VEGF. In human beings, VEGF not only can affect the expression and assembly of TJ proteins, but can also lead to the phosphorylation of TJ proteins (19, 28, 36). However, the direct effects of VEGF on the expression of CLN5 are still poorly understood. Further experiments in appropriate animal model and in vitro research will be required. Besides VEGF, it has been demonstrated that activation of VEGF receptor Type 2 and multiple redox-regulated signal transduction pathways are involved in Tat-induced alterations of CLN5 expression in the BBB (1). In this research, coexpression of VEGF, Flt-1, and PIGF were found in microvascular ECs of human HBs. These results imply not only that the overexpression of VEGF but also that the upregulated PIGF and Flt-1 may play an important role in the dysregulation of TJ proteins in microvessels of HBs. These results also support the direct relationship between VEGF expression and cyst formation in human brain tumors reported by other researchers (20, 32, 38).

In a normal brain, the astrocytic perivascular endfeet almost totally (more than 95%) embrace the abluminal surface of endothelium (34). Astrocytes play a key inductive role in the development of TJ and other specialized BBB phenotypes of brain endothelium (2, 40, 41). All components of the BBB are essential for the normal function and stability of the BBB (3). It has been found that microvessels in the brain will show loss of normal paracellular localization of CLN5 after transitory focal astrocyte loss (40). This process is correlated with focal vascular leak of dextran and fibrinogen. In HBs, there is a significant absence of astrocytic endfeet in microvessels. Without the induction of astrocytes, the microvascular ECs in HBs may lose some BBB phenotype of brain endothelium. This could also play an important role in the absence of TJs and the breakdown of the BBB in human HBs.

The cyst formation in human HBs is a very complex process that is determined by the balance between fluid extravasation and reabsorption. This process could be affected by many pathophysiological factors. Recently, we found that expression of aquaporin 1, a member of the water channel family, was significantly increased in HBs (7). This suggested that disturbed water homeostasis caused by abnormal expression of aquaporin 1 in the CNS may also play a role in cyst formation of HBs. This could partly explain why some brain tumors have a significant breakdown of the BBB, but no macroscopic cyst formation. Functional investigation and analysis of a larger number of HBs are necessary in the future to determine the exact mechanisms of cyst formation in HBs.

CONCLUSION

The continuity of TJs in the BBB is interrupted in human cerebellar HBs. Significant absence of astrocytic endfeet and TJs can be found in microvessels of HBs, which may lead to the breakdown of BBB in these tumors. These findings suggest that absence of TJs may play a role in cyst formation of HBs.

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COMMENTS

The authors investigate the role of endothelial tight junctions in the formation of cysts associated with hemangioblastomas. This is a very interesting hypothesis because the mechanism of cyst formation is unclear at the present time with this particular neoplastic lesion. This is a relevant clinical problem. At times, with incompletely resected lesions or tumors that are inoperable due to their proximity to the brainstem or optic pathways, radiation often fails to control the cyst despite controlling the solid portion of the tumor. Thus, a therapeutic modality is needed to control cysts that are recalcitrant to radiation when that is the appropriate method of treatment. The authors demonstrate conclusively that the tight junctions within the microvessels of these tumors were dysfunctional and interrupted in comparison to the control brain capillaries. There are a number of molecular constituents that comprise a functional tight junction, such as claudin, and in this study, it seemed as if claudin expression was significantly decreased in the endothelial cells of cystic hemangioblastomas. This can certainly affect the tight junctions and disrupt the blood-brain barrier, resulting in cyst formation. Other factors such as vascular endothelial growth factor (VEGF) also play a role in the functional nature of tight junction and expression of VEGF, along with Flt-1 and placenta growth factor, which were also found to be expressed in hemangioblastomas. They could contribute, along with claudin expression, to the dysfunctional nature of tight junctions in the microvessels of hemangioblastomas. However, whether this knowledge actually implies a direct correlation to the formation of cysts, and whether or not the expression profile of these molecular targets is altered after radiation-induced cyst control remains to be seen. This would certainly prove the concept proposed in this research. This is an important contribution to the literature with regard to the pathophysiology of cyst formation in hemangioblastomas, and with some of the newer small molecule inhibitors that are currently available. There may be a therapeutic correlate to the new information described in this report.

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Chen et al. report on the relationship between hemangioblastoma and the blood-brain barrier, and their study may possibily provide an understanding of the mechanism of cyst formation.

Their report opens up intriguing therapeutic possibilities to better understand pathophysiology. For example, given their findings regarding the over-expression of VEGF, there could be a role of VEGF inhibition, such as Avastin, a drug recently approved by the Food and Drug Administration for the long-term management of lesions in patients who may be inoperable or for use as a medical alternative to alleviating the mass effect of the cysts in hemangioblastomas.

The authors provided molecular insights that have potential therapeutic implications.

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Laboratory Investigation

Increased expression of aquaporin 1 in human hemangioblastomas and its correlation with cyst formation

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Key words: aquaporin 1, cyst, hemangioblastoma, water homeostasis

Summary

Aquaporins (AQPs) is a water channel family which facilitates the passage of water across cell membranes. Recently, expression of aquapporin 1 (AQP1) was found to be involved in not only water transport but also tumorigenesis. In present study, we analyzed the expression of AQP1 in 26 consecutive cases of human hemangioblastomas. Significant upregulation of AQP1 expression was found in hemangioblastomas compared with control brain ($P=0.002$). In hemangioblastomas, expression of AQP1 was predominantly localized on membranes of stromal cells. The expression level of AQP1 in cystic group of hemangioblastomas is much higher than that of solid group ($P=0.021$). Most hemangioblastomas showed a negative expression of AQP1 on endothelial cells. These results imply that increased expression of AQP1 in stromal cells may play a role in cyst formation and tumorigenesis of hemangioblastomas.

Introduction

Hemangioblastomas (HB) account for 1–2.5% of all primary CNS neoplasms and approximately 7% of primary posterior fossa tumors in adults [1,2]. In these tumors, cyst formation is a common and important clinical manifestation. Although it is well known that the majority of mass effect-producing symptoms in cystic HB patients always derive from the cyst rather than from the tumor causing the cyst [3], the molecular mechanisms for this cyst formation are still poorly understood.

Aquaporins (AQPs) is a water channel family which facilitates the passage of water across cell membranes. Up to now, 11 individual AQPs have been identified and cloned from mammals. They are ubiquitously distributed in tissues and may provide a key route for water movement in the brain [4–6]. In human brain, two AQPs have been identified: AQP1 and AQP4. AQP1 expression is confined to choroid plexus and it has been claimed to play a role in CSF secretion. AQP4 is expressed in astrocytic end-feet around microvessels and is related to brain water homeostasis and the development of brain edema [7–9]. Recently, increased AQP1 expression was found to be involved in not only brain edema in CNS but also tumorigenesis in colorectal cancer [10,11]. However, little is known about AQP1 expression in human hemangioblastomas. The purpose of this study is to analyze AQP1 expression in human hemangioblastomas and their relationship with cyst formation in hemangioblastomas.

Materials and methods

Patient population and imaging

We examined 26 consecutive cases of human hemangioblastomas (11 female and 15 male patients) treated in our institutions between 1980 and 2005. The mean age of the patients at the time of presentation to our institution was 40.9 ± 15.5 years. Of 26 patients, 8 were diagnosed von Hippel–Lindau disease (VHL) according to family histories.

All CT and MR images of the patients were evaluated separately by two authors (Y.C. and O.T.) for hemangioblastomas and associated cysts. The definitions of macroscopic patterns of hemangioblastomas utilized in previous studies have not been uniform. In this research, a tumor can be defined as cystic if all the following three criteria are followed: (1) presence of a hypodense/hypointense area on CT/MR, (2) intraoperative identification of the cystic elements, and (3) cysts of tumors making up at least one-third of the whole tumor volume [12,13]. According to this definition, hemangioblastoma patients were divided into cystic hemangioblastoma group and solid hemangioblastoma group.

Surgical tissue specimens

Twenty six paraffin-embedded hemangioblastoma specimens were obtained in patients undergoing therapeutic removal of tumors and used for immunohistochemical study. Eight fresh frozen samples collected between 1997

and 2005 were flash-frozen immediately after removal and stored at −136 °C. Histological diagnosis was confirmed through standard light microscopy evaluation of sections stained with H & E. All the tumor tissues in the present study were obtained from primary resections, and none of the patients had been subjected to radiotherapy or chemotherapy before resection. Control brain specimens were obtained from 5 epilepsy patients who were treated by temporal lobectomy.

Extraction of RNA and RT-PCR analysis

RNA was extracted using RNeasy kit (QIAGEN, Hilden, Germany) according to the supplier's protocol. RT-PCR was performed by using Ready-To-Go™ RT-PCR Beads (Amersham Biosciences Corp, Piscataway, NJ). The primer pair used to detect AQP1 mRNA was as follows: 5′-GTC TTC ATC AGC ATC GGT TC-3′ (upstream) and 5′-GTC GGC ATC CAG GTC ATA CT-3′ (downstream), with an expected PCR product of 701 bp. For the control gene encoding β-actin, the primers 5′-CTA CAA TGA GCT GCG TGT GGC-3′ (upstream) and 5′-CAG GTC CAG ACG CAG GAT GGC-3′ (downstream) were used, with an expected PCR product of 271 bp. After reverse transcription at 42 °C for 30 min, amplifications were performed for 30 cycles under the following conditions: denaturation, 94 °C for 30 s; annealing, 63 °C for 45 s; extension, 72 °C for 1 min. PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining.

