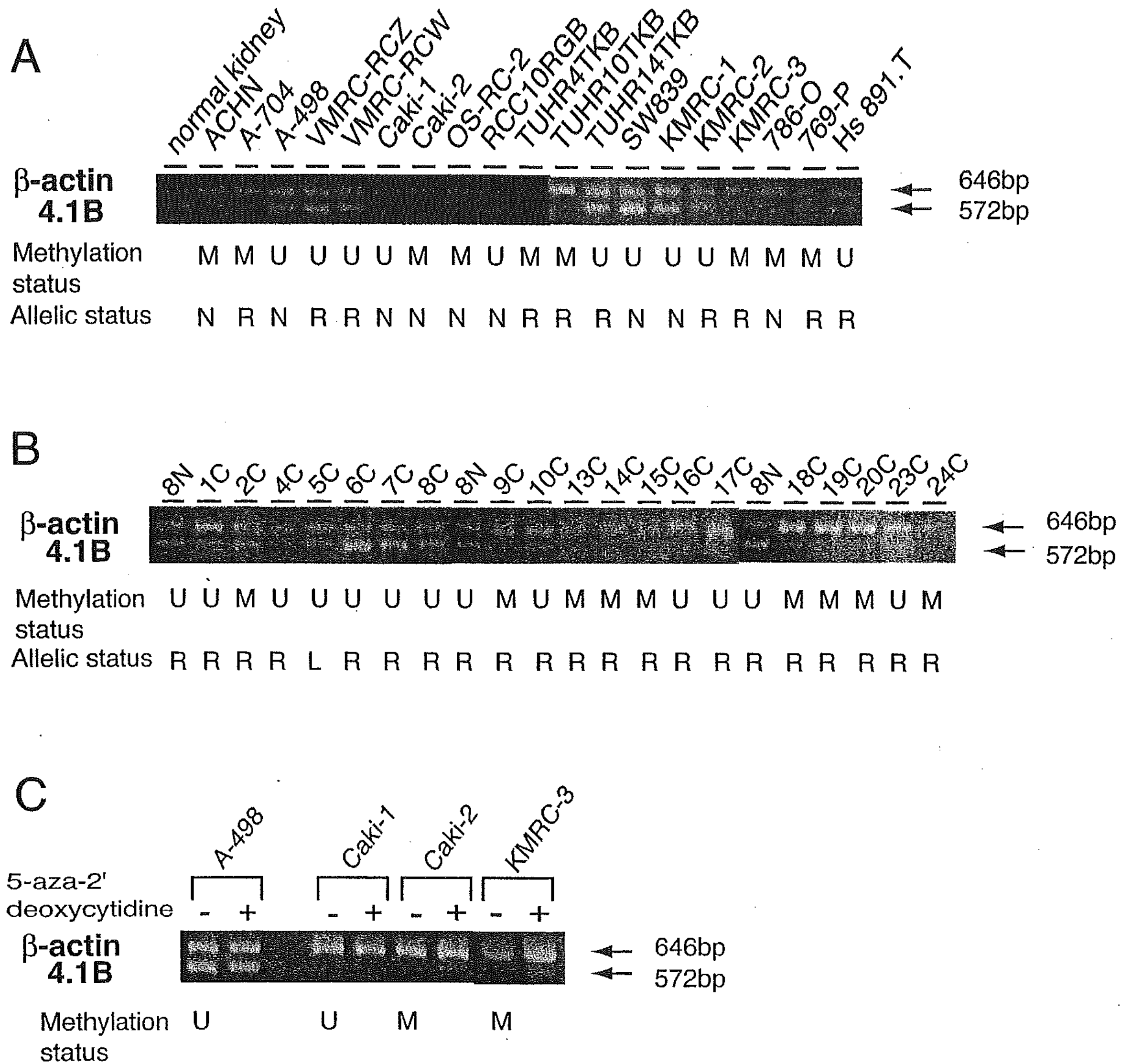

FIGURE 1 – Expression of the *4.1B* gene in RCC. (*a*) and (*b*): RT-PCR analysis of 4.1B and β-actin in RCC cell lines (*a*) and surgically resected RCCC (*b*). C and N in (*b*) indicate cDNA from a cancerous and noncancerous portion of the kidney, respectively. The results of methylation status determined 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′deoxycytidine (+) or PBS (−).

fite-treated DNA. As shown in Figures 2*a* and 2*c*, 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. 2*a* and 2*d*). Next, we examined the methylation status of the *4.1B* in surgically resected RCCC. As shown in Figure 2*e*, 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 RCCC showed hypermethylation. *4.1B* promoter methylation strongly correlated with loss of 4.1B expression in a subset of surgically resected RCCC examined ($p = 0.0063$, Fig. 1*b*, 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*): Bisulfate 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*): Bisulfate SSCP analyses of the cloned DNA fragments of known sequences (*c*), RCC cells (*d*), and surgically resected RCCC 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.
[1]Mann–Whitney *U*-test.–[2]Kruskal–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. 1*c*).

