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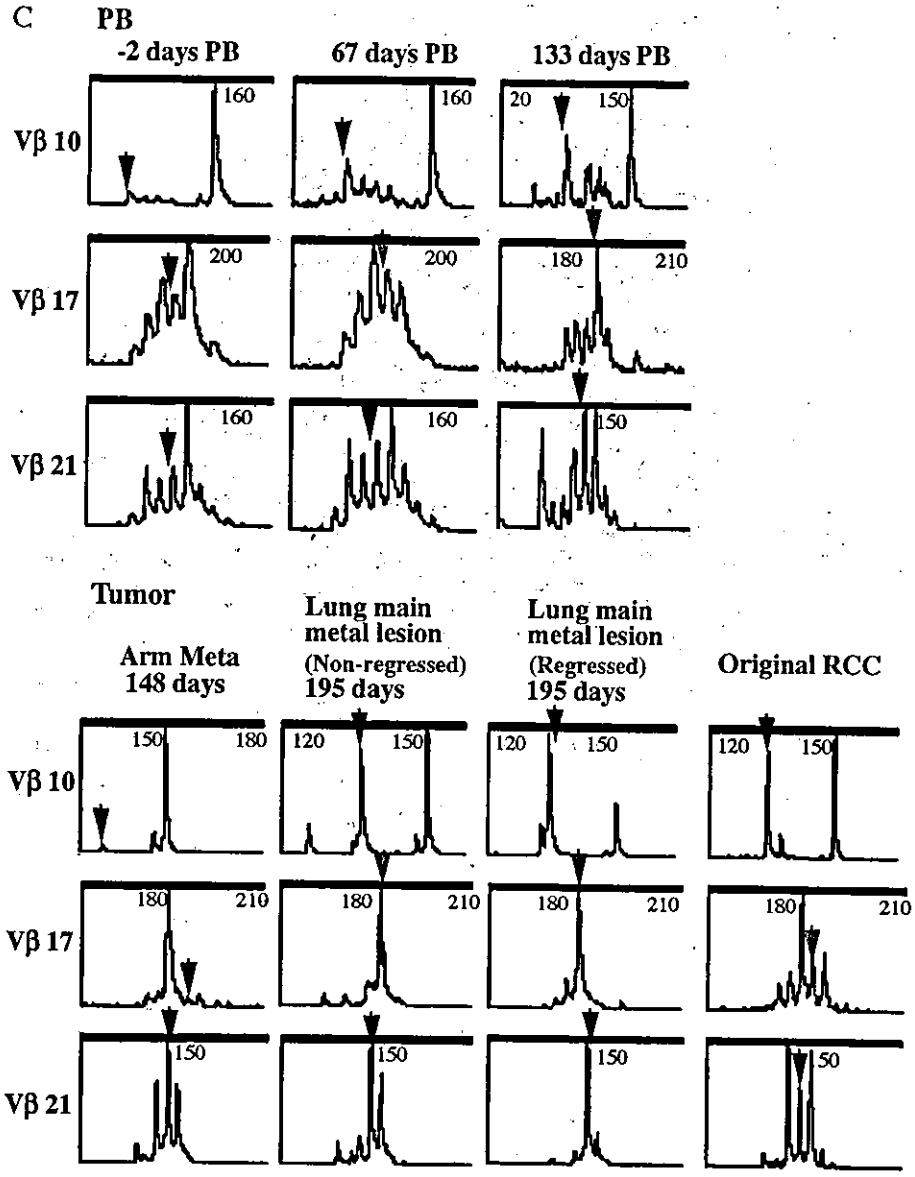

FIG. 5 (*continued*).

induced in these patients. Conversely, the reactions were also positive for NRC, most notably in Case 4, as judged by the skin reaction size (Table 2). The possible causes for this strong background DTH reaction include minimally residual xenoproteins of collagenase or trypsin in GVAX [14] and unknown common antigens existing between NRC and RCC, as the cells that infiltrated into the vaccination sites of RCC and NRC were phenotypically identical, although the number of the former cells was more prominent (data not shown). Thus, possible adverse events of autoimmune nephritis should be monitored carefully. Our follow-up observations over 3 years showed no remarkable renal dysfunction in Cases 2, 3, and 4. Also, Case 1 had no pathological changes associated with autoimmune nephritis in autopsy specimens 7 months after the vaccinations (data not shown). This long-term observation might support the safety of GVAX and its capability of inducing anti-RCC-specific immunity.

The significance of our effector-phase pathological analysis should be emphasized. We had a chance to

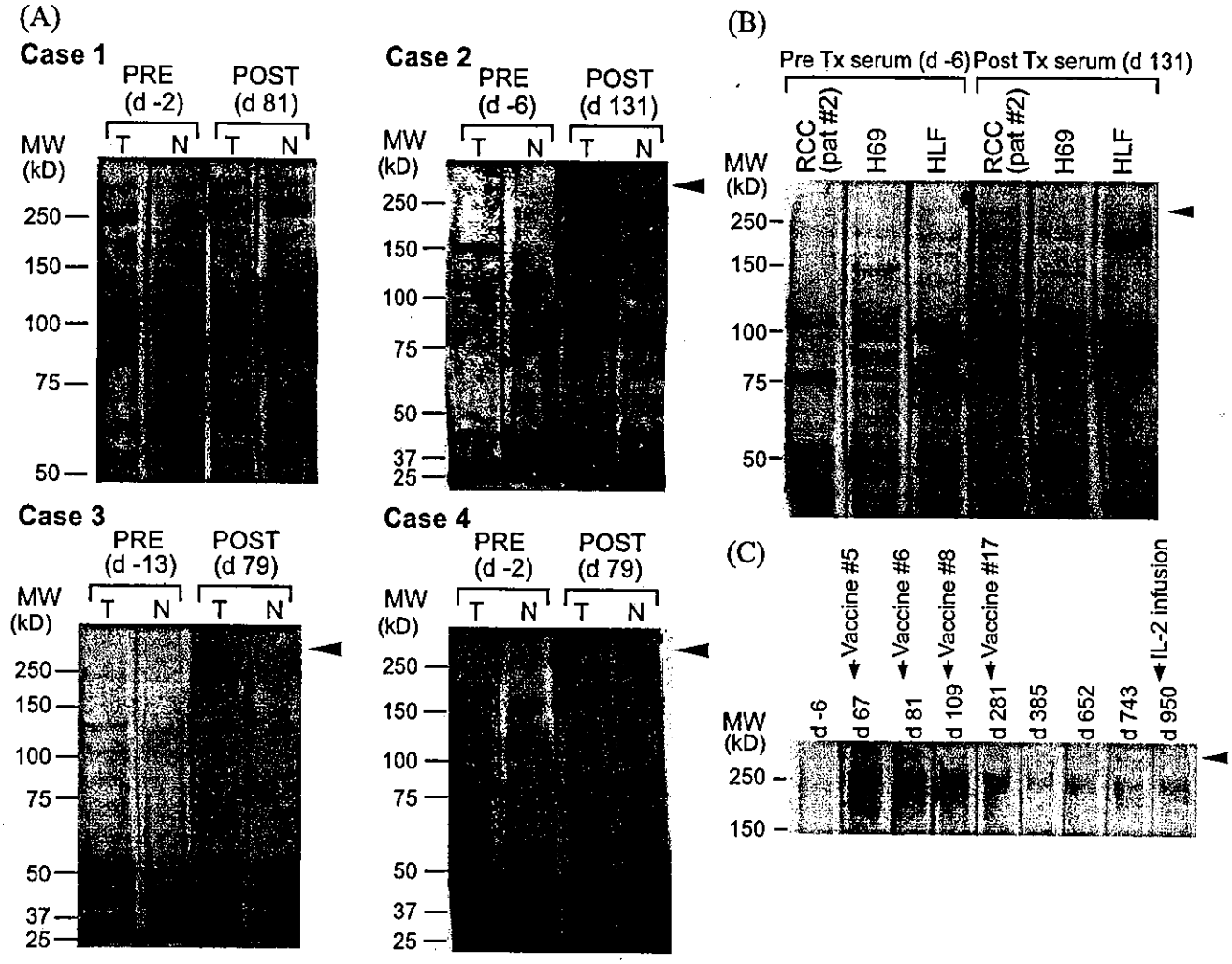

FIG. 6. Appearance of antitumor antibody responses in Cases 1 to 4, who received GVAX. (A) Comparisons of serum reactivity to autologous RCC proteins pre-GTx and post-GTx. Proteins extracted from cultured tumor cells (lane T) or normal kidney cells (lane N) were electrophoresed, transferred onto PVDF membranes, and detected with autologous patient sera (Cases 1 to 4). Sera were harvested before (PRE) and after (POST) the first vaccination (day 0). (B) Comparison of the serum reactivity to autologous RCC, H69 cells, and human lip fibroblasts (HLF). The reactivity to high-molecular-weight proteins was significant in autologous RCC and HLF, while it was weak in H69 cells. Autologous RCC from Case 2 (pat #2) was used. (C) Time course of changes in serum reactivity to high-molecular-weight proteins of approximately 250 kDa in Case 2. Immunoblotting of RCC proteins from Case 2 with autologous sera harvested at several postvaccination days (post-5th, 6th, 8th, and 17th vaccination and before the administration of low-dose IL-2).