Immunohistochemical analysis

All paraffin-embedded specimens had been fixed in buffered formalin/saline and processed into paraffin wax. Tissue sections (5 μm) mounted on MAS-coated slides (Matsunami Glass, Inc., Osaka, Japan) were deparafinized by treatment in xylene twice, rehydrated in a graded ethanol series and rinsed in phosphate–buffered saline (PBS), pH 7.4. Antigen retrieval was performed by boiling (750 W) in citric acid buffer (10 mM, pH 6.0) for 5 min in microwave oven. The sections were allowed to cool to room temperature in the buffer and then rinsed in phosphate buffered saline (PBS, pH 7.4). The endogenous peroxidase activity was blocked in 0.3% hydrogen peroxide in methanol for 30 min. Thereafter they were incubated with horse serum for 30 min (VECTASTAIN elite ABC KIT, Vector Laboratories, Burlingame, CA). Incubation with a polyclonal rabbit anti-AQP1 antibody (AB3065, Chemicon International, Temecula, CA) took place overnight at 4 °C followed by anti-rabbit biotinylated antibody and VECTASTAIN ABC Reagent (Vector Laboratories, Inc.). Then all slides were developed in 3,3′-diaminobenzidine tetrahydrochloride (Vector Laboratories, Inc.) for 4 min. After rinse in distilled water, sections were counterstained with hematoxylin. Finally, the sections were dehydrated, cleared in xylene and mounted in ENTELLAN (MERCK, Germany). Immunohistochemical results were judged by two histopathologists who were blinded to all clinical information on the specimens.

Immunofluorescence microscopy for AQP1 and CD34

After deparafinization, rehydration and antigen retrieval, tissue sections were incubated in donkey serum (Chemicon International, Temecula, CA) for 1 h at room temperature. Co-incubation with monoclonal mouse anti-CD34 (LS038, DakoCytomation, Denmark) and polyclonal rabbit anti-AQP1 antibody (Chemicon International) took place overnight at 4 °C. Sections were washed with PBS and incubated with Alexa Fluor donkey anti-mouse 594 and donkey anti-rabbit 488 secondary antibodies (Molecular Probes, Eugene, OR) for 1 h at 37 °C. Then all slides were covered with UltraCruz™ Mounting Medium (Santa Cruz Biotechnology, CA). Photomicrographs were obtained by AX80 microscope (Olympus Optical Co., Tokyo, Japan).

Western blots

Eight fresh frozen samples were homogenized in buffer (62.5 mM Tris–HCl, pH 7.6, 2% sodiumdodecyl sulfate, 10% glycerol, 1 mM dithiothreitol, 0.01% bromophenol blue and 2% mercaptoethanol) and centrifuged at 10,000 rpm for 5 min at 4 °C. Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA). After boiled for 2 min, 10 μg of each sample (microgram) were loaded onto 10% polyacrylamide Tris–HCl gels (BioRad) and run at 40 mA for 70 min at room temperature. Proteins were then transferred onto Hybond™-P PVDF membranes (Amersham Biosciences Corp). After incubation in blocking buffer consisting of 50 mM Tris–HCl (pH 7.4), 150 mM NaCl (TBS), 0.1% Tween 20, and 5% BSA for 1 h at room temperature, membranes were then rinsed once in TBS and treated with antibodies AQP1 overnight at 4 °C. Membranes were rinsed three times in TBS and 0.1% Tween 20 at room temperature, incubated for 1 h with alkaline phosphatase–linked anti-rabbit and anti-mouse immunoglobulin G (Sigma–Aldrich, Inc., St. Louis, MO). After rinsing in TBS-T buffer, membranes were incubated in BCIP/NBT (Sigma–Aldrich, Inc.).

Statistical analysis

Statistical difference in the differential expression of AQP1 between cystic and solid group of hemangioblastoma were evaluated by nonparametric Mann–Whitney *U*-test. All of the data were analyzed using a commercially available statistical package (SPSS, version 11.01; SPSS, Inc., Chicago, IL). A probability value less than 0.05 was taken as the level of significance.

Results

Demographic data

In this research, we analyzed 26 human hemangioblastomas. According to the preoperative CT, MR images and intraoperative findings, 17 hemangioblastomas (65.4%) showed a macroscopic cystic pattern (cystic group, Figure 1a). The macroscopic solid pattern (solid

group, Figure 1b) was found in 9 hemangioblastomas (34.6%).

In cystic hemangioblastoma group, 5 patients (29.4%) suffered from VHL disease. Three patients (33.3%) in solid group were diagnosed VHL disease. No significance was found between cyst formation of sporadic hemangioblastomas and cyst formation of VHL disease ($P > 0.05$).

RT-PCR analysis

We used RT-PCR to analyze the expression level of AQP1 mRNA in 8 cystic and solid hemangioblastomas by specific primers. Figure 2 shows a representative RT-PCR result of AQP1 expression in cystic and solid hemangioblastomas. In control brain, no expression of AQP1 was detected. In cystic hemangioblastomas, there was a dramatic upregulation of AQP1 expression and the expression level of AQP1 in cystic hemangioblastomas is higher than that of solid hemangioblastomas.

Immunohistochemical analysis of AQP1

To detect the expression and distribution of AQP1 in hemangioblastomas, immunohistochemical analysis of paraffin-embedded tissue sections was performed. In control brain tissue, antibody against AQP1 showed undetectable staining on neurons, glial cells and endothelial cells. Compared with control brain, a dramatic upregulation of AQP1 was observed in hemangioblastomas ($P = 0.002$). In cystic group, all 17 hemangioblastomas showed a strong expression on the membrane of stromal cells (Figure 3b). Compared with cystic hemangioblastomas, stromal cells showed a less expression of AQP1 in solid hemangioblastomas ($P = 0.021$). Most hemangioblastomas (76.9%) showed a negative staining on endothelial cells.

Immunofluorescence microscopy for AQP1 and CD34

In histopathology, hemangioblastomas are characterized by two main components: large vacuolated stromal cells and a rich capillary network. In order to confirm the distribution of AQP1 on stromal cells, we used double immunofluorescent staining to analyze the expression of AQP1 in 17 cystic hemangioblastomas. In control brain, only expression of CD34 was detected on endothelial cells. In hemangioblastoma, we detected a strong expression of AQP1 on membranes of stromal

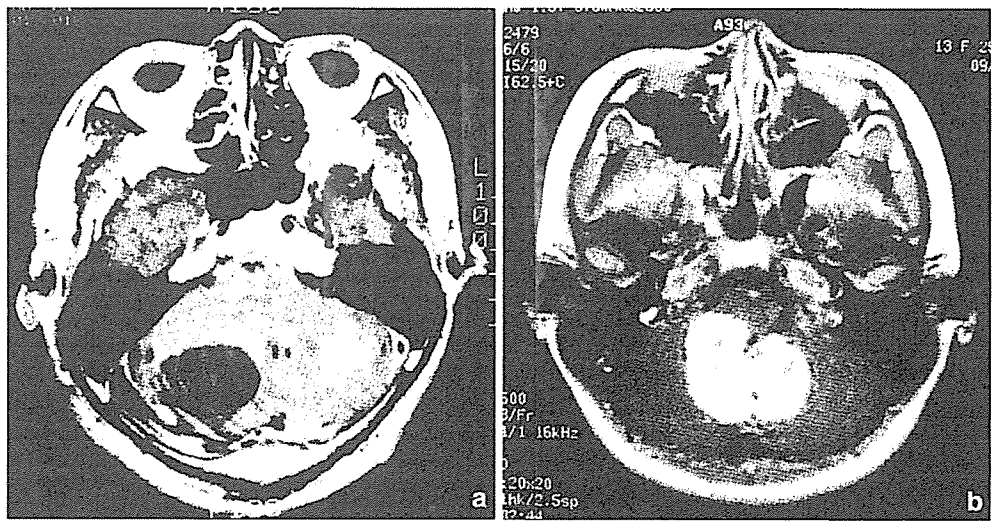

Figure 1. Classification of macroscopic patterns of hemangioblastomas. (a) Macroscopic cystic type of hemangioblastomas; (b) macroscopic solid type of hemangioblastomas.

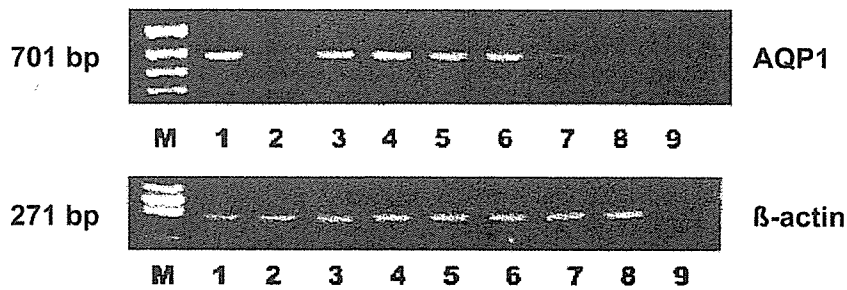

Figure 2. Representative RT-PCR results of human hemangioblastomas and control brain specimens. RT-PCR analysis revealed an upregulation of AQP1 expression (701-bp PCR product) in human hemangioblastomas, especially in cystic group of hemangioblastomas (lanes 3–6). No expression of AQP1 was detected in control brain (lane 2). Expression level of AQP1 in solid hemangioblastomas (lanes 7 and 8) is higher than control brain but much lower than cystic hemangioblastomas. Human β-actin (271-bp PCR product) was amplified as an internal control. Kidney mRNA was positive for both primer pairs (lane 1). Hemangioblastoma sample was analyzed without reverse transcriptase as a negative control (lane 9). M, marker.