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 3*a*, 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. 3*b*). 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. 3*c*). 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

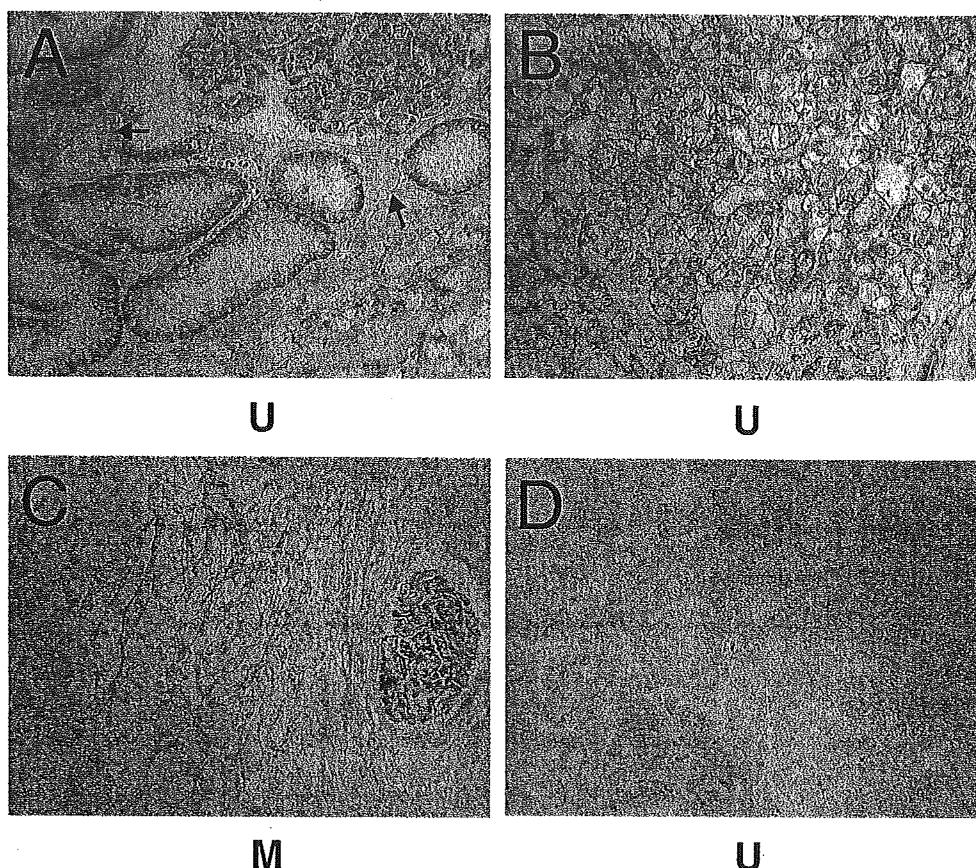

FIGURE 3 – Immunohistochemical analysis of 4.1B protein in human normal kidney (*a*) and surgically resected RCCC (*b*)–(*d*). (*a*) Expression of 4.1B is detected along the basolateral membrane of the proximal convoluted tubules and in the basement membrane of the glomeruli, but not in the distal convoluted 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, ×400.

diffusely in the cytoplasm, but not at the cell membrane (Fig. 3*d*). 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. 1*a* and 1*b*). In this regard, treatment of Caki-1 cells with 5-aza-2′-deoxycytidine did not restore 4.1B expression (Fig. 1*c*). These results suggest that some mechanisms other than promoter methylation, such as histone deacetyla-