investigate pathologically the RCC before and after vaccinations in Case 1 and have demonstrated the induction of tumor site-specific infiltration of predominantly $CD8^+$ T cells. This was associated with tumor apoptosis in postvaccinated biopsy and autopsy tumor specimens, whereas $CD4^+$ T cells predominated and tumor cell apoptosis was negligible in the original RCC (Fig. 3). Notably, these changes were demonstrated in the biopsy specimen that was obtained before IL-2 administration. These observations strongly suggest the induction of tumor-specific immunity by GVAX. Although GVAX could induce both the localization of $CD8^+$ cells within metastatic tumors and significant apoptosis, not all of the tumors showed regression. Thus, GVAX-induced antitumor immunity per se may not be sufficient for clinical efficacy.

We studied various parameters, as it is still unknown which immune factors can be used to predict the therapeutic efficacy of antitumor immune gene therapy. The results of our *in vitro* assessment of cytokine production were compatible to those reported by Soiffer *et al.* [13]. These cytokine profiles indicated the coordinate expression of gene products associated with both Th1 and Th2 cells and suggested that multiple lymphocyte effector mechanisms contribute to the potent antitumor immune response. The cytokines produced by

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these $CD4^+$ T cells activate eosinophils, as well as macrophages that produce both superoxide and nitric oxide. Both of these cell types then collaborate at the site of the tumor challenge to cause its destruction [13]. Our observation suggested that this Th2-dominant immunological response was particularly enhanced, namely, an *in vivo* immune shift from Th1 dominance to Th2 dominance was induced after repeated vaccinations and maintained. Previous studies of GM-CSF immune gene therapy assayed cytotoxicity using PBMC or TIL [13–15]. Kusumoto *et al.* reported that vaccination with irradiated autologous GM-CSF-producing melanoma cells appeared to increase the cytotoxicity against autologous tumor cells in five patients, although repeated vaccination appeared to decrease the CTL activity in two of these cases. They suggested that vaccination of these patients with autologous melanoma cells caused T cell anergy or tolerance [14] without demonstrating the precise underlying immune mechanism involved. In the present study, Case 1, with large lung metastases, which might have contributed to the observed immunological suppression, showed similar results. Although cytotoxicity assayed using PBMC gradually decreased in Case 1, our pathological findings in the metastatic lesion showed the predominant infiltration by CD8 T cells. These findings might support the limited predictability of the *in vivo* antitumor reaction using only traditional immunoassays using PBMC.

Recently, T cell receptor β chain repertoire analysis methods were reported to facilitate the detection of clonal T cell expansion in various biological specimens. As RCC is thought to be a tumor whose growth may be controlled by the immune response, characterization of T lymphocytes found in RCC patients may demonstrate this important issue [16,17]. Using CDR3 length pattern analysis, Puisseux *et al.* demonstrated a selective localization of oligoclonal T cell populations in malignant tissues after comparisons to the T cell repertoire in the tumor and in the autologous peripheral blood lymphocytes or normal adjacent kidney [16]. Importantly, in our clinical studies, the induction of oligoclonal expansion of T cells with the selected TCR in the peripheral blood, skin biopsy specimens from DTH sites, and tumors was demonstrated after vaccination. The reasons for the observed different clonal T cell expansions in the different tissues in our studies may arise from either a polymorphic T cell response to the same antigen or a different immunogenic environment [17]. Hanada *et al.* recently demonstrated the important role of posttranslational protein splicing in the immune recognition of self and foreign peptides using human RCC antigens, and this phenomenon may explain our results [29]. Although we could not prove directly that these oligoclonally expanded T cells responded to RCC antigens, our findings of the generation of MHC-restricted and TCR-mediated cytotoxicity against autologous RCC and the predominant infiltration of CD8 T cells and apoptosis in metastatic lesions [1] supported this possibility.

In addition to the enhanced antitumor cellular immunity, GVAX is thought to induce antitumor humoral immunity. Simons *et al.* measured increased titers of antibodies recognizing prostate tumor antigens in sera from patients vaccinated with GM-CSF-transduced autologous prostate tumor cells. New antibodies recognizing polypeptides of 26, 31, and 150 kDa in extracts from LN CapPCA cells were observed in three of eight patients following the final vaccinations [18]. Soiffer *et al.* reported similar observations, with antibodies recognizing different polypeptides, in melanoma patients [13]. In the present study, Western blot analysis identified RCC-derived polypeptides of 65 and 250 kDa. We are currently screening RCC cDNA expression libraries with our patients' sera using the SEREX method to look for RCC-specific antigens other than RAGE and G250 [30,31]. We have already cloned several candidate cDNAs and are studying their RCC specificities and the possibility of their future application in anti-RCC immunotherapy.

Currently, several candidate strategies to enhance the systemic anti-RCC immunity of GVAX can be considered. These include the coadministration of IL-2 to enhance basal antitumor immunity [3,5–7,32–34], allogeneic stem cell transplantation including nonmyeloablative stem cell transplantation to introduce allogeneic immunity [27], IL-12 or CD80 cDNA-transduced autologous tumor cells for the direct activation of CTLs, the blockade of CTLA-4/B7 interactions with monoclonal antibody to activate costimulation signals, and the functional activation of dendritic cells using HSP gp91 [35–38]. The administration of low-dose IL-2 as an anticancer immunotherapy has recently been introduced to decrease both the side effects and the cost of treatment [39–42].Our experience with three patients who were given GVAX followed by low-dose IL-2 would cast new light on anti-cancer immunotherapy, possibly by inducing tumor-specific immunity by GVAX, followed by enhancement of the broad antitumor immunity with systemic low-dose IL-2. In the present study, *in vitro* CTL analysis in these two patients supported the hypothesis that the antitumor CTL activity was maintained after administration of IL-2. The optimal duration of treatment with low-dose IL-2 in combination with GVAX remains to be determined by closely monitoring antitumor immunity both *in vitro* and *in vivo*.

PATIENTS AND METHODS

Selection of Patients

The details of the study design and methods of vaccine production were essentially the same as those reported by Simons *et al.* [13,14], except for modifications that were implemented according to the regulations for

clinical gene therapy announced by the Japanese government between 1995 and 1997. Briefly, patients with stage IV RCC (Union Internationale Contre le Cancer classification of 1997) were eligible. Chemotherapy, radiotherapy, systemic IL-2- or interferon-α-based regimens, or other investigational agents were also offered as treatment options to these patients. The following eligibility criteria were used: primary RCC in place with evaluable metastasis after nephrectomy; Eastern Cooperative Oncology Group performance status of zero or one; appropriate surgical candidate and estimated life expectancy of at least 6 months; no major surgery, radiotherapy, chemotherapy, immunotherapy, or immunosuppressive medications within 1 month prior to enrollment; age >18 years; absence of active infection, i.e., WBC count <4000/μl, platelets <100,000/μl, total bilirubin <1.5 mg/dl, and creatinine <2.0 mg/dl; HIV seronegativity; and no history of autoimmune disease. The exclusion criteria included age <20 years; pregnant or lactating women; double malignant tumors; surgery; local or systemic treatment with corticosteroids; immunotherapy; irradiation or anti-cancer drugs 1 month before registration; leukocytosis of unknown origin; history of systemic lupus erythematosis, sarcoidosis, rheumatoid arthritis, autoimmune hemolytic anemia, autoimmune thyroiditis, glomerulonephritis, or vasculitis; apparent infection requiring treatment before second stage; apparent brain metastasis detected on CT scan or MRI; postnephrectomy deep vein thrombosis or pulmonary embolism that required treatment; and opium or alcohol abuse. The study was reviewed and approved by the Committee on Clinical Investigation and Institutional Gene Therapy Ethical Committee, The Institute of Medical Science, University of Tokyo, in April 1998, and by the Joint Committee of the BioScience Committees of the Ministry of Health, Labor, and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology in August 1998.