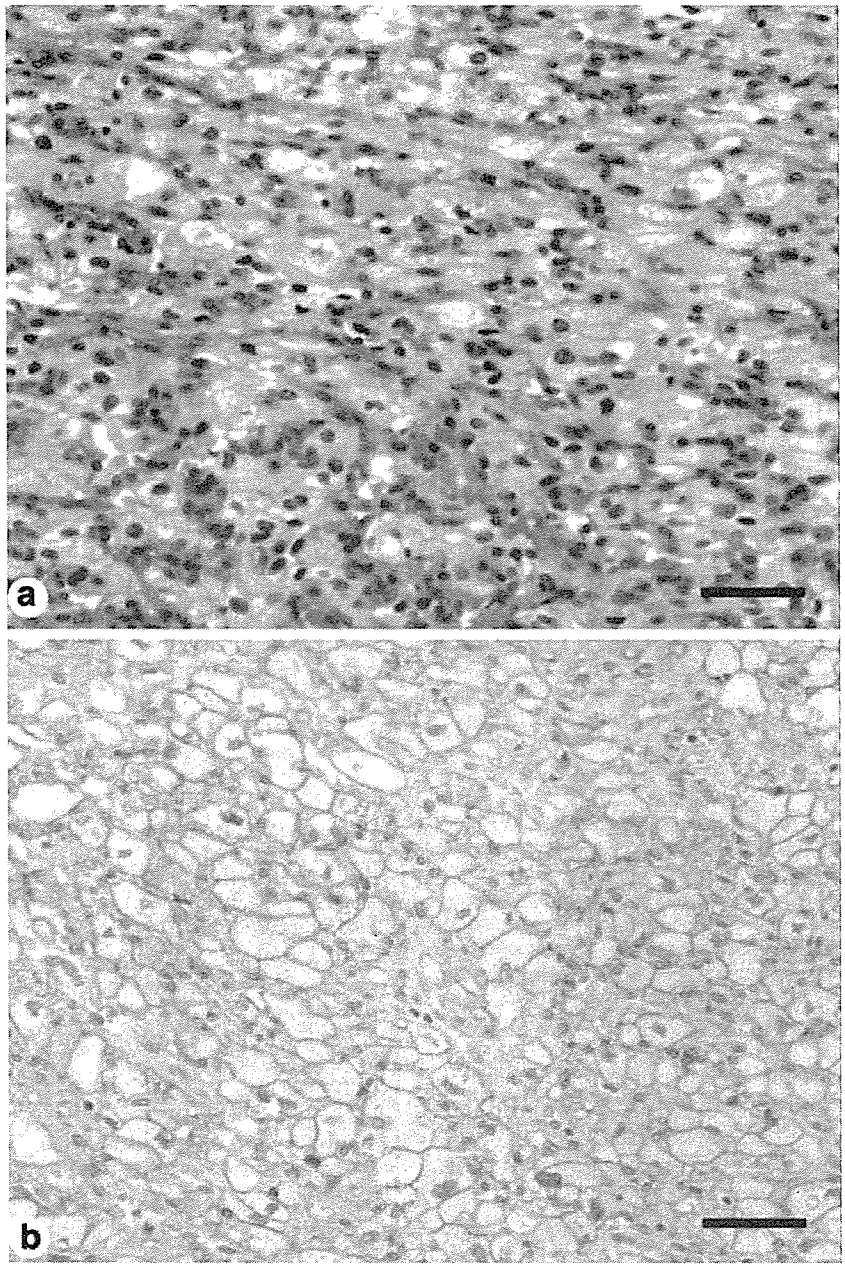

Figure 3. Photographs revealing representative immunohistochemical results of AQP1 expression in hemangioblastomas and control brain specimens. (a) Typical histological characteristics of cystic hemangioblastoma with large vacuolated stromal cells and a rich capillary network (hematoxylin and eosin). (b) Significant upregulation of AQP1 expression on membranes of stromal cells in cystic hemangioblastomas. No expression of AQP1 was found on microvascular endothelial cells. Scale bars, 25 μm.

cells around microvessels labeled by CD34 (Figure 4). All cystic hemangioblastomas showed a strong expression of AQP1 on the membrane of stromal cells.

Western blot analysis

In order to investigate the expression level of AQP1 protein in cystic and solid hemangioblastomas, we used Western blot to confirm our previous results in 8 fresh frozen hemangioblastomas. In Figure 5 no expression of AQP1 protein was detected in control brain. Compared with control brain, the expression level of AQP1 protein was increased both in cystic and solid hemangioblastomas. In solid hemangioblastomas, the expression level of AQP1 protein was much less than that of cystic hemangioblastomas. This result was in accordance with our previous experimental results.

Discussion

Aquaporin 1 (AQP1) is an integral membrane protein at the plasma membrane with six transmembrane domains that functions as a constitutive channel for water transport. The expression of AQP1 is generally limited to fluid secreting and absorbing tissues in the human body that demonstrate an enhanced permeability to water in comparison with other tissues [14]. In normal brain, expression of AQP1 was found only on epithelial cells of choroids plexus and it may play an important

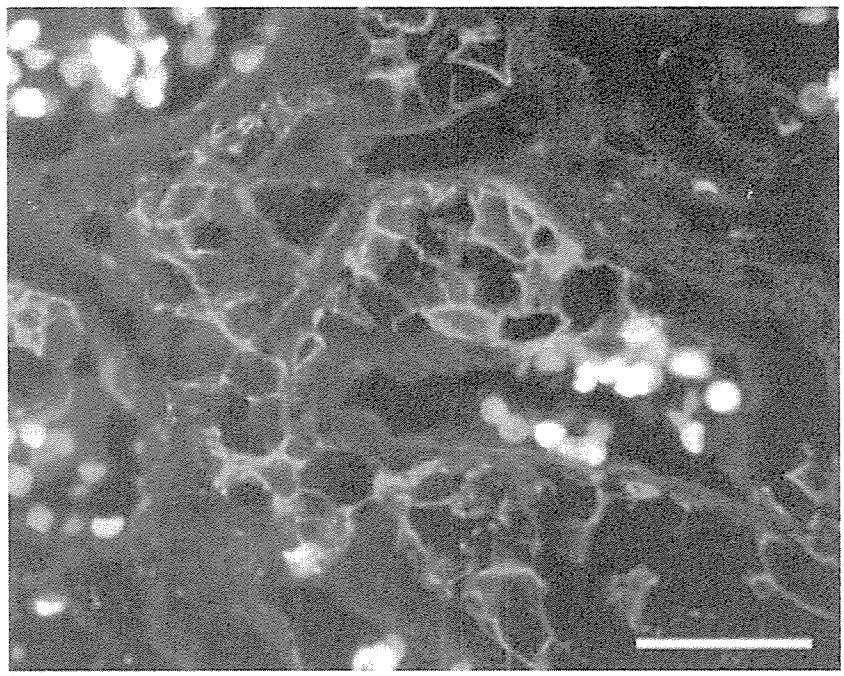

Figure 4. Photographs showing immunofluorescence in cystic hemangioblastomas stained with AQP1 (green) and CD34 (red). In hemangioblastomas, AQP1 was strongly expressed on membranes of stromal cells. Positive CD34 was detected on microvascular endothelial cells of hemangioblastomas. Scale bars, 50 μm.

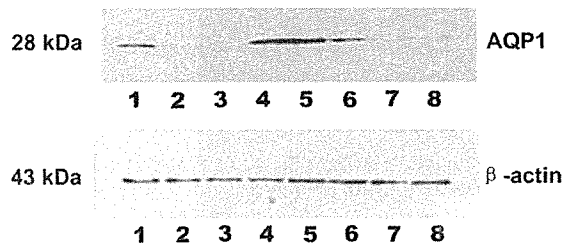

Figure 5. Western blot analysis of AQP1 protein expression in human hemangioblastomas and control brain. No expression of AQP1 was detected in control brain (lanes 2 and 3); the expression level of AQP1 in cystic hemangioblastomas (lanes 4–6) was much higher than that of solid hemangioblastomas (lanes 7 and 8); lane 1, positive control from kidney.

role in CSF secretion [7–9]. Recently, upregulation of AQP1 was reported in human gliomas and metastatic brain tumors [10,15,16]. Increased AQP1 expression in these tumors is related to the formation of brain edema. However, no information is available about AQP1 expression in hemangioblastoma at present. These prompted us to examine AQP1 expression in human hemangioblastomas.