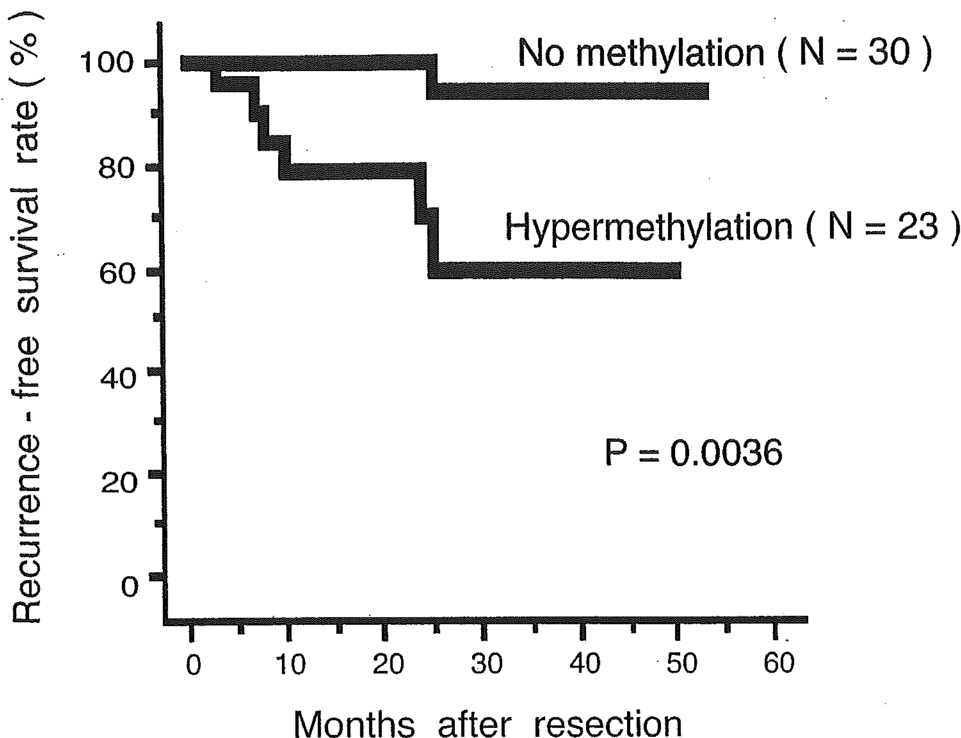

FIGURE 4 – Recurrence-free survival of the patients who received complete resection of RCCC with hypermethylated and unmethylated *4.1B* promoters. Intervals between the primary surgical resection and the metastatic recurrence at the lung, bone, liver, or pancreas are plotted in the Kaplan–Meier analysis. 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		
	p-value	Relative risk	95% confidence interval	*p*-value
4.1B 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

[1]U, 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.

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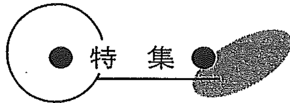

悪性脳腫瘍治療の新しい展開

診断と治療の変遷

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はじめに

膵がんは治療の最も難しいがんとして広く認識されている。しかし，さらに難しいがんが脳実質に発生する膠芽腫であることは一般には知られていない。膵がんの5年生存率は17.1%[1]と最近になってやや向上してきているのに対し，膠芽腫はがんのなかで最も難治性の腫瘍で7.0%と，この30年間治療の向上は認められていない[2]。脳実質に発生し神経細胞を破壊しながら増殖するこのがんは，文学的にいえば人間の人間たるゆえんを特徴付ける意識（心）を破壊してしまうことになり，悲惨さはこの上ない。この超難治がんに対して，これまでの研究・診療面での発展・進歩の過程を振り返ってその足跡をみることは，現在の進んだ医学のなかで取り残された領域である脳実質性悪性腫瘍の治療向上に必ず役立つものと考えられるので，筆者の経験を中心に記述することとする。

I．脳腫瘍の診断学の進歩

1950年代から1975年くらいの脳腫瘍の診断は神経症候の把握が第一であり，進行性症状増悪，これが重要所見であった。増大する占拠性病変により生じる頭蓋内圧亢進症候として当時把握できる3大症候は頭痛，鬱血乳頭，嘔気（嘔吐）であり，この症候は正に頭蓋内占拠性病変診断の基本的な知識であった[3,4]。これらの症候をとらえた時に，まず脳腫瘍が疑われ，次の検査に進むプロセスがとられた。そのプロセスは，局在診断をするために神経所見をいかに正確にとらえるかが重要であり[5]，その正確さは医師の経験と知識の蓄積によってなされていた。さらに得られた局在診断から，摘出手術を行うのに必要な腫瘍の悪性度診断，局在の確認，腫瘍血管の多寡，脳実質性か脳実質外かを判定することが必要となる。この先は身体に侵襲を加えなければできない検査をすることになる。それが脳血管撮影[6]であり，今ではほとんど使われなくなったが気脳検査[7]，脳室撮影である。この他に脳波診断，超音波診断，放射線同位元素診断[8]，髄液検査などがあった。1970年代前半までは正にその時代であり，脳血管撮影をいかに正確に読影できるかが，診療に大きな影響を与えたわけである。この時代に登場したのが神経放射線学者であり，卓越した学者の出現でこの学問は飛躍的進歩を遂げた。1971年，イギリスのハンスフィールドにより実用化されたCT画像診断は，1974年代以降日本にも導入され，脳腫瘍の診断学は飛躍的に進歩した[9,10]。その当時の脳外科医にしてみれば，正に診断分野における革命的変化であった。

1．コンピュータ断層画像診断検査の出現

CTの出現によって，脳腫瘍の診断は飛躍的に進歩した。従来奥深い知識の集積と経験を必要とした診断学が，脳の構造とともに腫瘍を描出するこの技術によって深遠なる経験と知識を要した診断学から，具体的かつ客観的診断学へと発展した。その結果，医療者および受診者ともに腫瘍の局在と大きさが明確に認識できるようになった。一方で，手術による摘出率の記載も従来の主観的記述から，より客観的な提示を余儀なくされた。このことが外科医の認識を変える大きな要因となり，手術による摘出率の向上を来した。結果的には，短期生存率に関する限りにおいて治療成績の向上をもたらしたわけである[11]。

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Brief Review of the Neuro Oncology for Recent Three Decades in Japan: Kazuhiro Nomura (*National Cancer Center Hospital*)