Study Design

Patients were enrolled from September 1998 to May 2001. Eligible patients were nephrectomized after giving their initial informed consent. The second informed consent was obtained after safety confirmation tests, which included negative tests for microbial contaminants such as bacteria, fungi, mycoplasma, RCR, and endotoxin, and when sufficient production (>40 ng/10^6 cells/24 h) of GM-CSF was detected in the GM-CSF gene-transduced RCC. The vaccination schedule of GVAX, including additional vaccinations, is described precisely under Vaccine preparation and administration. Peripheral blood was obtained (as per NIH Recombinant DNA Advisory Committee and Food and Drug Administration guidelines) for detecting RCR before treatment, after vaccination, monthly for 3 months, every 3 months for the next 9months, and then yearly [14]. Long-term follow-up, including periodic evaluation for autoimmune disease and tumor progression, was performed.

Clinical Evaluation

The patients received daily physical examinations and periodic laboratory tests, which included hematological parameters and liver, renal, and immunological functions, prior to and after the vaccinations. The metastatic lesion volumes were measured using CT (lung, liver, bone, brain), MRI (liver, bone, brain), and thallium or technetium scintigraphy (whole body). Unenhanced helical CT images that covered each lesion were obtained during a single breath-hold. The thickness of the slices ranged from 3 to 10 mm, depending on the lesion size. The data were transferred to a workstation (Advantage Windows; General Electric Medical Systems, Milwaukee, WI, USA) to calculate the tumor volumes. Low-density areas, which represent lung parenchyma, were excluded at a threshold of −400 HU, and lesion sections were selected manually from the remaining areas of each slice. The lesion volume was calculated with a 3-D utility on the workstation for Cases 1, 2, and 3. The lesion in Case 4 was calculated as the sum of the perpendicular diameters of all lesions measured by CT scan, due to difficulties in measuring small multiple tumors volumetrically.

Vaccine Preparation and Administration

The methods used for autologous RCC vaccine preparation and MFGS-GM-CSF gene transfer at the Clinical Cell Processing Facility of the Institute of Medical Science Hospital at the University of Tokyo have been described previously [13]. The procedure complied with good manufacturing practices. Primary cultures were established and transduced at the first passage. Following *in vitro* expansion, the vaccine cells were irradiated at 150 Gy to prevent clonogenic survival *in vivo* after vaccination. GM-CSF production was determined using a GM-CSF ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Genomic integration of the GM-CSF cDNA into the patients' autologous RCC cells was determined by the standard Southern blotting method using MFG-GM-CSF plasmid DNA to determine the copy number, as described elsewhere [43]. The tests for microbial contaminants, i.e., bacteria, fungi, mycoplasma, RCR, and endotoxin, were all performed by BioReliance Corp. (Rockville, MD, USA). The vaccine cells were stored in liquid nitrogen until use. On the day of vaccination, 4×10^7 viable cells were administered intradermally in the first injection, and thereafter, 2×10^7 cells were administered at least five times at 2-week intervals, which was considered to be a superior vaccination schedule as described by Soiffer *et al.* [16]. Each patient was carefully screened for eligibility according to the inclusion criteria by the Institutional Review Board (IRB) of the Institute of Medical Science, University of Tokyo. The IRB permitted additional

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administration of vaccine every 2 weeks when the yield of cells was higher than the 1.4×10^8 cells required for the six scheduled administrations and in cases in which the patient's physical condition was acceptable after further informed consent was obtained. The vaccinated sites were biopsied for microscopic examination at 3 and/or 7 days after every second vaccination.

Toxicity Assessment and Pharmacokinetic Analysis of Serum GM-CSF Levels

The levels of toxicity were graded using the National Cancer Institute's cancer common toxicity criteria for clinical trials. Toxicities were identified by medical history, physical examination, and review of the laboratory studies performed. Patients' sera were frozen in 1-ml aliquots at −80°C until the day of testing. The serum GM-CSF levels were determined for all collection time points by enzyme-linked immunosorbent assay using the Biotrak human GM-CSF ELISA system (Amersham International Plc., Amersham, UK) according to the manufacturer's protocol.

Histological Studies

Six-millimeter punch biopsies were removed from the intradermal injection sites on days 3 and/or 7 following the first vaccination. Prevaccination skin biopsies were obtained for comparison. Similarly, skin biopsies were also taken for evaluation of the DTH reaction 48 h after intradermal inoculation of RCC cells and NRC. Surgically removed and autopsy materials were used for the histological evaluation of tumors and tumor-infiltrating cells. Biopsy materials were fixed in 10% buffered formalin, embedded in paraffin, stained with H&E, and labeled with antibodies to CD3, BMP (rabbit antiserum to human myelin basic protein; DAKO Corp., Carpinteria, CA, USA), AE1/AE3 (pooled mAbs to human epithelial keratin, IgG1 subtype; Boehringer Mannheim, Indianapolis, IN, USA), S100 (rabbit anti-cow S100; DAKO), CD68 (anti-human macrophage CD68 mAb, IgG3-subtype; DAKO), HLA-DR (clone LN3, IgG2a subtype; Lab Vision Corp., Fremont, CA, USA), CD3 (clone PS1, mAb, IgG2a subtype; Novocastra Laboratories, Newcastle, UK), CD4 (clone 1F6, mAb, IgG1 subtype; Novocastra Laboratories), CD8 (clone 1A5, IgG1 subtype; Novocastra Laboratories), and CD20cy (B cell marker, clone L26, mAb, IgG2a subtype; Lab Vision Corp.). For the evaluation of tumor apoptosis, the TdT-mediated dUTP–biotin nick end-labeling (TUNEL) method was applied using an ApopTag Kit (Intergen Co., Purchase, NY, USA).

Delayed-Type Hypersensitivity Testing

To evaluate the cell-mediated immunity status of each patient before and after treatment, DTH testing was performed using seven common recall antigens (Multitest CMI; Connaught Laboratories, Swiftwater, PA, USA) according to the manufacturer's instructions. Reaction scoring was also performed according to the manufacturer's instructions. The patients were tested simultaneously for reactivity to autologous, irradiated cultured RCC cells and NRC. The autologous RCC cells and NRC for DTH testing were prepared and stored in liquid nitrogen according to the same procedure used for vaccine cell production omitting GM-CSF transduction. During storage, sterility testing for bacteria, fungi, mycoplasma, and endotoxin was carried out at the Department of Laboratory Medicine, Institute of Medical Science, University of Tokyo. PBMC were isolated using the standard Lymphoprep (d = 1.077; Nycomed Pharma AS, Oslo, Norway) density gradient centrifugation method. These cells were washed three times with HBSS, counted, and injected intradermally at 10^6 cells/0.2 ml. DTH reactions were observed 48 h after each DTH injection, i.e., 1 week before the first vaccination and 1 week after the second, fourth, and sixth vaccinations in all four patients.

Tumor Tissues, Peripheral Blood, and Skin Biopsies from Patients

Single-cell suspensions of tumor tissues were obtained from biopsied or autopsied (Case 1) tumor specimens that were minced mechanically and treated with collagenase and DNase. RCC cells and TILs were separated by density gradient centrifugation, as described elsewhere [24]. Heparinized peripheral blood samples (20 ml) were drawn from patients every other week before vaccination. For follow-up, samples were also drawn when the patients permitted. Patients' sera were frozen at −80°C until use for Western blot analysis. PBMC were isolated as above. PBMC and TIL (5×10^6 cells/tube) were cryopreserved using a programmable freezer and stored in liquid nitrogen. In addition, the cell pellets were frozen in liquid nitrogen until used for RNA extraction. Skin biopsies obtained from the DTH reaction site (6 mm in diameter) were cut into pieces measuring approximately 1 × 1 mm and rapidly frozen in liquid nitrogen until used for RNA extraction.