In histopathology, hemangioblastomas are characterized by two main components: large vacuolated stromal cells and a rich capillary network. The stromal cells represent the neoplastic component of hemangioblastoma [17–19]. In hemangioblastomas, significant upregulation of AQP1 expression was detected on membranes of stromal cells. This upregulated expression of AQP1 in stromal cells suggested a very active water metabolism and water movement in these neoplastic cells of hemangioblastomas. In other tumors, AQP1 has been demonstrated to play a role in tumorigenesis and tumor growth [11,20]. Ectopic expression of full-length cDNA of AQP1 can induce many phenotypic changes characteristic of transformation, including cell proliferation enhancing activity and anchorage-independent growth [11,21]. During the cell cycle, as cell volume needs to increase rapidly by absorbing water from the outside with a minimal amount of energy, tumor cells may require expression of AQPs for high metabolic turnover or tumor-specific metabolic pathways needed for survival [11]. Therefore, increased expression of AQP1 may help tumor cells in hemangioblastomas gain advantages for replication and it may play a role in the progress of hemangioblastomas.

The exact mechanism for the upregulation of AQP1 in these stromal cells is still unknown. Recently, MAPK signaling pathways have been implicated in regulation of AQP1 expression [22,23]. The blockade of any one of ERK (extracellular signal-regulated protein kinase), p38 kinase, or JNK (Jun N-terminal kinase) signaling pathway by a specific inhibitor significantly reduced hypertonicity-induced AQP1 expression in mIMCD-3 cells [23]. It is speculated that the increased AQP1 expression may be related to dysregulation of MAPK signal-transduction pathway in these tumor cells. However, further research is necessary to determine the molecular mechanism for regulation of AQP1 in these tumors.

In hemangioblastomas, cyst formation is an important clinical manifestation. It is commonly associated with cerebellar, brain stem, and spinal hemangioblastomas. The majorities (60–70%) of all hemangioblastomas are cystic masses and about 40% are solid [24,25]. Moreover, the pace of enlargement is much faster for cysts than for hemangioblastomas themselves [3,26]. As the result, it is the cysts that mainly cause the mass effect-producing symptoms in patients of cystic type [3]. At present, the

molecular mechanism for cyst formation is still poorly understood. These years, discovery of the aquaporin family of water channel proteins has provided us new insights into the molecular mechanisms of water movement in human body. In human brain, AQPs provide a major pathway for water transport. Both the expression and distribution of AQPs are important for water homeostasis in CNS. In hemangioblastomas, increased expression of AQP1 on membranes of stromal cells suggested an active water movement between stromal cells and surrounding interstitium. This abnormal distribution of AQP1 and active water transport of tumor cells may make fluid easily collected in surrounding interstitial space.

In gliomas, increased AQP1 expression has been shown to be related to brain edema [10,15,16]. Interestingly, it has been demonstrated that the development of cysts is related to brain edema in gliomas [27]. There is histological evidence of a gradual liquefaction of the spongy edematous tissue to form the cyst fluid [27]. This increased expression of AQP1 in combination of the blood–brain barrier breakdown may play a role in cyst formation of hemangioblastomas.

Cyst formation in human hemangioblastomas is a very complex process which is determined by the balance between fluid extravasation and reabsorption. This process could be affected by many other pathophysiologic factors. In our research, microvessels in some hemangioblastomas were also found to have a significant absence of astrocytic end-feet (data not shown). In human brain, aquaporins on astrocytic end-feet have been demonstrated to be related to fluid reabsorption [28]. They are also critical for water homeostasis in CNS. Therefore, this absence of astrocytic end-feet may further promote cyst formation in hemangioblastomas. This could partly explain why some brain tumors have an increased expression of AQP1 but there is no macroscopic cyst formation. Further studies and analysis of a larger number of cases are required to determine their relationship with cyst formation of brain tumors.

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Phase II study of nimustine, carboplatin, vincristine, and interferon-β with radiotherapy for glioblastoma multiforme: experience of the Kyoto Neuro-Oncology Group

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Object. This Phase II study was performed to determine the safety, tolerability, and efficacy of combining nimustine (ACNU)–carboplatin-vincristine-Interferon-β (IFNβ) chemotherapy.

Methods. Ninety-seven patients with Karnofsky Performance Scale scores of 50 or greater were enrolled in the study. Nimustine (60 mg/m^2), carboplatin (110 mg/m^2), vincristine (0.6 mg/m^2), and IFNβ (10 μg) were administered on Day 1 concomitant with radiotherapy (63 Gy); vincristine (0.6 mg/m^2) and IFNβ (10 μg) on Days 8 and 15; and IFNβ alone (10 μg) three times per week throughout the course of radiotherapy. Fifty-six days after radiotherapy ended, the time schedule for chemotherapy was reset and ACNU, carboplatin, vincristine, and IFNβ were again administered on the new Day 1 and vincristine and IFNβ on the new Days 8 and 15. This course was repeated every 56 days. Instances of nonhematological toxicity were rare and mild. During the course of radiotherapy, the percentages of patients who experienced Grade 3 toxicity were 14% with neurocytopenia and 7% with thrombocytopenia. Seven percent of all adjuvant chemotherapy cycles following radiotherapy were associated with Grade 3 toxicity, as manifested in neurocytopenia or thrombocytopenia. No instance of Grade 4 toxicity was observed. The median duration of progression-free survival was 10 months (95% confidence interval [CI] 8–12 months) and the median duration of overall survival was 16 months (95% CI 13–20 months).

Conclusions. The combination of ACNU-carboplatin-vincristine-IFNβ chemotherapy and radiotherapy is safe and well tolerated, and may prolong survival in patients with glioblastoma multiforme.

Key Words • **glioblastoma multiforme** • **chemotherapy** • **nimustine** • **carboplatin** • **vincristine** • **interferon-β**

Glioblastoma multiforme is one of the most devastating malignant neoplasms, destroying a person's cognitive functions and personality and leading to death. Outcomes in patients with GBMs have changed little during the last three decades. The median survival time following cytoreductive surgery and radiotherapy is less than 1 year, the percentage of surviving patients at 2 years is less than 10%, and long-term survival is rare.[3] Although the effect of chemotherapy has been thought to be marginal, a metaanalysis of 12 clinical trials in which individual patients' data were examined showed a small but clear improvement in survival due to chemotherapy.[3,6] A new drug, temozolomide, was recently developed and greeted with anticipation; however, data on median survival times following its administration have not yet proved to be satisfactory.[21] We are currently exploring a novel synergetic combination of drugs and radiation therapy in the treatment of malignant gliomas.

The availability of chemotherapeutic agents differs throughout the world. In Japan temozolomide will not be available until the end of 2006 pending government approval. Both BCNU and CCNU are also not available. Nimustine, which was developed as one of the chloroethylnitrosoureas, has been widely used for Japanese patients

Abbreviations used in this paper: ACNU = nimustine; BCNU = carmustine; CCNU = lomustine; CI = confidence interval; CR = complete response; CTV-1 = initial clinical target volume; CTV-2 = boost CTV; GBM = glioblastoma multiforme; IFNβ = interferon-β; ITT = intent-to-treat; KNOG = Kyoto Neuro-Oncology Group; KPS = Karnofsky Performance Scale; MR = magnetic resonance; OS = overall survival; PFS = progression-free survival; PR = partial response; PTV-1 = initial planning target volume; PTV-2 = boost PTV; RPA = recursive partitioning analysis; RTOG = Radiation Therapy Oncology Group.

with malignant gliomas.[24] Combined treatment with ACNU plus vincristine or IFNβ has also been used in daily medical practice for the treatment of malignant gliomas.[13,28] The safety profiles of these treatments have been accepted.

In 1998, a metaanalysis of published reports on the treatment of high-grade astrocytomas found some efficacy in the use of nitrosoureas, platinums, vincristine, and IFNβ.[9] Some of the reports analyzed demonstrated promising results for combinations of nitrosoureas, platinums, and vincristine in children harboring primitive neuroectodermal tumors and those with low-grade gliomas.[16,17] Currently, GBM continues to be a devastating and uncurable disease. Nitrosoureas, platinums, vincristine, and IFNβ have shown some effect on these tumors. In this study, we selected a combination of four drugs (ACNU, carboplatin, vincristine, and IFNβ), rather than temozolomide or BCNU, based on the findings of these reports and the availability of chemotherapeutic agents in Japan. In our preclinical study, the combination of the four drugs we selected displayed some additive and synergistic effects on glioma cell lines during an in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.[25] We selected potentially optimal doses for the four drugs based on findings in other reports.[13,16,17,28] Using our selected dose settings we performed a feasibility study rather than a Phase I study to confirm the treatment's safety and efficacy. Results of our feasibility study involving 21 patients with malignant gliomas demonstrated a response rate of 45% and an 8% rate of Grade 3 or 4 toxicity.[1] We therefore performed this Phase II study for further evaluation of the treatment.

Clinical Material and Methods

This study was performed by the KNOG, which comprises healthcare workers in hospitals affiliated with Kyoto University. The study was started in August 2000 and was closed in March 2004. All data were collected and analyzed at the KNOG data management office. The protocol was reviewed and approved by the local ethics committees at each hospital.

Study Objectives

The primary end points of the study were safety and tolerability. The secondary end point was the duration of overall survival.