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2．MRIとPETの出現

1971年，英国のダマジオンがMRIの特許の申請を経て，CT出現から数年遅れでMRIが脳疾患の診断に導入された[12]。MRIはCTよりもさらに軟部組織の診断にその機能を発揮し，CTでは境界の不明瞭な浮腫の範囲など明確に表示することができるようになった。さらにMRIの進歩は急速であり，diffusion weighted imagingなどにより髄液と区別の難しいcysticな腫瘍に対しても診断が容易になった。一方，PETの出現は核医学画像診断として生体の糖代謝，アミノ酸代謝あるいは血流など生理，生化学的現象を画像でとらえられるようになった。現在，主な応用は神経膠腫の再発あるいは腫瘍壊死の判定などであるが，その特性から近い将来には腫瘍の生化学的面からの腫瘍の特性などの解析，化学療法剤の腫瘍内分布や感受性の解析，治療効果判定などにも大きく貢献するはずである。

3．機能MRIと脳磁図(magnetoencephalography: MEG)

一方，手術に際しては脳の機能分野と腫瘍の局在との関係は不可欠な情報となる。教科書的解剖図譜による機能局在は，個々の症例にとって必ずしも一致したものではなく，個人差が認められることがわかってきた。さらに，腫瘍の存在によって脳の機能分野が変移するので，実際の手術においては患者個々人における機能分野の確認が必要となる。従来は腫瘍を摘出するに当たって機能障害を引き起こさないか否かの判断は，CT出現以後も教科書的図譜と術者の勘と経験によるところが大きかったわけであるが，最近のこの分野の進歩はさらに著しく，機能MRI (fMRI)[13]の登場は術前に機能分野，特に運動野の同定を可能とした。また，電気生理学的検査法としてハンスバーガーによって発見された脳波は，1970年代には脳の磁場を測定するsuper-conductive quantum interference device (SQUID) が開発され，極めて正確に脳内電流の発生源，誘発磁場の検査を可能とし，究極の成果として脳磁図(MEG)が開発された。MEG[14]を用いた大脳皮質における神経活動の把握，さらにMRIのdiffusion tensor imaging (DTI) を利用したfiber tracking[15]法も開発され，現在では錐体路などの神経線維路の画像描出が実用化しつつある。このことは脳機能の存在部位を一括したアトラスで知るだけでなく，治療の対象となる個々人における解剖図を確認できるメリットがあり，治療する側も受ける側も治療結果が正確に予測できるためたいへん有益である。これらの実際については，それぞれの項において解説されているので参照されたい。

4．画像診断の進歩と手術摘出率

前述したように画像診断は手術後の摘出結果を明確に提示できることにより，術者に緊迫感を与え，全摘出を合併症なく実行する意欲を喚起することとなった。しかも，腫瘍の局在から脳機能局在部位，神経線維路との関係に至るまで提示可能になった結果，摘出が機能損傷なくして行えるのか否かなどの正確な術前検討を可能とした。さらに術中の機能マッピングモニタリング法[16]の導入により，脳外科の手術はより安全に，より効果的に実施できるようになってきている。

5．画像の進歩と放射線治療

画像の進歩はコンピュータの進歩と平行している。これらの技術は当然のことながら悪性脳腫瘍の放射線治療にも応用され，著明な進歩，発展を遂げている。

stereotactic radiosurgery (treatment)[17]の実施は正にその具現化である。従来の多門照射による正常脳への放射線の障害を極力少なくするという方式の急速な展開は，強度変調放射線治療(IMRT)[18]の世界を実現し，局所治療をさらに有効なものにするべく臨床研究が進められている。すでに導入されたガンマナイフ[19]はその有効性と安全性から，従来の放射線治療の概念に革新的考えを導入する契機となったものと考えられる。

この他，過去において術中照射による治療，放射線同位元素を用いた組織内照射による治療[20]などが行われてきたが，これらの効果については未だ確実な有効性は確認されていない。むしろその適応はstereotactic radiosurgery (-therapy) の導入以来，縮小傾向にある。これらの放射線治療は高精度集中治療によって，従来晩発生放射線障害の防御のため腫瘍に十分な照射量にて治療できなかった状況を打破して，効果的治療が進められる可能性が高くなってきている。

II．脳腫瘍の成長解析と治療

1．脳腫瘍の増殖動態に関する研究

がんは無制限に増殖する細胞群からなるいわゆる，正常細胞の増殖形態を逸脱した細胞の集団であるとの概念を修正した研究が脳腫瘍に関して行われたのが1970年代に入ってからである。その成果は今でも悪性の指標として利用されている増殖細胞の標識率である[21]。現在ではモノクローナル抗体を用いた簡略な方法(MIB-1標識率など)で行われているが，初期には放射性同位元素，H^3あるいはC^{14}標識チミジンを用いて行われた。この成果の多くは，日本から米国に留学し，カリフォルニア大学サンフランシスコ脳腫瘍研究所にて活躍した故星野孝夫教授の功績によるものである[22]。