Assessment of Lymphocyte Proliferation and Cytokine Production

On the day of the assay, the cryopreserved samples were thawed. PBMC (1×10^5 cells/well) were cultured in the presence of irradiated (150 Gy) GM-CSF-transduced autologous tumor cells (1×10^4 cells/well) plus IL-2 (40 U/ml), in 96-well flat-bottomed plates. RPMI 1640 medium with L-glutamine (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD, USA) and gentamicin was used as complete medium. On day 3 or 6, culture supernatants (100 μl/well) were collected from each well to determine the cytokine levels, and fresh medium was added. The cultures were then pulsed with [^{3}H]thymidine (0.5 μCi/well; DuPont-NEN, Boston, MA, USA) for a final 18 h and harvested on a Micro 96 harvester (Skatron, Lier, Norway), and the incorporated radioactivity was measured using a

microplate counter (Micro Beta Plus; Wallac, Turku, Finland). ELISAs for human IFN-γ, IL-5, and IL-10 were performed using ELISA kits (OptEIA; BD-Biosciences, Boston, MA, USA) according to the manufacturer's protocols.

Cytotoxicity Assay

To prepare effector cells, cultures with IL-2 and irradiated GM-CSF-transduced autologous tumor cells as described above were prepared in 96-well round-bottomed plates and the plates were cultured for 7 days. On the day of the assay, aliquots of 100 μl of the culture medium were removed from each well and then labeled target cells (5×10^3 cells/100 μl/well) were added. To label the target cells, single-cell suspensions of cultured autologous or allogeneic RCC cells, autologous NRC, and K562 cells were incubated with $Na_2^{51}CrO_4$ (100 μCi) for 1 h at 37°C and washed three times prior to use. For blocking experiments, F(ab′)2 anti-CD3 mAb prepared as described previously [44] was added to a final concentration of 10 μg/ml at the start of the assay. The plates were incubated at 37°C for 6 h, the supernatants were collected using a Skatron cell harvester system (Diversified Equipment Co., Lorton, VA, USA), and the radioactivity was measured using a γ counter. Spontaneous release (SR) and maximal release (MR) were measured in the supernatant of target cells alone with 100 μl of either medium or 10% Triton X-100 (Sigma, St. Louis, MO, USA). The percentage specific cytotoxicity was calculated using the following formula: % cytotoxicity = experimental release − SR/MR − SR × 100.

Analysis of the TCR β Repertoire

Total RNA was isolated from PBMC and homogenized tumor tissues using Trizol reagent (Invitrogen) with a cryo-press crusher (Microtech Nichion, Tokyo, Japan). TCR β repertoire analysis was performed as described previously [45]. Briefly, TCR β cDNA was synthesized using C-oligonucleotides (5′-CGGGCTGCTCCTT GAGGGGCTGCG-3′) with AMV reverse transcriptase (Invitrogen). The TCR cDNA was amplified by 40 cycles of PCR with each of the 24 Vβ 5′ primers (Vβ 1-w24) and the Cβ 3′ primer in PCR buffer containing 1 U of Hot Start *Taq* polymerase (AmpliTaq Gold; Applied Biosystems, Foster City, CA, USA). The products were subjected to Southern blot analysis using a ^{32}P-labeled Cβ probe. Different samples of each Vβ product were compared after quantifying the autoradiographs by densitometry BAS-2000II (Fuji Photo Film Corp.). To refine CDR3 size analysis, the Vβ–Cβ PCR product was copied in a 10-cycle run-off reaction with a fluorescence-labeled Cβ primer. The labeled PCR products were electrophoresed on a DNA sequencer (ABI Prism 377; Applied Biosystems) in the presence of a fluorescent size standard and analyzed with a DNA fragment size program (GeneScan; Applied Biosystems).

The PCR products of the CDR3 fragment were cloned into the pCRII-TOPO vector system (Invitrogen). Thirty colonies containing the insert fragment were selected at random and sequenced using an ABI Prism Cycle Sequencing Kit (Applied Biosystems) and an automatic DNA sequencer ABI 373 (Applied Biosystems). The amino acid sequence of the CDR3 region was deduced using the software GENETYX-MAC v10.1.4 (Software Development Co., Ltd., Tokyo, Japan).

Detection of Antitumor Antibodies

The antitumor antibodies appearing in patients' sera were detected by Western blot analysis according to the standard procedure with some modifications [18]. Briefly, humoral antitumor immune responses were evaluated using the reactivity of the tumor cell lysate and sera from the patients. Autologous RCC and NRC were extracted in lysis buffer containing 20 mM Tris–HCl at pH 7.6, 1% NP-40, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 500 units/ml aprotinin (Calbiochem, La Jolla, CA, USA). A fibroblast cell line of human lip origin, which was established in our laboratory, and a small-cell lung carcinoma cell line, H69, were used as irrelevant control cells. Cell lysates were denatured, reduced in SDS sample buffer with 2-mercaptoethanol, and then electrophoresed on 7.5% polyacrylamide minigels (Bio-Rad Laboratories, Hercules, CA, USA). The proteins were transferred onto Immobilon membranes (Millipore, Bedford, MA, USA) and the blots were stained with Ponceau S solution (Sigma) for visualization. After destaining with TBST (0.1% Tween 20–Tris-buffered saline) and blocking with 5% nonfat dried milk in TBST overnight, the blots were probed with diluted (1:300) patient sera for 2 h. Horseradish peroxidase-conjugated rabbit F(ab′)2 anti-IgG Ab (DAKO, 1:3000 dilution) was added for 1 h, and the blots were developed with an ECL kit (Amersham Biosciences, Piscataway, NJ, USA).

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

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ABSTRACT

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

RESULTS

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

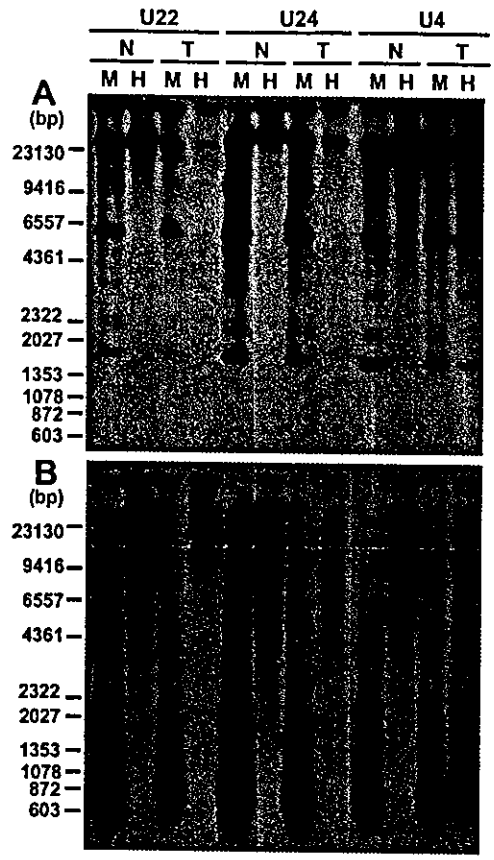

FIG. 1. Examples of Southern blotting for satellites 2 (*A*) and 3 (*B*) in cases of urothelial carcinoma. Methylation status was assessed by digesting DNA with *Msp* I (*Lane M*) and *Hpa* II (*Lane H*). DNA fragments were separated by electrophoresis, transferred to nitrocellulose membranes and hybridized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

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

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

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

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

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

TABLE 1. *DNA hypomethylation on pericentromeric satellite regions and clinicopathological parameters in urothelial carcinomas*