Patient Eligibility

Patients were enrolled in this study if they met the following criteria. 1) The patient was 18 years of age or older and harbored a histologically proven supratentorial GBM. All histological slides were reviewed by an independent central neuropathologist (Y.N.) and categorized according to the World Health Organization classification system.[10] 2) No previous antineoplastic therapy had been given for the brain tumor. 3) The KPS score had to be 50 or higher. 4) The patient's neutrophils must have been at least 1500/μl, the platelet count at least 100,000/μl, the creatinine concentration 1.7 mg/dl or lower, the transaminase and alkaline phosphatase levels no higher than 1.5 times upper normal limits, and the creatinine and bilirubin levels no higher than 1.25 times upper normal limits. 5) The patient could not be pregnant or have a history of a malignancy or uncontrolled infection. 6) Written informed consent had to be obtained from the patient in accordance with the principles of the Declaration of Helsinki and the rules of good clinical practice.

Radiotherapy Regimen

Radiotherapy was started between 7 and 21 days after surgery. A megavoltage x-ray generated by a linear accelerator was focused on the CTV-1, which was defined as the enhanced tumor volume shown on T_1-weighted MR images together with a 2.5-cm margin and edema surrounding the tumor, which was shown on the preoperative T_2-weighted MR image. The CTV-2 was defined as the enhanced tumor volume found on preoperative T_1-weighted MR images along with a 0.5-cm margin. The PTV-1 and the PTV-2 were determined by adding a 0.5-cm margin to both the CTV-1 and CTV-2. A total dose of 63 Gy was delivered in 35 daily fractions of 1.8 Gy over a 7-week period. The PTV-1 received 50.4 Gy in 28 fractions and the PTV-2 received 12.6 Gy in seven fractions (Fig. 1).

Chemotherapy Regimen

Chemotherapy was begun the day before the start of radiotherapy (Day 1). The ACNU (60 mg/m^2), carboplatin (110 mg/m^2), vincristine (0.6 mg/m^2), and IFNβ (10 μg) were administered on Day 1. Vincristine (0.6 mg/m^2) and IFNβ (10 μg) were given on Days 8 and 15, and IFNβ (10 μg) alone was given three times per week, on alternate days, throughout the course of the radiotherapy. Fifty-six days after the completion of radiotherapy, the chemotherapy regimen was reset and ACNU (60 mg/m^2), carboplatin (110 mg/m^2), vincristine (0.6 mg/m^2), and IFNβ (10 μg) were administered on the newly reset Day 1 and vincristine (0.6 mg/m^2) and IFNβ (10 μg) were given on the new Days 8 and 15. This treatment course was repeated every 56 days from the reset Day 1, provided that all the hematological toxicity from the previous course of chemotherapy had resolved to Grade 2 or less, and all nonhematological toxicity had recovered to either Grade 0 or 1. If sufficient recovery had not occurred, the subsequent course was delayed until these criteria were met.

No dose escalation was allowed. A dose reduction of 30% for toxicity was permitted. Only two dose reductions were allowed, and patients experiencing Grade 3 toxicity of any type after two dose reductions were removed from the study.

Response Evaluation

The response to treatment was assessed using a modification of the Macdonald criteria.[12] We compared baseline contrast-enhanced MR images, which were obtained 1 week before every course of chemotherapy, while considering changes in physical findings on the neurological examination and changes in the dose of the steroid. In brief, a CR was defined as the disappearance of all enhanced tumors at least 1 month apart, with no corticosteroid therapy and no neurological deterioration. A PR was defined as a greater than 50% reduction in the size of the lesion (product of the two largest perpendicular diameters), which was maintained for at least 1 month without neurological deterioration or an increased dose of corticosteroids. No response

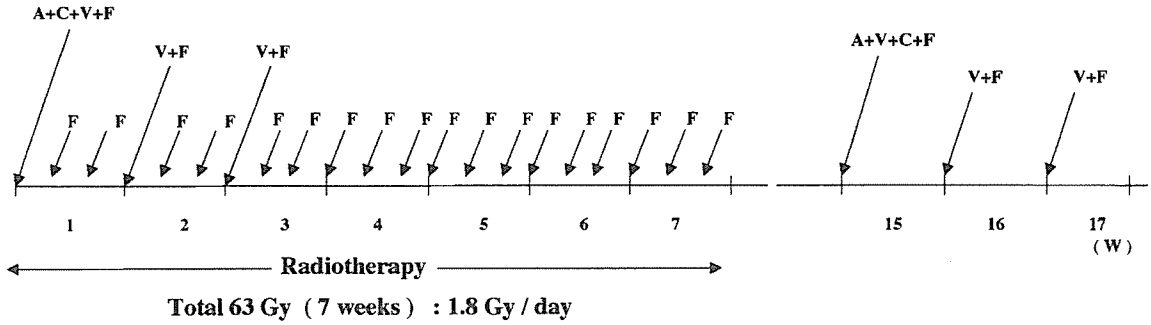

FIG. 1. Time line showing the combined treatment of ACNU-carboplatin-vincristine-IFNβ chemotherapy and radiotherapy. A = ACNU; C = carboplatin; F = IFNβ; V = vincristine; W = week. Doses of each drug are given in *Clinical Material and Methods*.

was defined as either no change in tumor size after 1 month or a change in tumor size after 1 month that did not qualify as a CR, PR, or progressive disease. Progressive disease was defined by the following: a greater than 25% increase in the size of the lesion or new tumor on MR images, a deterioration in the patient's neurological status, or a stable neurological status in response to an increased dose of steroids.

Toxicity of Treatment

Toxicity monitoring was performed in patients throughout all cycles, according to the National Cancer Institute Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/reporting/ctc_archive.html). Safety parameters, including all laboratory and hematological abnormalities, findings of the neurological examination, and adverse events, were reported by local investigators. Weekly hematological and serological testing was required.

Statistical Analysis

Overall survival and PFS were calculated from the time of surgery until death, disease progression, or the last follow-up examination according to the Kaplan–Meier method. The 95% CIs were calculated in the following manner: mean survival time $\pm$ 1.96 $\times$ the standard error of the mean. The results of the survival analysis were reported for all patients enrolled in the study. Some prognostic factors were explored using a multivariate Cox proportional hazards model and the log-rank test. These statistical analyses were performed with the aid of JMP software (version 3.1; SAS Institute, Inc., Cary, NC).

Results

Patient Characteristics

Between July 2000 and March 2004, 97 patients with newly diagnosed supratentorial GBMs were enrolled at 16 hospitals participating in the KNOG. Seven patients were ineligible, not treated, or incorrectly treated; the reasons for this included treatment refusal (three patients), chronic hepatitis (one patient), and ineligible histological diagnosis (three patients: two with anaplastic oligodendrogliomas and one with an anaplastic astrocytoma). All but two patients received radiotherapy. All follow-up data were collected by the main KNOG. The demographic and baseline clinical characteristics in the patients treated using this protocol are listed in Table 1.

At enrollment, the median age of the original 97 patients was 55 years (range 19–70 years). Fifty-eight patients (60%) were men and 74 (76%) had a KPS score of 70 or better. Complete resection was achieved in 26 patients (27%), incomplete resection in 59 (61%), and biopsy sampling in 12 (12%).

Response to Treatment

Sixty-two (66%) of 94 patients with histologically confirmed GBMs were found to harbor measurable residual tumor masses after the first surgery (Table 2). The results of surgery were CR in four cases (6%) and PR in 17 (27%). The overall response rate (CR and PR) was 34% (21 of 62 patients). Outcomes of surgery demonstrated on MR images were confirmed by an independent central review of all patient data. On review, all responding patients were on a

TABLE 1

Characteristics in 97 patients in the ITT population

Parameter	No. of Patients (%)*
age (yrs)	
median	55
range	19–70
<50	23 (24)
≥50	74 (76)
sex	
male	58 (60)
female	39 (40)
KPS score	
90–100	37 (38)
70–80	37 (38)
50–60	23 (24)
antiepileptic prophylaxis w/ enzyme-induced drugs	97 (100)
extent of surgery	
complete resection	26 (27)
incomplete resection	59 (61)
biopsy	12 (12)
histological diagnosis	
GBM	94 (97)
anaplastic oligodendroglioma	2 (2)
anaplastic astrocytoma	1 (1)

* Unless otherwise specified.

TABLE 2

Responses to treatment in 62 patients with GBMs who had measurable enhanced masses on MR imaging after surgery

Parameter	No. of Patients (%)
CR	4 (6)
PR	17 (27)
stable disease	35 (56)
progressive disease	6 (10)
objective response (CR & PR)	21 (34)

stable regimen of corticosteroids or were taking no corticosteroids at the time of the best response.

Duration of Survival

According to our ITT plan, 97 patients were enrolled in the study. At the time of this analysis, 59 patients had died. The median follow-up duration of was 24 months, with a minimum follow-up period of 6 months for surviving patients. On the basis of Kaplan–Meier estimates, the median survival for the ITT population was 16 months (95% CI 13–20 months; Fig. 2). At 1 year 68% of the patients were alive and at 2 years 24% were alive. The median duration of PFS was 10 months (95% CI 8–12 months). The duration of PFS at 6 months was 69% (95% CI 50–85%) and that at 12 months was 33% (95% CI 15–40%). The median duration of survival in the eligible patient population was also 16 months. There was no difference in 1- and 2-year survival times between the ITT and eligible patient populations.