まず増殖細胞群の測定により，最も悪性とされる膠芽腫でもすべての細胞が分裂，増殖しているのではなく，増殖細胞の占める率は悪性グリオーマで約30%程度に

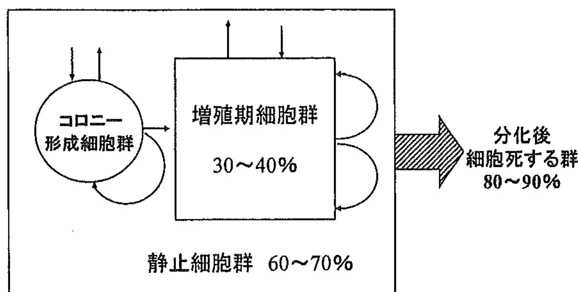


図 1 脳腫瘍の細胞動態

すぎないことを実証した。残りの 70%の細胞は静止期にあるが，この細胞群は常時，静止期に存在するのではなく，交互に増殖期，静止期の間を移行しているものである。

また，放射線のエネルギーの違う C^{13} と H^{3}チミジンを時間差を付けて標識する二重標識法によって，S 期の長さを測定する簡便法の開発，この成果によって細胞世代時間を経時的に追跡せずに測定可能とした（図 1)。

こうした研究の結果，悪性脳腫瘍の成長解析のパラメータとして細胞世代時間 2〜3 日，S 期 8〜10 時間，G_2+M 期約 3 時間と計測され，細胞周期の多くは G_1期で占められることも判明した。

このころ，多くの細胞周期依存性抗癌剤（cell cycle phase specific drug）と呼ばれる 5-FU，MTX などは，このうち S 期に作用して増殖抑制効果を発揮すると分類され，nitrosourea 系の薬剤などは細胞周期非依存性抗癌剤（non-cell cycle phase specific drug）と分類されたわけである。その結果，70%近い静止期細胞で構成される悪性脳腫瘍は，この non-cell cycle phase specific drug を使用することで効果が期待できるとされてきた。

1）血液脳関門

正常脳に認められる血液脳関門（blood brain barrier: BBB）の存在は脳実質性脳腫瘍，すなわち多くの低悪性度の神経膠腫において保たれている場合が多く，膠芽腫においてもその辺縁部では血液関門は保たれていると報告されている[23]。Levin らは抗癌剤と BBB との関係に関する研究を重点的に行い，その通過にはオクタノールと水との溶解性の比，log p 値（log octanol solubility/water solubility）が 0.37 に近いほど BBB をよく通過するという実験結果を報告した[24]。薬剤の面からみると，イオン化しない場合には水溶性薬剤で分子量 180 以下，脂溶性では 450 以下であれば BBB を通過し得るとされている。たとえば，BCNU は分子量 234，log p=1.53，CCNU 234，p=2.83，ACNU 309，p=0.92，PCNU は p 値が 0.37 の薬剤であると報告されている。さらに drug delivery system には，血清蛋白との結合性にも関係した種々の因子が絡んでくることは確かで，この分野の研究も進展している。

また，髄注投与も頭蓋内疾患に対して行われるが，この場合，浸透圧によってのみ薬剤が脳実質内に到達するもので，水溶性の薬剤でラットの実験では約 1.5 cm しか到達しない。脂溶性では，髄液から逆に BBB を通過して血管内に流れてしまうため脳（脳腫瘍）内にとどまる時間が短くて，深部までの到達は見込めないとされている。さらに脳実質に長時間とどまることは薬剤の作用時間が長いことでは長所であるが，神経毒性からすると逆に欠点になることも考慮した治療研究が必要である。

2．放射線治療と化学療法

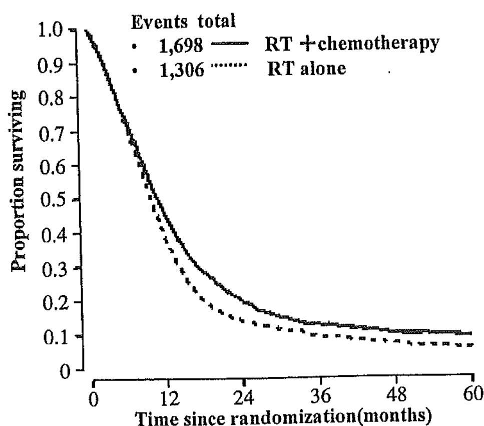
放射線治療は外科と匹敵する重要な治療である。最近では放射線治療単独で治癒可能な悪性腫瘍が確認されている。効果を期待できる治療である。細胞周期の研究が報告されるに伴って，細胞を放射線治療感受性のある G_2期に集積させる力のある化学療法剤を併用した放射線治療によって相乗効果を狙った臨床試験が行われた。高倉らによって実施された多施設共同研究[25]では，ACNU（100 mg/m 6 週ごと）併用放射線治療（2.0 Gy×30 腫瘍照射量）が放射線照射単独よりも，固形腫瘍のサイズ縮小効果で前者が 47%，後者が 15%と有意であることを実証した。しかし生存期間中央値は前者が 14 か月，後者が 12 か月と有意差は認められなかった。