Tissue Specimens	No. Analyzed	No. Hypomethylation (%)	p Value (chi-square test)
	Satellite 2		
Histological grade			
G1–2	15	2 (13)	
G3–4	12	9 (75)	0.0012
Invasion depth			
Superficial (pTa, pT1)	11	1 (9)	
Invasive (pT2–4)	16	10 (63)	0.0055
Histological structure			
Papillary	21	6 (29)	
Nodular	6	5 (83)	0.0161
	Satellite 3		
Histological grade			
G1–2	15	3 (20)	
G3–4	12	9 (75)	0.0043
Invasion depth			
Superficial (pTa, pT1)	11	2 (18)	
Invasive (pT2–4)	16	10 (63)	0.0228
Histological structure			
Papillary	21	7 (33)	
Nodular	6	5 (83)	0.0297

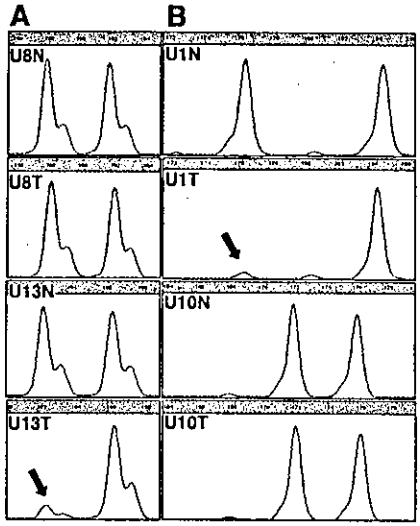

FIG. 2. Examples of results of allelic status analyses in cases of urothelial carcinoma. U8 and U13 DNA samples were amplified for D9S747 (*A*), while U1 and U10 samples were amplified for D9S775 (*B*). Genotypes derived from noncancerous U8N, U13N, U1N and U10N tissues, and corresponding U8T, U13T, U1T and U10T cancerous tissues are shown. Allele size in bp is indicated on top of horizontal axis. In all 4 noncancerous samples PCR products showed polymorphism, indicating that these cases were informative. U8T for D9S747 and U10T for D9S775 were classified as retention of alleles because signal intensity for tumor alleles was not changed significantly relative to matched normal alleles. LOH was identified when signal intensity for tumor allele was decreased by more than 50% relative to matched normal allele, that is in U13T for D9S747 and U1T for D9S775 (arrows).

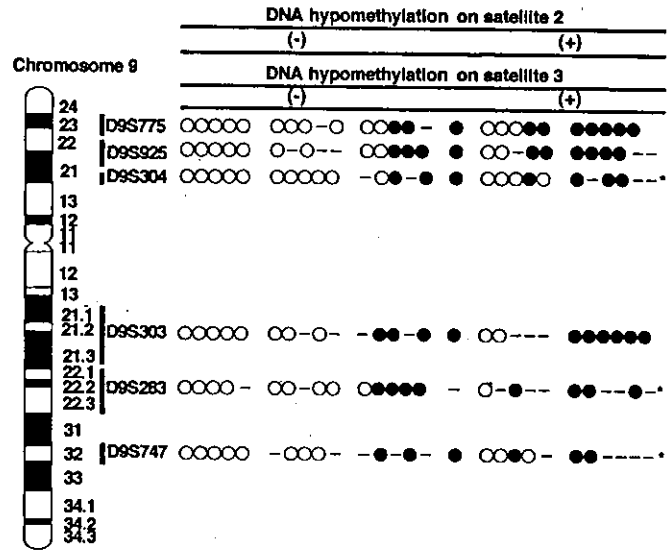

FIG. 3. Allelic status of each locus in urothelial carcinomas. Vertical lines indicate each carcinoma. Open circles indicate retention of 2 alleles. Filled circle indicate LOH. Bar indicates uninformative case. Asterisk indicates replication error. −, negative. +, positive.

TABLE 2. *LOH on chromosome 9 in urothelial carcinomas*

Locus	No. Analyzed	No. Informative	No. LOH (%)
9p			
D9S775	27	24	10 (42)
D9S925	27	21	10 (48)
D9S304	27	22	7 (32)
Any on 9p	27	26	11 (42)
9q			
D9S303	27	20	10 (50)
D9S283	27	18	8 (44)
D9S747	27	17	6 (35)
Any on 9q	27	26	12 (46)
Any on chromosome 9	27	27	14 (52)

hypomethylation on pericentromeric satellite regions significantly correlated with the presence of LOH on at least 1 locus on chromosome 9 in urothelial carcinomas (chi-square test p = 0.0098 and 0.0034 for satellites 2 and 3, respectively, table 4).

DISCUSSION

DNA hypomethylation on satellites 2 and 3 was observed frequently in urothelial carcinomas but it was extremely rare in noncancerous tissues, suggesting that DNA hypomethylation on satellites 2 and 3 is associated with urothelial carcinogenesis. We have previously reported that DNA hypomethylation on satellites 2 and 3 is a frequent and early event during hepatocarcinogenesis,[18] whereas it is rare in colorectal and stomach cancers.[19] These and the current findings suggest that DNA hypomethylation on pericentromeric satellite regions is organ specific during human carcinogenesis. In the current study DNA hypomethylation correlated with tumor aggressiveness (eg histological grade and invasion depth), indicating that it may participate in the malignant progression of urothelial carcinomas. In addition, DNA hy-

TABLE 3 LOH on chromosome 9 and clinicopathological parameters in urothelial carcinomas

Parameters	No. Analyzed	No. LOH+ (%)	p Value (chi-square test)
Histological grade			
G1–2	15	5 (33)	
G3	12	9 (75)	0.0512
Invasion depth			
Superficial (pTa, pT1)	11	4 (36)	
Invasive (pT2–4)	16	10 (63)	0.1817
Histological structure			
Papillary	21	8 (38)	
Nodular	6	6 (100)	0.0074

TABLE 4 DNA hypomethylation on pericentromeric satellite regions and LOH on chromosome 9 in urothelial carcinomas

Chromosome 9 LOH	Hypomethylation		p Value (chi-square test)
	Neg.	Pos.	
Satellite 2			
Neg.	11	2	
Pos.	5	9	0.0098
Satellite 3			
Neg.	11	2	
Pos.	4	10	0.0084

pomethylation was associated more frequently with nodular invasive carcinomas showing an aggressive clinical outcome than with papillary carcinomas. Nodular invasive carcinomas arise from their precursor lesions, that is widely spreading flat carcinoma in situ, and rapidly invading suburothelial tissues, whereas papillary carcinomas usually remain noninvasive for a long period, even after recurrence in the bladder following cystoscopic resection.[13]

LOH on chromosome 9 was detected in more than half of the cases and in these cases rather large regions of 9p and/or 9q were lost, consistent with other reports that loss of an entire chromosome arm is frequent (fig. 3).[11] The observed high incidence of LOH on chromosome 9 in urothelial carcinomas may indicate the existence of tumor suppressor genes important for urothelial carcinogenesis on this chromosome.[11] DNA hypomethylation on satellites 2 and 3 significantly correlated with LOH on chromosome 9 in urothelial carcinomas. After the induction of DNA hypomethylation in cultured cells by treatment with 5-azacytidine, a DNA methyltransferase inhibitor, chromosomal recombination occurred between satellite regions.[3] In patients with ICF syndrome DNA hypomethylation on satellites 2 and 3, and multiradiate chromosomes composed of chromosomes 1, 9 and 16 are characteristic.[2] During hepatocarcinogenesis DNA hypomethylation on satellite 2 significantly correlates with chromosome 1 q-arm copy gain with pericentromeric break points.[8] By analogy with these findings DNA hypomethylation on satellites 2 and 3 could be the underlying molecular background for the frequently observed LOH on chromosome 9 in urothelial carcinomas.

DNMT3b has been identified as a DNA methyltransferase specifically targeting satellites 2 and 3 during mouse development.[20] In human hepatocarcinogenesis over expression of DNMT3b4, a splice variant of DNMT3b that lacks methyltransferase activity and competes with the major variant in normal liver tissues, DNMT3b3, for targeting to pericentromeric satellite regions, results in DNA hypomethylation on these regions.[21] Although further studies are needed to understand the molecular mechanism causing DNA hypomethylation on satellites 2 and 3 during urothelial carcinogenesis, this hypomethylation may have a role in the development and progression of urothelial carcinomas by inducing chromosomal instability. These data highlight the practical significance of correction of DNA methylation status for the prevention and/or therapy of urothelial carcinomas.