Treatment Delivery

The median time from surgery to the start of radiotherapy plus chemotherapy was 15 days (range 9–21 days). All but two patients received the planned 63 Gy of radiotherapy; the other two patients underwent salvage surgery due to tumor progression before radiotherapy could be completed. The majority of patients completed their radiotherapy within 7 weeks. All additional delays in the course of radiotherapy were due to holidays. One patient was ineligible due to a hepatic dysfunction. In the late stage (5–6 weeks) of radiotherapy IFNβ was discontinued in five patients because of Grade 3 thrombocytopenia.

Chemotherapy following radiotherapy was administered in 89 patients for a total of 257 cycles. Dose reductions occurred in 21 (8.2%) of these cycles. The primary reason for dose reductions was hematological toxicity (21 [100%] of 21 cases). Sixty-two (24%) of the 257 cycles were delayed; 82% of these delayed cycles were due to hematological toxicity. Almost all others were delayed because of scheduling conflicts.

Safety and Tolerability

Toxicity levels were recorded for all enrolled patients by using the National Cancer Institute Common Toxicity Criteria (version 2.0). Our chemotherapy regimen was generally well tolerated. Grade 4 hematological toxicity was not observed in any patient.

During the phase of combined radiotherapy and chemotherapy, Grade 3 neutropenia occurred in 14 patients (14%)

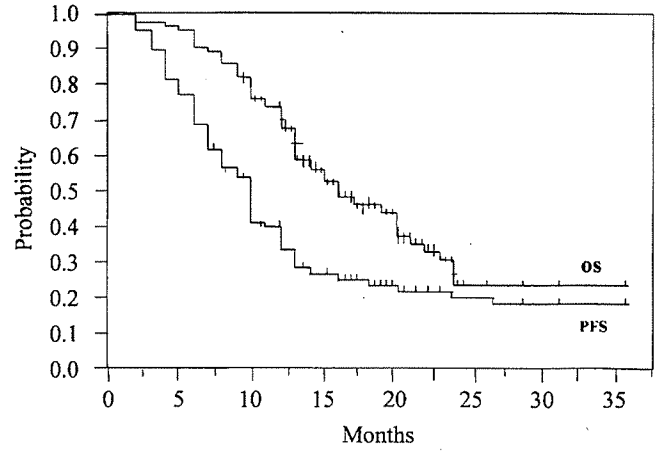

FIG. 2. Kaplan–Meier estimates of PFS and OS in patients in the ITT population who harbored GBMs.

(Table 3) and Grade 3 thrombocytopenia in seven patients (7%).

During the phase in which chemotherapy was administered after radiotherapy, Grade 3 neutropenia occurred in four patients (4%) and Grade 3 thrombocytopenia in three patients (3%; Table 3). No Grade 4 toxicity was observed in any patient. Lack of appetite and nausea sometimes persisting for 1 or 2 days were identified, although the best antiemetic agents were made available to patients in this study. Grade 3/4 pulmonary fibrosis, which is often observed in chemotherapy involving BCNU, was not observed. Fever, which is due to IFNβ, was well controlled by prophylactic antifebrile medication. Periphery sensory neuropathy (Grade 3/4), which is often caused by vincristine, was not observed. Twenty-seven patients were alive with follow-up times longer than 18 months. Five patients experienced neurological deterioration with a recent disturbance in memory without any apparent sign of leukoencephalopathy due to late radiation toxicity.

Prognostic Factors

We separately analyzed the PFS and OS of both ITT and eligible patient populations as they related to prognostic factors. Because there was no significant difference in outcomes, we only report the results for the ITT population (Table 4). A multivariate Cox proportional hazard model was used to test the effect of patient age, KPS score, and the extent of surgery on PFS and OS (Table 4). With respect to PFS, the extent of surgery and the KPS score were close to a level of significance, although patient age was not as significant. With respect to OS, the extent of surgery, the KPS score, and patient age were close to a level of significance.

Comparison of Survival According to RPA Criteria

We analyzed our data according to the prognostic classes of the RPA, which are based on the RTOG database of patients with malignant gliomas[19] (Table 5). The median duration of survival in this study may suggest a favorable prognosis in all RPA classes in the RTOG database. For our patients in RPA Classes III and IV, outcomes seem better but not significantly better, because the patient population in

TABLE 3

*Rates of toxicity in the ITT patient population and throughout all chemotherapy cycles**

	RT w/ Chemotherapy (97 patients)					Adjuvant Chemotherapy (257 cycles)				
	Toxicity Grade					Toxicity Grade				
Toxicity Parameter	0	1	2	3	4	0	1	2	3	4
anemia (%)	39	46	13	2	0	72	18	9	1	0
neutropenia (%)	12	52	22	14	0	12	59	25	4	0
thrombocytopenia (%)	21	40	32	7	0	41	33	23	3	0
nausea (%)	25	59	26	0	0	31	54	15	0	0
vomiting (%)	95	5	0	0	0	97	3	0	0	0
pulmonary fibrosis (%)	97	3	0	0	0	99	1	0	0	0
transaminase (%)	86	11	2	1	0	89	7	2	2	0
creatinine (%)	92	6	2	0	0	94	4	2	0	0
sensory neuropathy (%)	95	3	2	0	0	90	8	2	0	0
fever (%)	92	7	1	0	0	93	6	1	0	0

* RT = radiotherapy.

our study was small and the 95% CIs were wide in range and indefinite in part. With respect to patients in RPA Classes V and VI, our patients' disease may have had a better prognosis, considering that the value ranges of 95% CIs in this study were better than those in the RTOG database.

Discussion

During the last two decades, chemotherapy has had little effect on outcomes in patients with GBMs. The value of radiotherapy was confirmed in randomized trials in the late 1970s and has been considered the standard treatment.[26,27]

Trials of radiotherapy combined with chemotherapy in newly diagnosed cases of GBM have been reported. Outcomes in patients with GBMs have been reported following radiation treatment combined with topotecan;[5] with tirapazamine, a hypoxia-selective cytotoxin;[4] with RSR13, a synthetic allosteric modifier of hemoglobin;[11] with combined procarbazine-CCNU-vincristine treatment;[14] and with BCNU and cisplatin.[7] Nevertheless, the median survival times in patients in all these studies were less than 1 year and unsatisfactory. Recently, temozolomide was developed and its effects were anticipated, but thus far the median duration of survival following its administration is not satisfactory.[21]

Temozolomide, BCNU, and CCNU are presently unavailable in Japan. In our country ACNU has been used in patients with malignant gliomas for approximately two decades,[24] and ACNU plus vincristine or IFNβ is widely used in daily medical practice.[13,28] On the basis of previous reports and drug regulation in Japan, we selected a regimen in which four drugs—ACNU, carboplatin, vincristine, and IFNβ—were combined rather than a regimen including temozolomide or BCNU. We therefore initiated this Phase II study to obtain further evaluation of this therapy after we had performed a preclinical study[25] and a feasibility study.[1]

The incidences of Grade 3 or 4 toxicity in our study were few and rare. Although myelosuppression was a dose-limiting side effect in this regimen, its toxicity was not as cumulative and reversible and was usually resolved by a one-dose-level reduction or a treatment delay up to 1 week. Although pulmonary fibrosis is a possible severe toxicity in chemotherapy regimens containing BCNU, the incidences of Grade 3 or 4 pulmonary toxicity were rare in this ACNU–containing regimen. Periphery sensory neuropathy (Grade 3/4), which is often caused by vincristine, was not observed. Setting a low dose for the drugs in this study may be a major reason for these safety profiles.

Results of our study showed that the objective response rate was 34%, with 57% disease stabilization. In another

TABLE 4

*Prognostic factors for PFS and OS**

	PFS			OS		
Variable	HR	95% CI	p Value†	HR	95% CI	p Value†
patient age (yrs)						
70–50 vs 49–18	1.05	0.61–1.82	0.8033	1.40	0.72–2.93	0.32
70–60 vs 59–18	1.16	0.67–1.98	0.56	1.79	0.95–3.34	0.058
KPS score						
100–90 vs 80–50	0.68	0.39–1.16	0.14	0.72	0.40–1.41	0.4
100–70 vs 60–50	0.69	0.32–1.31	0.26	0.58	0.76–3.13	0.26
extent of surgery						
complete vs biopsy	0.72	0.47–1.04	0.1013	0.53	0.29–0.93	0.0187
complete vs incomplete	0.72	0.50–0.99	0.04	0.47	0.27–0.73	0.0008

* HR = hazard ratio.
† According to the log-rank test.