この後，生塩らによる ACNU+放射線治療（40〜60 Gy）対 ACNU+tegafur+放射線治療との第III相多施設臨床試験では前者 30.1%，後者 50.1%と腫瘍縮小効果を認めたが，やはり生存期間には有意差は認められなかった[26]。この後の本邦での臨床試験は，いずれも小規模のものであり比較対照にならない報告が多い。なお，米国での BCNU 併用の放射線治療は 303 例の悪性神経膠腫に対し，無治療群，BCNU 単独群，放射線治療単独群，BCNU+放射線治療併用群の 4 群に分け，第III相臨床研究を実施した。その結果，それぞれの生存期間中央値は 14，18.5，36，34.5 と手術のみ群に対し，放射線治療併用群が有意な生存期間延長を示した。しかし，BCNU 追加による上乗せ効果は認められなかった。大規模臨床研究として実施された BCNU を選択的に内頸動脈に投与する臨床試験では，結果的には正常脳に対する白質脳症を来す頻度が高くなることが判明し中途で中止になった[27]。

3．化学療法単独での効果

化学療法単独での効果は，米国の Levin らによって PCV（procarbazine, CCNU, vincristine）療法が第II相試験として 1980 年に報告され奏効率 29%を得た[28]。

一般に脳腫瘍に対する化学療法の臨床試験は，症例の絶対数が少ないために大規模試験が困難であり信頼でき



Patients at risk

RT +chemotherapy	1,698	720	295	149	96	68
RT alone	1,306	456	155	86	45	28

図 2 高悪性度神経膠腫に対する化学療法の効果（メタアナリシスの結果）[29]

るデータをとり難いのであるが，2002年のStewartらの報告によれば12文献，3,004例の症例を用いたメタアナリシスの結果，1年生存率は放射線治療後に化学療法を行った群で46%，放射線治療のみの群では40%（95%CI 3～9），生存期間では2か月の延長が期待できるとしている（図2）[29]。しかし臨床試験全体をみると，ヨーロッパでの研究成果も含めて，再発悪性神経膠腫に対しては，約3～6か月の延長効果を認めるにすぎない。

この30年間で，特筆に価する化学療法は乏突起神経膠腫に対するPCV療法であり，優れた効果が再認識されている。特に悪性乏突起細胞主の効果は著しく，染色体欠失1p，19qの存在は化学療法効果良好群として報告されている。最近ではテロモゾマイドの臨床試験が始まり，間もなく終了するはずである。これらのことについては化学療法の新展開での記述を参照されたい。

4．血液幹細胞移殖の併用による超大量化学療法

幹細胞移植を応用した超大量化学療法も最近，髄芽腫，胚芽細胞腫の再発例には応用されている。これらの新しい治療の開発には，やはりしっかりしたプロトコールが必須である。外科医の実施している化学療法は何かと非難の対象になるが，専門家として実施するにはそれなりの研修と知識の集積が必要であることはいうまでもない。今までの経験と予測に頼った治療はすでに終焉を迎えたと考えて，確実な知識と技術をもって行う必要がある。

5．免疫療法

免疫療法は昔から隆盛期を迎えたり，それが衰退したりの繰り返しである。約20年前にはinterferonが出現し，免疫力強化を含めた大きな期待が寄せられたものである。しかし，脳腫瘍に関する限りにおいて，β interferonの効果は膠芽腫に対しわずか14%（8/57例）の奏効率しか得られていない[30]。期待したほどの力がないことがわかり，現在では放射線，他化学療法剤との併用で少数の施設で実施されている。約20数年前では免疫療法はOK-432，PSKなどとともにBCGの臨床応用が研究されていた。BCGに関しては，皮内注射による免疫能賦活に効果ありとして進められた[31]。しかし，これらの臨床研究は単発的なものであり，何ら臨床試験としての意味をもったものでなく単なる臨床で使用したという段階を超えていない。サイトカインによる治療として，TNFやIL-2などの報告もあるが，その効果も著明な効果を示したとされる症例が数例紹介された程度で影を潜めてしまっているのが現状である。

遺伝子治療の代表的臨床試験は米国で行われ[32]，HSV-tdkウイルスを用いた研究である。第III相試験まで至ったが，生存期間に有意差を認めることができずに消退している。

現在，臨床に利用されている治療は名古屋大学脳神経外科にて実施されている，リポゾームを用いたinterferon遺伝子の導入による治療ぐらいである。これは日本発の治療でありその成果が期待されている。これらの遺伝子治療の課題は，いかに目標の細胞に遺伝子を繰り込むかであってその方策が現在は検討されている。特に遺伝子を細胞に運んでいくベクターの開発に重点は移ったようである。安全で効果のあるベクターが開発されることを切に期待する。

まとめ

以上，悪性脳腫瘍の診断・治療の変遷について通覧したが，最近では分子標的薬剤が一般のがん治療の分野では効果を発揮している。しかしこういった最新の薬剤も悪性脳腫瘍治療分野においては，現在までのところ期待されたほどの効果は報告されていない。その原因などが明らかにされ，この難病である神経膠腫治療のブレークスルーとなるような事実が発見されることを期待する。

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脳腫瘍の診断と治療
―最新の研究動向―

脳腫瘍取扱い規約の概要

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脳腫瘍取扱い規約の概要

Overview of the general rules for clinical and pathological studies on brain tumors, published by the Committee of Brain Tumor Registry of Japan, and the Japanese Pathological Society in 2002