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オムニ・マネジメント

OCTOBER 2004

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特集

企業経営を脅かす「ハラスメント」の最新事情

「辞めろ！」がパワハラ常套句。尊厳の自覚と毅然たる態度を
[illegible] 取締役会長 岡田康子

セクハラ対策はいまや「配慮義務」。何もしなければ「違法」に
[illegible] 金子雅臣

防止の決め手は企業レベルと管理者レベルの二段階対応
早稲田大学大学院 … 専門職大学院教授 梅津祐良

〈特別掲載〉国際モダンホスピタルショウ2004／キーノートスピーチから
患者さんと家族の診療を念頭に、良好な医師患者関係の構築を
国立がんセンター中央病院院長 野村和弘

〈特別掲載〉国際モダンホスピタルショウ2004／ITフォーラムから
守秘だけでは困難な時代、自己情報の上手なコントロール・活用を
東京大学大学院 … 助教授 山口隆

社団法人 日本経営協会
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特別掲載①

患者さん主体の診療を念頭に、良好な医師患者関係の構築を

国際モダンホスピタルショウ2004／キーノートスピーチ「セカンドオピニオンの推進」から

国立がんセンター中央病院
病院長
野村　和弘

スタートは医療費抑制策

皆さんが病院に行って、「進行がんです、今の医療ではもう手がつけられません」と言われた場合にどうされますか。誰に相談しますか。大変な事態です。「先生はそう言われますが、自分を納得させるため、他の先生の意見を聞いてみたい」ということが言えますか。

社会では、いろいろな医療不信が渦を巻いています。患者が医師から聞いたことをますます信用できなくなっています。もっと何かいい治療法があるのではないかと当然みんな考えている。しかしながら、ドクターの側でも疾患によっては、どこに行ったら一番良いのかわからない状況なのです。従って一口にセカンドオピニオンと言っても非常に難しい問題が含まれています。本日は、患者さんとの信頼関係を育むためにセカンドオピニオンはどうあるべきかについてお話します。

最初に、セカンドオピニオンがどのように形成されてきたのかその経緯を話します。

外国の例ですが、手術の適応範囲というのはドクターによって随分と違います。カナダのオンタリオ州の44郡で人口10万人に対する外科手術実施件数を見てみますと、子宮摘出術では、郡によって200例から800例と随分と差があることが分かります。この理由としては、手術で取ってしまうのが一番だというようなカナダの人たちの国民性の問題が考えられます。外科医も手術を推進する方向で進めています。こういった国民性やドクターの考えは米国も同じです。米国の人口１人あたりの手術件数は日本の３倍です。その手術料は、ドクターが自分で決められる自由料金です。

こうした状況から、米国では1970年代に医療費抑制を目的として、手術適応について他の医師の意見を求めることを保険会社が患者に要求しました。１人のドクターだけではなく、もう１人のドクターの意見を入れて、両方のドクターが同意見であれば保険が使えるという形にしたのです。このような背景があってセカンドオピニオンが急速に普及して行ったわけです。それが1980年代以降になり、患者さんの権利意識の高揚と医療の透明性の要求がだんだんと高くなり、医療費と関係なく定着するようになったということです。

セカンドオピニオンとは

では、日本におけるセカンドオピニオンはどうあるべきかいうと、『セカンドオピニオンは治療方針を決めるにあたって、主治医とは別の医師の意見を聞くしくみである。従って患者さんの自己決定のお手伝いであり、インフォームド・コンセントを支える一助であり、主体はあくまでも患者さんとその主治医の信頼関係に基づく共同作業である』ということになります。

ですから、例えば、受けている治療がおかしい、訴訟してやりたいとか、医師が信用できないからといったことでセカンドオピニオンを求めるのは、セカンドオピニオンの域を脱していると考えられます。訴訟であれば、別の手続きがありますし、医師が信用できないのであれば、医師を変えればよいわけです。

セカンドオピニオンの概念に基づく医療サイドと患者サイドの共通認識として求められ

ることは、「東京都特定機能病院や都立病院の共通認識」としても謳われています。

主治医は十分に説明した上で、セカンドオピニオンにも積極的な姿勢が必要である。主治医が病気を患者さんによく説明する。その上で、さらに詳しい意見を聞きたいというのであれば、積極的にそれを支援する。その場合、主治医は速やかに可能な限りの資料を提供する。一方、セカンドオピニオンにおいては、治療行為（検査、投薬、処置）を行なわない。基本は、主治医とセカンドオピニオン側の診療の意見の差があるかどうかといったことを主治医に知らせ、患者さんとの間のコミュニケーションをよくするということです。

治療法に関するセカンドオピニオンが多い

このような共通認識の下に、セカンドオピニオンを施行できるのかどうかを見るために、国立がんセンターの例をお話します。

がんセンターでは、15年以上前から各科初診外来でセカンドオピニオンの対応をしていましたが、多忙な外来時間内の対応は難しい面があるとして、1989年には医療相談室を設け、これをがんセンターの役割の１つと位置づけました。設立当初は、家族からの相談に限りました。告知がなされない場合に患者さんが同席すると、相談が困難になってしまうためです。患者さん本人からの相談は通常の診療で対応していました。

その相談内容はというと、大きく３つに分かれます。１つは現在の治療が最良か否かの相談です。特にがんの告知がなされていない患者さんは、さまざまな治療を受けていてもどんどん悪くなってしまう、どうしてなんだろうといった不信感を抱いて来ます。２つめが転院の希望。３つめが最後の宣告を受け納得したいというものです。いろいろなところで最後の治療法がないと言われたが、がんセンターではどうか。それによって納得した自分の人生を送りたいというようなことでした。

データでこれを見ると、治療法についての

「訴訟目的や医師不信によるセカンドオピニオンは、本来のあり方を逸脱している」

セカンドオピニオンを求めて来られた人が約73%。転院受け入れの希望が約38%です。これらの人たちも、主治医からいろいろ話を聞いているのですが、さらに何かいい治療法がないのかということで来られます。主治医への不信や不満という相談はわずか４％です。

また、セカンドオピニオンを求めて来る際に資料を持参できなかった人は20%ぐらいで、ほとんどの人は主治医から情報をもらってきているわけです。このことからも、主治医への不信感ではなくて、主治医がそういうけれども、まだ他に何かないのかということで来られるのが実情だということが分かります。

医療相談の結果としては、元の病院での治療を継続しようという人が約32%。こういうふうな形でこれからの人生を送るようにしたらいいのではないかという話を聞いて、ホスピス・緩和ケアを紹介されたのが25%。当院で受け入れたのが18%、他の病院を紹介したのが３％弱です。

がんセンターにおけるセカンドオピニオンの要点は、まず主治医の説明を補足することで納得してもらうということです。次に末期がんであれば、特殊なケアの方法を指導します。これは、主治医が終末期のケアについて情報不足で知らない場合が多いので、それを補う説明をする方向で進めています。その結果、初対面で30分程度の面談でも納得した治療の方向づけに役立っていると考えられます。

今なぜセカンドオピニオンなのか

それでは、今なぜセカンドオピニオンが要

請されるのかについて説明します。

1つは、国民の自己権利意識の高揚があります。自分の身体の治療は自分で決めていくという意識です。この意識を高揚させるものとして、インフォームド・コンセントの一般化があります。それと、治療選択肢の増加。従来は手術が先決でしたが、今は複数の治療法があります。加えて価値観の多様化。現在の自分のがんであれば、どういう治療をしたらどうなって、治療をしなかったらどうなるか。それを知った上で、自分で治療法を選択する。その後の人生設計や、それに対しての価値観は人によってさまざまだからです。

もう1つは、医療の不透明性に対する不信感です。新聞やテレビなどメディアで、どこで医療事故があった、どこに医療過誤があったというようなことがいろいろと報じられています。そういうものに対して、それを正確に判断する情報が欠如しているのが現状です。さらに最近は病院のランキング化、安全ランキングや治療成績ランキングといったものもたくさん出ています。ところがこのランキングが精度が高いのか、公的に認められるような検証をした上で出ているのかといったことがほとんどわからない。そうすると、何を信じたらいいのか、頭の中がますます混乱してしまうというわけです。