TABLE 5

*Median duration of survival in patients in the present study and in the RTOG malignant glioma database**

RPA Class	No. of Patients	Median Survival Time (mos)	
		Estimate	95% CI
III			
RTOG 90-06	105	17.5	15.6–20.2
present study	12	24	8–NA
IV			
RTOG 90-06	240	11.5	10.8–12.7
present study	29	20	11–24
V			
RTOG 90-06	150	7.4	6.2–9.1
present study	33	17	13–24
VI			
RTOG 90-06	23	2.7	1.7–4.5
present study	18	13	8–14

* Data from the RTOG 90-06 study are found in the paper by Scott, et al.
Abbreviation: NA = not accessible.

study in which BCNU was used with cisplatin, the response rate was 42% with 53% disease stabilization.[8] The response rate in our patients and the percentage of disease stabilization were almost equal to data obtained in the study of BCNU plus cisplatin; however, the median duration of survival in our patients was better. When safety profiles and disease stabilization can be assessed over a prolonged period, the findings will support our present findings on survival. Although the response rate is an important factor, a static phase over a long duration may be more important than tumor reduction.[23] Because other reported chemotherapy regimens have been almost aggressive (that is, they seem to have toxic side effects), they may be harmful to patients by suppressing immune system functions and including toxic side effects and cachexia. This viewpoint is supported by the observation that immunotherapy often prolongs survival times despite no tumor reduction.[22]

We performed a feasibility study instead of a Phase I study. The doses for the drugs used in this regimen are acceptable optimal doses, judging from the response rates and the incidence and grades of toxicity in this study.

In general, patient age and performance status and the extent of surgery are thought to be important prognostic factors for GBMs. The extent of surgery was close to significant in this study (Table 4). In a recent study investigators reported that the type of surgery was not prognostic; in another report investigators found that it was somewhat prognostic, although not statistically significant.[2,20] The extent of resection is still a controversial issue.[18]

Although comparisons among studies have to be approached with caution, the median duration of survival in patients in our study may be promising and better than those associated with other reported regimens. We compared our results with data on RPA prognostic classes in RTOG trials of malignant gliomas,[19] which are viewed as a popular and reliable historical control. The median duration of survival in our study suggests a favorable prognosis in all RPA classes of the RTOG database (Table 5). In a comparison with recent promising reports, the 16-month median survival in this study compares favorably with the 15.7-month time found in the Phase II study of temozolomide with radiotherapy[2] and the 17.3-month duration in the Neuro-Oncology Working Group 01 trial of ACNU plus teniposide with radiotherapy[15] in patients with GBMs. With regard to patient comfort, temozolomide may be better because it is administered orally whereas our regimen is administered by a venous route. Nevertheless, both the efficacy and toxicity of the treatment given in this study compare favorably with those in studies of radiotherapy with temozolomide. The results of this study convey a positive meaning by showing that a regimen not containing temozolomide or BCNU can provide promising results in the treatment of gliomas.

Conclusions

This Phase II study was performed to determine the safety, tolerability, and efficacy of ACNU-carboplatin-vincristine-IFNβ chemotherapy combined with radiotherapy for patients with GBMs. Ninety-seven patients with GBMs and a KPS score equal to or higher than 50 were enrolled in the study. No Grade 4 toxicity was observed. The median duration of PFS was 10 months (95% CI 8–12 months), and the median duration of OS was 16 months (95% CI 13–20 months). This treatment is safe and well tolerated, and may prolong survival in patients with GBMs.

Disclaimer

The authors have no financial interest in any drug or procedure described in this article.

Appendix

The following hospitals participate in the KNOG study: Kyoto University, Kitano Hospital, Shimizu Hospital, Kyoto National Hospital, Himeji National Hospital, Tenri Yorozu Hospital, Takeda Hospital, Osaka Red Cross Hospital, Kishiwada City Hospital, Fukui Red Cross Hospital, Kurasiki Central Hospital, National Cardio-Vascular Center, Saiseikai Noe Hospital, Kizugawa Hospital, Hikone City Hospital, Shizuoka Preferectual Hospital, Saisekai Nakatsu Hospital, Hamamatsu Rousai Hospital, Tsukaguchi Preferectual Hospital, Kobe Metal Hospital, Nagahama City Hospital, Takatsuki Red Cross Hospital, and Kobe Central City Hospital.

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Image of the Month

Cases with Carcinomatous Meningitis and Cerebral Infarction

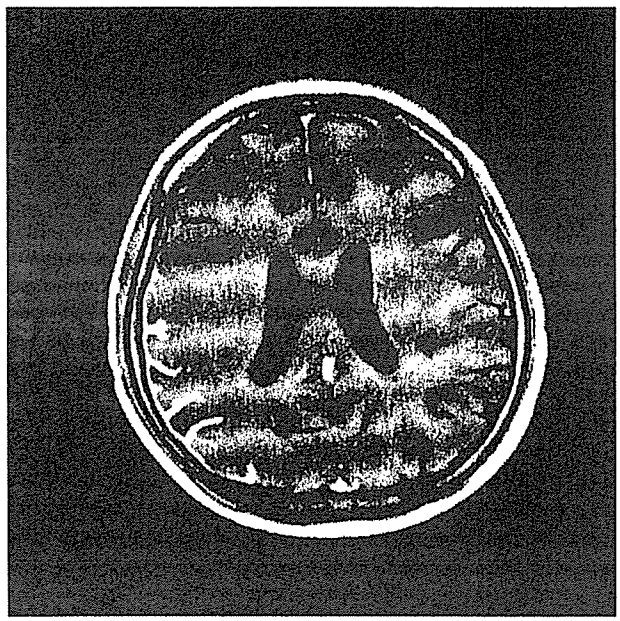

Figure 1.

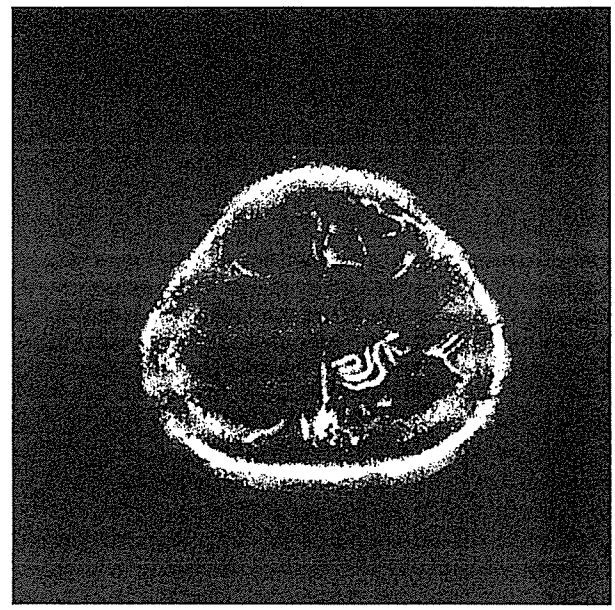

Figure 2.

A 34-year-old female with a history of breast cancer complained of headache, nausea and left hemiparesis. Magnetic resonance imaging (MRI) with Gd-DTPA showed an enhancement of the cerebral sulci (Fig. 1). Lumbar-puncture examination revealed that the number of atypical cells (779/3) and the protein concentration (776 mg/dl) increased in the cerebral spinal fluid (CSF). She was diagnosed as carcinomatous meningitis and the treatment with intrathecal application of methotrexate was performed.

A 49-year-old female with a history of ovarian cancer complained of sudden onset of weakness of the right lower limb. MRI with Gd-DTPA a few days after attack showed the enhancement of the limited area in the cerebral gyrus (Fig. 2). The results of CSF examination were normal (cells < 3/3, protein < 30 mg/dl) even with three times of the examinations. She was diagnosed as cerebral infarction and the right hemiparesis was improved without any specific treatment about two weeks after the onset of the symptom.

The differential diagnoses among cerebral infarction, carcinomatous meningitis and brain tumor are sometimes difficult in cancer patients. The cerebellar corteces and sulci are more enhanced on MRI with Gd-DTPA compared to the cerebral sulci in patients with carcinomatous meningitis. The infarct area is also enhanced with Gd-DTPA on MRI ~24 h after the onset and it is sometimes misdiagnosed as a brain tumor. The history of neurological symptoms and the results of CSF are very important for the differential diagnosis of these diseases.

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総説

悪性グリオーマに対する化学療法
—大規模臨床試験とテーラーメイド治療—

渋　井　壮一郎

Chemotherapy for Malignant Gliomas: Randomized Controlled Study and Taylor-made Therapy

by

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In order to establish the standard therapy for malignant gliomas, the JCOG-Brain Tumor Study Group was organized in 2002 and started a phase II/III study. It is investigating the efficacy of procarbazine+ACNU+radiation compared to ACNU+radiation which is considered to be the standard therapy for malignant gliomas. Patients with astrocytoma grade 3 or 4 are randomized into two groups postoperatively. Patients in Arm A receive intravenous injection of ACNU on day 1 and day 36 of radiotherapy. Those in Arm B receive oral procarbazine for 10 days before injection of ACNU. Procarbazine is reported to reduce O^6-methylguanine-DNA methyltransferase (MGMT) activity and enhance the anti-cancer effect of nitrosoureas.

Recently many studies have been started to overcome chemo-resistance. A trial of the individualization of the treatment, the so-called taylor-made therapy, is one of the challenges for treatment. Loss of chromosome 1p and 19q is considered to be closely related to chemo-sensitivity in anaplastic oligodendrogliomas. Procarbazine+CCNU+vincristine (PCV) therapy is very effective in tumors with 1p and 19q losses compared to those without these losses. This is one of the epoch making findings in the field of chemotherapy for malignant brain tumors. MGMT is a DNA repair enzyme which reduces the anti-cancer effect of nitrosourea. In order to overcome this chemo-resistance nitrosourea or drugs which reduce the MGMT activity such as procarbazine or O^6-benzylguanine are used for those tumors expressing MGMT. However, even in Taylor-made therapy prospective randomized studies under good quality control and quality assurance are essential to establish an evidence-based treatment.