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Key words 脳腫瘍取扱い規約，WHO分類，脳腫瘍病理，脳腫瘍登録頻度，小児・成人の登録別頻度

はじめに

脳腫瘍取扱い規約[1]は1991年，京都国際会議場で開催された脳腫瘍全国統計委員会において悪性脳腫瘍取扱い規約作成委員会の設立が出発点となる．委員には脳腫瘍全国統計委員会より8人(委員長　高倉公朋東京女子医大教授)，日本病理学会より長嶋和郎教授(北海道大学)，中里洋一教授(群馬大学)2人の推薦を受けて発足した．翌年1992年の夏，第1回脳腫瘍取扱い規約作成委員会が開催され，悪性のみではなく良性を含めた全脳腫瘍を対象として決定され，執筆者の選出が行われた．特に画像診断については神経放射線学に造詣の深い前原忠行教授(順天堂大学)の協力を得ることになった．編集方針としてWHO分類を用いること，規約内容の全体的統一を図るため，原稿について企画委員会でレビューし執筆者に修正要請を行うことが決定された．1993年の京王プラザでの脳腫瘍全国統計委員会では上記のことが承認され実行に移された．1995年の4月と6月に病理部門の編集会議が行われ，掲載される組織写真の再検討，修正などが行われ，9月初版の運びとなった．

その後，1999年7月，Lyonで採択された脳腫瘍の新しいWHO分類に準拠して内容を改訂し，診断，治療内容ともに新しい知見を補足して2002年に改訂版が刊行された．

その内容は3部からなり，第1部では脳腫瘍の一般的事項を，脳腫瘍の診断，治療について総論的に記し，参考のために脳腫瘍全国集計調査の資料解析による治療成績を掲載した．第2部では病理組織分類とそのカラーアトラスを簡潔，明解に記し，日本における病理診断の全国的統一を期待して掲載された．第3部においては日本における脳腫瘍の診断と治療として，それぞれの腫瘍の特徴と説明，治療についても当時の共通認識とされた治療法について記された．

第1部　脳腫瘍取扱い規約

a. 脳腫瘍の種類と頻度

日本における原発性脳腫瘍の発生頻度は人口10万人に対し8〜10人程度(最近の報告では11〜12人)と考えられ，規約に記載されている1993年までの資料では神経上皮性腫瘍(広義の神経膠腫：グリオーマ glioma)が全脳腫瘍の28%，続いて髄膜腫が26%，下垂体腺腫17%，シュワン細胞腫11%で，この4種で約80%を占める．しかし脳腫瘍の疫学(本誌のI章1)にて記述したように，1990年を境に髄膜腫が頻度としては1位になり，1996年では，その割合は27.8%，神経膠腫は2位で22.8%である．図1には1984〜96年に集計された約5万例の症例の登録頻度割合が示されている(図1)．表1に

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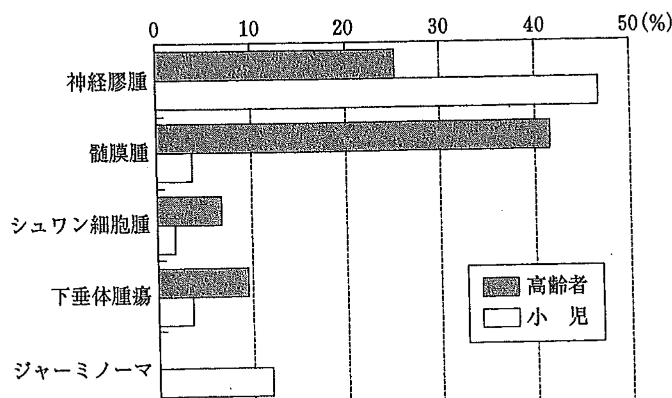

図1　脳腫瘍の種類と年齢区分別登録頻度

表1　脳腫瘍の種類と登録頻度
（全国脳腫瘍統計 1984-96）

脳実質外腫瘍	髄膜腫	26.8％
	下垂体腺腫	17.9％
	神経鞘腫	10.3％
	頭蓋咽頭腫	3.5％
脳実質内腫瘍	神経膠腫	24.9％
	膠芽腫	9.0％
	星細胞腫	7.5％
	悪性星細胞腫	4.8％
	その他	3.6％
	胚細胞腫	2.9％
	悪性リンパ腫	2.9％

は理解を容易にするため，脳実質内腫瘍と実質以外と分類したが，WHO分類にはこの分類は採用されていない．更に，小児，高齢者層それぞれでの代表的脳腫瘍登録頻度を検討すると，髄膜腫が高齢者に圧倒的に多いことがわかる．逆に小児には神経膠腫が圧倒的に多い（図1）．同様に神経膠腫のみを取り上げて分析してみると，膠芽腫は高齢者に圧倒的に多く，小児にはまれであることがわかる（図2）．高齢者の約60％に達する膠芽腫は治療が困難であり，解決されるべき多くの課題を残している．

b．脳腫瘍の診断と病期分類

病期分類は，治療効果を的確に決定するうえで重要である．良性の脳腫瘍においては，手術の難易度を示す良い分類となり，術者の技術の達成度をみるためにも重要である．しかし，神経膠腫においては，その病期分類が確立されていない．その理由の一つに，脳のもつ複雑な機能分野との関係がある．むしろ進展度による手術の困難性よりも，機能欠損を来すことなく，いかに手術を施行するかの方が困難度は高い．脳の機能を把握する方法は簡単ではなく高度な術中管理を必要とする．そのために，幾つかの分類が提案されるが，広く利用され成果を上げるまでに至っていない．取扱い規約の中ではこのような意味で，今まで報告され，比較的脳外科医に知られた分類について掲載してある．これらは，将来更に改善され，実際の治療に応用されるようにならなければならない．