すなわち、国民への信頼できる医療に対する正確な情報が欠如しているために不信感を助長しているのです。

セカンドオピニオンの問題点

次に、セカンドオピニオンを行なうにあたっての問題点を述べます。

基本的な問題としては、不安の解消に対応できる医療制度が整っていないことです。風邪や発熱などといったときにはかかりつけ医に行っても、がんとか他の大きな病気のときは、大学病院や大きな病院に行かないといけないのではないかという風潮が社会的に蔓延しています。一方、かかりつけ医はいつも診ていて患者さんの家族関係や状況などをよく知っている反面、医療情報が不足していることが多く、そのため説明をしっかりできない。つまり、医療機関の役割分担がきちんとされていないわけです。

医療サイドにおける問題としては、1つは良好な医師・患者関係、患者さん中心という視点の欠如。2つめはコミュニケーションスキルの稚拙さ。3つめがパターナリズムです。今まではパターナリズムが基本にあってドクターも患者さんも良しとしていたところがあります。4つめが多様な価値観への無理解と相手の気持ちに対する配慮が欠如していることです。5つめが業績至上主義、画一的な評価。大学などで教授になるには研究論文の数が決め手となり、実際に患者さんと接して治療してどのくらいの成果をあげたかということが業績としてあまり評価されないということです。6つめとして医療連携に関するアピール不足があげられます。

患者サイドの問題としては、まず、医療の不確実性への理解不足があります。手術をすれば必ず危険を伴います。ところが、今までは手術をすれば絶対に治ると信じていたわけです。そこに手術をして亡くなってしまったら、これは医療過誤ではないかというようになってしまう。治療する上で、そういうことが起こってしまうことはあり得るのです。そういうことを踏まえて、物事を判断する訓練がまだされていないということです。さらに、コスト意識の不足があげられます。日本は皆保険で自己負担費用が少ないため、自分が健康管理をするのにいくら使ったのかという意識が足りないということです。また、大病院嗜好、医療機関の役割分担と医療連携への理解不足も問題点としてあげられます。

医療面接教育の導入を

このような問題をどのように解決していけばいいのか。

第1に医学教育における医療面接教育。こ

れを医療教育に絶対に取り入れなければいけないということです。すでに大学でも取り入れて積極的にやっているところがあります。しかし、そこで教育されたドクターが育って実際に診療に携わるまで、最低10年かかります。もちろん、教育されたドクターを見て周りのドクターも感化されていい方向に行くとは思います。さらに問題は、医療面接教育をするには、まずは教育者を養成しなければならないというわけです。

第2に知識の伝達からトレーニング的側面の重視。覚えろ、覚えろということではなくて、患者さんを前にして、どういうふうに患者さんの気持ちを察し、いかに患者さんの身になって診療を進めていくかを身につけるということです。第3が医療面接に関する適切な評価方法の確立です。

先ほどかかりつけ医の役割について少し触れましたが、かかりつけ医とは全人的、包括的、継続的に責任をもって国民の健康管理を担当するのであって、それが行なえるのはかかりつけ医に他ならないわけです。とはいえ、かかりつけ医のところに行っても、かかりつけ医が信頼できる説明をしてくれなかったら、これは何の意味もないのです。そうならないためには、かかりつけ医に十分な情報を提供することが必要です。そして、かかりつけ医に地域医療を自分が担っているという自覚をもって勉強してもらうことが重要です。

国民にも情報はどんどん公開すべきです。この情報は一般の人にはわからないだろうと制限する必要はないと思います。勿論、理解し易いようにすべきですが、それを見るのは個人の自由です。わからなければ、かかりつけ医に聞くなどして勉強すればいいわけです。

標準的治療法の確立が重要

正確な情報を提供するために不可欠なことは、標準的治療法の確立です。かかりつけ医も、どういう治療法が最良か、あるいは患者さんに紹介するにあたってどのドクターがいいのかわからない理由の1つは、標準的治療法が確立されていないからです。ドクターによって治療法が違うのではなく、この段階だったらこの治療が標準だということがかかりつけ医にもわかるようにすればいいわけです。患者さんもインターネットなどで調べれば、主治医が進めている治療法がこういうことで、これでやればいいんだといったことが確信できればいいわけです。そうすれば、患者さんの不信感や不安感がなくなります。そういったことが非常に大事です。

さらに標準的治療法の中では、個人プレーを廃止する。ドクターが私だったらこうしてあげると言ったり、薬を使用するにしても、EBM（科学的根拠に基づいた医療）に基づくデータではなく、例えば今まで何人かに使用したら、ほとんどの人がよくなったというだけのデータで話をする。それでは医療は成り立たないのです。また、最新の効果的医療の臨床研究と普及。これは国でも力を入れています。臨床研究事業に取り組んでおり、新しい標準的治療を確立しようと進めています。普及方法も併せて取り組んでいます。

そして、診療の質の適正評価とその公表。国の病院選択として、評価基準をしっかりと確認して、それを公表する。それによってかかりつけ医も国民も判断できるようにしなければいけないと思います。

2階建て方式の説明を

そうすると、セカンドオピニオンにおける説明内容としては、将来的には2階建て方式が考えられます。

1階部分は一般論、標準的治療になります。ここについては、患者さんがかかりつけ医と話をして納得してもらえればそれでいいわけです。他の情報があれば、患者さんが自分で見て納得するというようなこともあると思います。2階部分は個別的事項となります。これは、各専門分野の最新情報や専門知識などをもった専門病院や大学病院でないと説明で

きない場合です。そこに初めてセカンドオピニオンの重要性が出てきます。

患者さん主体の診療を念頭に

すでにお話しましたが、理想的なセカンドオピニオンとは、主治医と患者さんの間で良好な医師患者関係が築かれていること。その上で第三者の意見を聞くことにより、より強固な信頼関係が築かれ、納得して治療を受けることができることです。

それを実現するには、やはり患者さん主体の診療の実施というものを医療者側が常に頭において実施していく。そのためのセカンドオピニオンであるということを念頭に進めなければいけないわけです。

そうすることで、患者さんにとっては、病気や治療に関する理解が深まり、信頼し納得して治療を受けることができる。新たな視点、価値観、治療の目標設定を得ることができる。自分が主役であることが実感できるという効用が生まれてきます。こういった効用を生かすためには、患者さんには自分が主役であるという認識を強めていただきたい。自分が主役である、自分に権利があるということは、同時に義務も生じますから、そのことも知っていただく必要があります。

主治医サイドとしては、ポジティブに全て考えていくことが大切です。患者さんとより強固な信頼関係の構築ができますし、地域や分野を越えた専門家との交流ができるようになります。さらには見落としの予防にもつながり、自らの知識を再認識することも、各専門分野の最新情報に触れることもできます。こういう効用を考えて、患者さんにセカンドオピニオンを求めますかと自分から進んで聞いていくことも大事です。

セカンドオピニオンの落とし穴

最後にセカンドオピニオンの落とし穴について述べます。

セカンドオピニオンを求めに行っても、セカンドオピニオンをしてくれるドクターが必ずしも最高の医療者とは限らないことが、ままあります。特にいくつもの治療法がある場合、そのドクターが慣れている治療法を強調することは当然あるわけです。その中で、患者さんが何を選択したらいいか、情報を得たいがために、難しい問題が出てきます。

診療が進んだ段階では、主治医の方が患者さんの身体、心を含めて深く理解しているはずです。先にも触れた通り、セカンドオピニオンを求めに行っても、そこで即決をしたり、正しい指針を出すのは大変難しいことです。

患者さんはこうした難しさを理解してセカンドオピニオンを受けにいくことが必要です。さらに、セカンドオピニオンの普及をビジネスチャンスととらえ、患者さんの弱みにつけ込もうとする業者の存在があります。こういう問題をどうするか。やはり非営利を原則とした社会制度の整備を考えていかなければならないと思います。