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Key words: malignant glioma, chemotherapy, randomized controlled study, tailor-made therapy

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はじめに

悪性グリオーマはきわめて治療の困難な疾患であり，他臓器の悪性腫瘍の治療成績が向上している中で，30年来生存率の改善がみられていないというのが現状である．浸潤性発育を示すため，その境界が不明瞭であり，手術的に全摘することが困難であることや，放射線治療および化学療法に抵抗性を示すことなどがその原因の一つになっている．放射線治療は線量を増やすことでその効果は高まるが，同時に放射線による脳障害や二次発癌の危険性が増加する．化学療法においては，血液脳関門の存在による薬剤到達性の障害，化学療法剤に対する各

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Table 1 Levels of evidences and grade of recommendation[9)]

Level	Type of evidence
I	Evidence obtained from meta-analysis of multiple, well-designed, controlled studies; or from randomized trials with low false-positive and low false-negative errors (high power).
II	Evidence is obtained from at least one well-designed experimental study. Randomized trials have high false-positive and/or false-negative errors (low power).
III	Evidence is obtained from well-designed, quasi-experimental studies such as nonrandomized, controlled, single-group, pre-post, cohort, time, or matched case-control series.
IV	Evidence is obtained from well-designed, nonexperimental studies, such as comparative and correlational descriptive and case studies.
V	Evidence is obtained from case reports and clinical examples.
Grade	**Grade for recommendation**
A	There is evidence of type I or consistent findings from multiple studies of types II, III, or IV.
B	There is evidence of types II, III, or IV, and the findings are generally consistent.
C	There is evidence of types II, III, or IV, but the findings are inconsistent.
D	There is little or no systematic empirical evidence.

種耐性遺伝子や O^6-methylguanine-DNA methyl transferase (MGMT) 酵素などの耐性機構の存在などが問題となっている.

本稿では, このように多くの問題点を持つ悪性グリオーマに対する治療法を開発していくための臨床試験のあり方と近年注目されているテーラーメイド治療について概説する.

悪性グリオーマの標準治療とは

悪性グリオーマの治療を考えるうえで, まず必要なことは, 現時点での標準治療は何かということである. 標準治療とは,「科学的証拠 (エビデンス) によって裏付けされたデータに基づく現時点で最も良好な治療成績を期待できる治療法」といえる. つまり, 生存期間が最も長いというだけでなく, 治療に伴う有害事象が少なく, 良好な quality of life (QOL) を保てなくてはならない.

このような標準治療が確立されていくためには, 最もエビデンスレベルの高い臨床試験である第III相試験を経て, その有効性・安全性が証明されていかなければならない. 臨床試験は, 通常, 薬剤の忍容性・薬物動態・薬力学などを調べる第I相試験, 比較的少数例を対象とした初期臨床試験である第II相試験, 多数の症例を対象とした無作為 (ランダム) 化比較試験の第III相試験に分けられる. 第III相臨床試験は, American Society of Clinical Oncology (ASCO) によるエビデンスレベルの評価によれば, 最も推奨度の高いレベル1に属し, 第III相試験により, 統計学的に生存期間の長い, あるいはより有害事象の少ない治療法が標準治療として確立されていく[9)] (Table 1).

悪性グリオーマ治療におけるエビデンス

現在までに行われた臨床試験で悪性グリオーマ治療上エビデンスとなりうるものは何か. これについては本誌において篠田ら[15)]が詳細に報告しており, 代表的なもののみを紹介する.

開頭手術においてランダム化による前向き (prospective) 試験は不可能である. 巨大な悪性グリオーマによる頭蓋内圧亢進症状を呈する患者に対し, 生検にとどめるか全摘を目指すかという informed consent (IC) をとることは事実上できないからである. また, 全摘群に入っても実際の手術現場では不測の事態により部分摘出に終わることも少なくない. そのため, 開頭手術については後ろ向き (retrospective) 試験にならざるをえない. Winger ら[21)]は, 悪性グリオーマ患者の予後改善因子として, 年齢・治療開始までの期間・術前 performance status (PS)・組織診断・放射線治療・先行する分化型グリオーマの他に手術摘出度を挙げている. さらに, 全摘手術は亜全摘や部分摘出よりも予後がよく, いかなる程度の摘出でも生検に比べ予後がよいと述べている. 他の多くの報告も開頭手術の優位性を指摘しており, 脳腫瘍全国統計においても同様な結果が得られており, 最も悪性度の高い glioblastoma においても手術的摘出度に応じて生存率の向上がみられている[5)] (**Fig. 1, Table 2**). しかしながら, 手術に対して否定的な報告もあり, 推奨グレードとしては C に属するものといえる.

放射線治療について, Anderson[1)]は 108 例の glioblastoma に対する比較試験を行い, 手術単独では1年生存割合が0%であったものが, 45 Gy の照射を加えることにより 28% に上昇したと報告している. さらに Walker

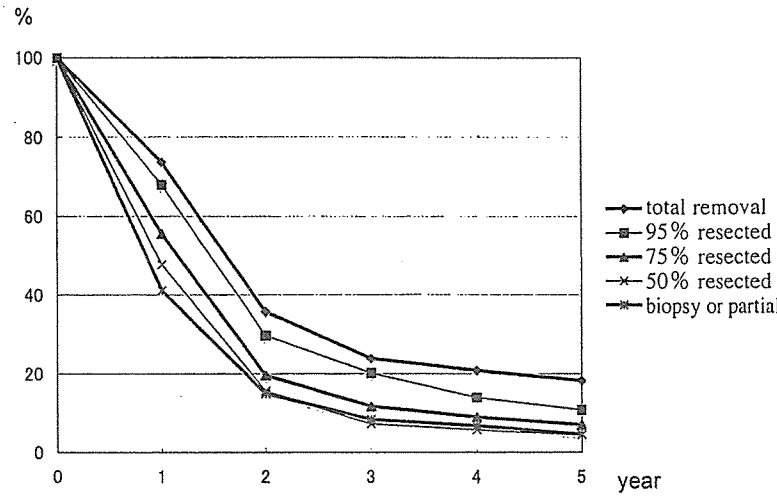

Fig. 1 Survival curves of glioblastoma by surgery

Table 2 Results of Mantel chi-square test

	biopsy or partial	50% resected	75% resected	95% resected	total removal
biopsy or partial		ns	＊＊＊	＊＊＊	＊＊＊
50% resected	ns		＊＊＊	＊＊＊	＊＊＊
75% resected	＊＊＊	＊＊＊		＊＊＊	＊＊＊
95% resected	＊＊＊	＊＊＊	＊＊＊		＊＊
total removal	＊＊＊	＊＊＊	＊＊＊	＊＊	

＊＊＊；$P<0.01$　＊＊；$P<0.05$　ns；not significant

Better survivals are obtained by total or 95% removal of the tumors compared to less than 75% removal. ($P<0.001$)

ら[19]は，照射量を 45 Gy から 50 Gy，55 Gy，60 Gy と高め，それに伴い生存期間中央値が，18 週，13.5 週，28 週，36 週，42 週と有意に延長したと述べている．化学療法との併用については，悪性グリオーマの術後補助療法として，「methyl CCNU 単独経口投与」，「放射線単独照射」，「放射線および BCNU 静脈内投与」，「放射線および methyl CCNU 経口投与」などに分けたランダム化試験を行い，BCNU 併用療法による放射線照射の生存が最も優っていたことから，米国での標準治療として今日まで用いられることになった[4)8)20)]．

国内でのランダム化試験としては大規模なものが少なく，Takakura ら[17]の「放射線単独」と「ACNU 併用放射線照射」の 2 群による第Ⅲ相試験が唯一といえるものである．この試験結果では，奏効率に有意差があったものの生存割合での有意差がなかったが，欧米での BCNU を用いた治療成績を参考に，国内での標準治療とされている．また，BCNU や ACNU などの nitrosourea 系抗癌剤を併用した放射線治療と単純放射線治療との 12 のランダム化試験のメタアナリシスでも化学療法併用群の 1 年生存が 46%で，放射線単独の 40%に比べ有意に高く，2 年生存も 15%に対し 20%であったことから，現時点での悪性グリオーマに対する標準治療は開頭手術による可及的摘出および術後 nitrosourea 系抗癌剤を併用した 60 Gy の放射線治療といえよう．

悪性グリオーマに対する JCOG 臨床試験

国内における標準治療確立のための動きとして，現在，日本臨床腫瘍研究グループ（Japan Clinical Oncology Group；JCOG）による臨床試験が始まっている．多施設共同臨床試験においては，各施設から集められる臨床データの信頼性が問題になる．一定の基準を満たし客観性を持った信頼できるデータを集めるためには，データの質を保ち，監視していく機構が必要である．JCOG は，欧米の Southwest Oncology Group（SWOG），Radiation Therapy Oncology Group（RTOG），European Organization for Research and Treatment of Cancer（EORTC）などの悪性腫瘍に対する多施設共同試験グループによる臨床試験の方法論を参考に作られた国内最大の臨床試験グループである．中央機構は，JCOG 代表者，データセンター，各種委員会および臓器別研究グループから成り，2002 年に第 13 番目の臓器グループとして脳腫瘍研究グループが