掲載されている分類は，UICCのTM分類，その元になったと思われるAANS/CNS Joint Tumor Sections Staging Formである．そのほか，下垂体腫瘍，聴神経腫瘍（シュワン細胞腫）などの既報告の分類を掲載しているが，将来的にはそれぞれの腫瘍に対し標準的手術法を示す必要がある．

効果判定法に関する記載は，1975年の厚生省がん研究助成金判定基準によるもので，脳腫瘍の効果判定基準が掲載されているが，現在では，国際基準としての，RECIST（Revised Evaluation Criteria in Solid Tumor）基準が汎用されているので注意する．規約では2方向測定にて縮小率を算出しているが，RECISTでは，1方向測定を採用している．その結果，評価は最大径の合計が30％の縮小をもって効果ありとする

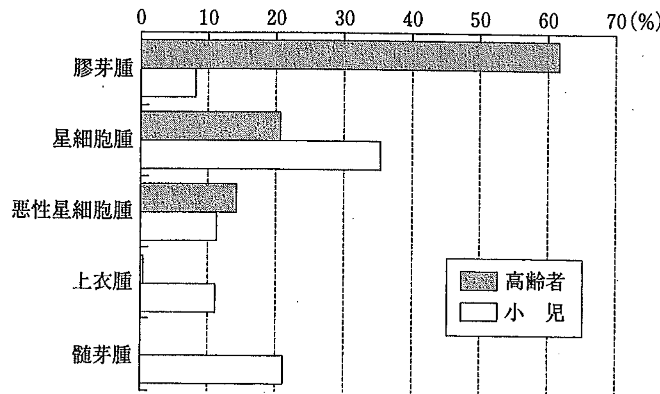

図2　神経膠腫の種類別と年齢区分別登録頻度

(2方向では50％縮小)．また，1方向20％の増加で増悪と判定する．

c．脳腫瘍の登録

脳腫瘍全国登録の均一性を確保するために，調査用紙の記入法について規約で説明をしている．調査用紙は，第1頁(表2)の調査用紙Aに個人識別項目があるが，個人情報保護法に準拠して実施するためにこの項目は削除され，施設ナンバーとして登録されることになる．従来は，重複登録例を氏名，生年月日を調べることによって除いていたが，重複登録を排除できなくなるために，5年に1度の調査に変更することになった．

調査用紙Bにおいては，治療を調査する．手術摘出率，放射線治療，化学療法，免疫療法，遺伝子治療も記入できるようになっている．また，術前，術後のperformance stateを記入することでQOLへの影響を検索できるように工夫されている．続いて，2頁(表3)中の，調査用紙Cには原発性脳腫瘍の診断，局在，肉眼所見，病理診断の記入，調査用紙Dには転移性脳腫瘍に関する同一の調査項目がある．調査用紙Eが予後調査として活用される．脳腫瘍登録を精度の高いものにするために，統計調査用紙は時々改訂されて今日まで至っているが，1969年からのデータをそのまま生かして継続登録できるような工夫はされている．最近では，2000年にWHO病理組織分類が改定されたことによる修正として，従来の組織分類との整合性を保つために組織対応表を掲載した(表4)．このコードは，従来登録された症例について自動的に従来の組織コードが新コードに変換されるようになっている．更に病理組織について，大分類と細分類を採用して，大分類で探し，その中に細分類を並べることによって，たくさんの病理分類の中から探し出す煩わしさを軽減している．また，調査協力施設へのインセンティブを維持するために，調査収集データの論文への利用を可能としている．具体的には，調査資料利用依頼の申請書(表5)を送付することによって，統計利用審査委員会での審査を受け，論文作成に利用できる仕組みになっている．また，この仕組みはデータを私物化して使用するために起こる，問題の回避にもなっている．

第2部　病理組織診断・カラーアトラス

病理診断の均一性を保つために，日本病理学会からの代表による力作である．これらは腫瘍の病理学的な診断のための特徴を明確に記載し，可能なかぎりの精細な病理所見を鮮明なカラーで掲載している．他に類を見ない貴重な病理組織写真は，執筆者，協力者の賜物である．さて，分類はWHO分類に準拠しているが，WHO分類は神経組織腫瘍分類であるため下垂体腺腫や嚢胞性病変などは包括していない．しかし本規約では，特別に項を設けて嚢胞性病変，下垂体腫瘍と下垂体炎，頭蓋骨および隣接する軟部に発生する腫瘍ないしは腫瘍様病変，周囲組織よ