それから、セカンドオピニオンを推奨する立場の人たちの多くが健常人、あるいは自ら考えて選択する「強い患者」です。ところが、それに加われない、肉体的、精神的、経済的にさまざまな不自由さを抱え、自ら選択しその結果を引き受ける能力にゆとりがなくなっている患者さんの存在もあるわけです。そういう主張ができない人たちの存在にも配慮して進めていかなければいけないのです。弱者が取り残されるようなセカンドオピニオンというのは、問題があります。

まとめ

よりよいセカンドオピニオンのために、患者サイドはかかりつけ医を利用し、医学の限界を知る。治療選択には、社会的、家庭的、人生観など自分を中心に考え、結論付けて行くこと。医療サイドには、標準的治療法の確立と周知、説明と同意のスキルを早急に身につけること。行政サイドでは、医学教育の再検討と、医療の現状についての普及と啓蒙が大切です。これらのことを強調しておきます。

キーノートスピーチ

「セカンドオピニオンの推進」
～患者さんとの信頼関係を育むために～

国立がんセンター中央病院　病院長　野 村 和 弘

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キーノートスピーチ

〈平成16年7月・東京都〉

セカンドオピニオンの推進
～患者さんとの信頼関係を育むために～

国立がんセンター中央病院 病院長　野村　和弘

皆さんおはようございます。本日はセカンドオピニオンの推進というタイトルで患者さんとの信頼関係を育むために，セカンドオピニオンが意味を持つのだという方向でお話させていただきたいと思っております。

1. はじめに

皆さんが病院に行って「進行がんです。もう今の医学では手がつきません」と言われた場合どうされるでしょう。だれに相談しますか？　かかりつけ医がいますか？　かかりつけ医の先生はちゃんと情報を持っていますか？　大変な事態です。それでも「先生はそうおっしゃるけど，まだ違う意見があるかもしれません。先生資料をいただけますか。ほかの医師の意見を聞いてみたいのです」。こう言えますか？　セカンドオピニオンにはいろいろな問題が混在しています。今，世間では，医療不信が渦巻いています。先生から聞いたことがますます信用できなくなる。みんなもっとなんか良い方法があるんじゃないかと考える。私もそうです。けれども専門のドクターでさえも，どこへ行ったらよいか分からない，正確な情報がないという時代なんですね。その辺のところをこれから詰めていかなければならないと考えています。

例えば，私は脳外科医なんですが，聴神経腫瘍という脳腫瘍があります。聴神経というのは耳で聴いた音を脳に伝える役目をする神経です。その神経にできる腫瘍です。それが例えば2㎝くらいになって見つかった。そうしてドクターからその腫瘍の手術をするという説明を受けた。「手術でほとんど良くなるでしょう。ただ聴力が落ちる可能性は五分五分です。今はガンマナイフという治療法もあります。ガンマナイフで治療をすれば手術しなくてもコントロールできそうです。しかし現状では，放射線であるガンマナイフの障害について10年あるいは20年後にどんな障害が出るかは明確ではありません。しかし手術と同じくらいの成果はあげられると思います。どちらを選びますか？」こう聞かれたときに，皆さんどうします？

ドクターが手術を得意とすれば「私なら手術が良いと思いますよ。ガンマナイフでやっても将来再発することがあるかもしれないし。これが数%の確率であるでしょうから」というふうに言うかもしれません。手術でも再発の確率はあります。だけど手術は実際に目で見て取りますから，手術後はすぐ腫瘍はなくなるわけです。しかし数%の再発の可能性は残ります。ところがガンマナイフでは腫瘍は治療直後はそのまま残るわけです。だんだんと小さくなるかもしれませんが，そのまま残った状態でしばらくいます。ひょっとしてその中に生き残った細胞があるとまた大きくなってしまう。それが数%の確率。さらにガンマナイフをやったあと，再発時の手術の困難さ。そういう意味から手術の上手な人は「手術がいいんじゃないですか？　私がやれば大丈夫です」というふうに言うかもしれない。大丈夫だといっても2，3%は障害の起こる危険はあるし，まれには命の危険もあるということです。

そこで「それではどちらにするか皆さん自分で決めてください」と言われたらどうします？　ドクターサイドにしてみると，例えば，脳神経科の

コングレス，これは脳外科医が集まって議論する学会ですが，ここで「こういう腫瘍がある患者さんが来た場合どうしますか」と聞いたんですね。みんなボタンで回答をババーンと押すわけです。そうすると80%の人が手術をする。ガンマナイフではなくて手術すると回答しました。「それではあなたが患者さんだったらどうしますか」。またバーッと押すわけですよ。そうすると60%の人が手術をすると言うんですね。なぜでしょう？

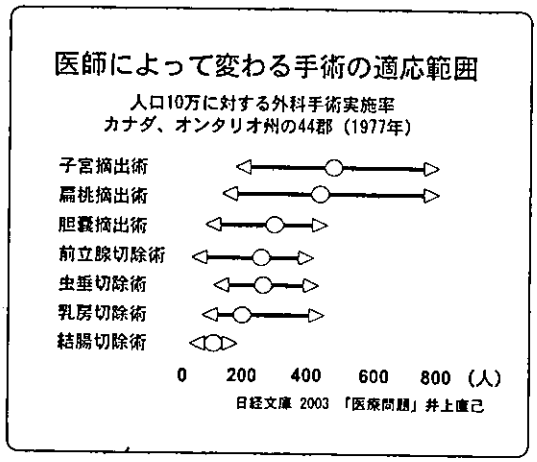
自分が自分の手術をするなら自信も持てるけど，他人にやらせたんじゃとてもじゃないけど心配だっていうのが20%くらいあるんでしょうか。つまりドクターでもそのくらい差があるのですね。どうやって責任あるセカンドオピニオンを得て治療を決めるのがいいか，非常に難しいことです。

2. セカンドオピニオンの歴史

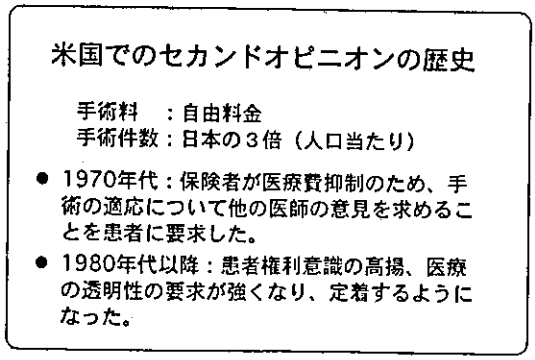
さて，そんな疑問を皆さんに投げかけましたけれど，セカンドオピニオンがどういうかたちで形成されてきたかを古い時代を見ながら，お話をさせてもらいます。

スライド1に書いてありますけれど，手術の適応は各ドクターによって随分違うのです。これはカナダのオンタリオ州，この州は44郡あるのですが，そこで人口10万人比率で外科手術，実施率を見てみたわけです。そうすると例えば子宮摘出術を見ると，あるところは10万人に対して200例。ところがほかのところでは800例。こんなことがあるんでしょうか。800例の郡では何かがんの状態が違うのでしょうか。ほかの郡の4倍も手術しなければならないような，そういう状況があるのでしょうか。そんなことは常識的には考えられないことです。こうした土壌は，1つは国民性の問題にあります。米国などの国々では，手術で切除する，これがいちばんの治療と考える国民性があります。外科医のほうも手術をして切除すればそれで治るという考えが強く，そういう方向に進んでいくわけです。

（スライド2）米国も同じような状況です。これらの国は手術料は自由料金になっていて，ドクターが自分で決められる。こんな状況があって，比率にすると手術件数は日本の3倍くらい行われています。それで1970年代，保険者が医療費抑制を意図して手術の適応について，担当医だけでなくほかの医師の意見を求めることを患者に要求したのです。保険者である保険会社のほうとしては，なるべく払いたくない。適正な医療についてのみ払う。そこで複数のドクターの意見を聞き，両者とも手術適応ありということであれば保険で払いましょうということにしました。その結果，このセカンドオピニオンという制度が否応なく進んだわけです。なぜならこれは強制ですから。ドクターもそうしなければお金が入ってこない。患者さんは保険料を払っても，そうしなければ医療費を保険から出してもらえない。そういうことで始まった制度なのです。それが時代とともに患者さん



スライド1



スライド2