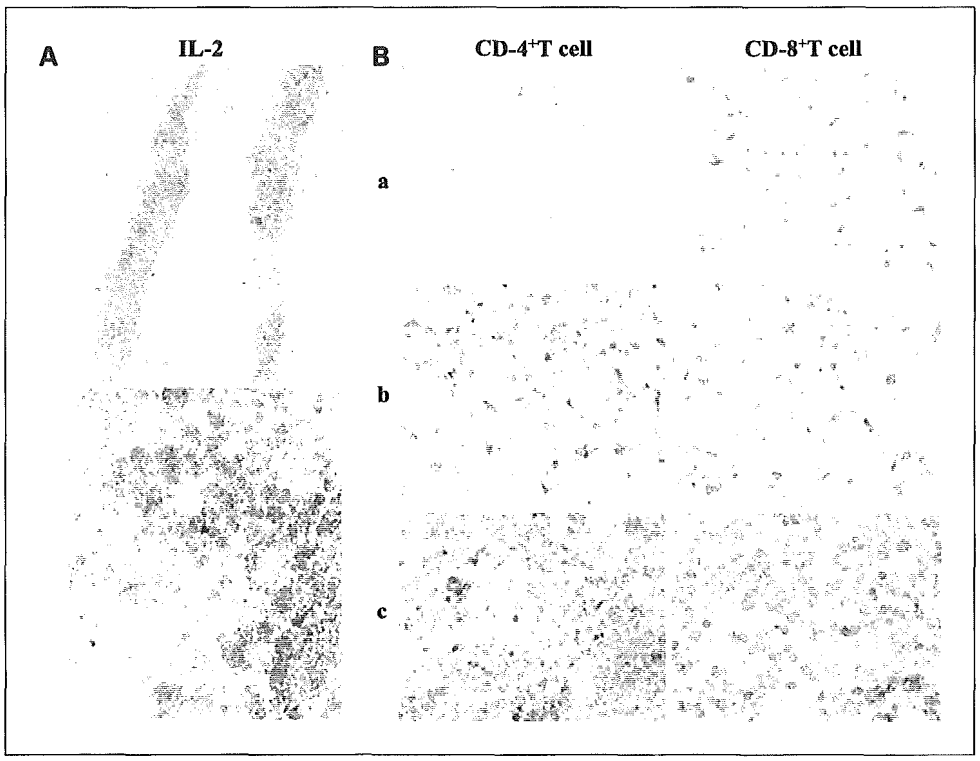

Fig. 6. *A*, immunohistochemical analysis of the expression of IL-2 in the 9L brain tumors treated with i.c. administration of hIL2-SeV/ΔMΔF. IL-2 protein is diffusely expressed. *B*, immunohistochemical analysis of the expression of CD4 and CD8 antigens in rats treated with i.c. administration of lacZ-SeV/ΔMΔF and s.c. vaccination of irradiated 9L cells (*a*), i.c. administration of hIL2-SeV/ΔMΔF alone (*b*), and i.c. administration of hIL2-SeV/ΔMΔF combined with the vaccination (*c*; ×200 magnification). Diffuse and dense infiltrations of $CD4^+$ Tcells and $CD8^+$ Tcells were observed in the tumors that were treated with i.c. administration of hIL2-SeV/ΔMΔF and the vaccination.

The receptor for SeV is sialic acid bound to gangliosides, which is present on most cell types including the human glia and glioma cells (22, 23). It may be altered by infiltration of the host cells in the xenograft models (24) and this probably affects the infection efficiency of hIL2-SeV/ΔMΔF and resultant efficacy.

Regarding the safety in clinical application, the biological features of SeV, such as the lack of integration into the cellular genome and the lack of homologous recombination between different SeV genomes, are desirable. Furthermore, we utilized both *M* and *F* genes-deleted SeV (SeV/MF) to enhance its safety. We previously constructed an *F* gene-deleted SeV (SeV/F; ref. 13) and an *M* gene-deleted SeV (SeV/M; ref. 14). *F* gene deletion made the SeV vector nontransmissible and *M* gene deletion worked well to make SeV incapable of forming particles from the infected cells. Although simultaneous deletions of these two genes in the same genome resulted in combining both advantages and contributed to increase the safety of the SeV vector, SeV/ΔMΔF still retains high levels of infectivity and gene expression *in vitro* and *in vivo* (i.e., similar to the wild-type SeV; ref. 15). These characteristics of SeV/ΔMΔF are considered suitable for the clinical application in gene therapy. In contrast, a theoretical obstacle for the clinical application could be the presence of antibodies against the human parainfluenza virus type I, which are known to cross-react with SeV HN proteins (4). However, it is currently not known whether a respiratory infection with human parainfluenza virus type I in the past will interfere with the transduction process of SeV vector at distant site, such as the central nervous system.

In conclusion, the present study showed that the nontransmissible recombinant SeV vector provided efficient transduction of the target genes into i.c. glioma cells. The i.c. administration of hIL2-SeV/ΔMΔF could induce a substantial production of IL-2 protein to induce the proliferation and expansion of peripherally activated, tumor-specific T cells. The therapeutic efficacy obtained by sufficient gene transfer with wide distribution and the high-grade safety of the nontransmissible recombinant SeV vector warrants clinical trials to evaluate its usefulness for human glioblastoma.

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Cathepsin D Is a Potential Serum Marker for Poor Prognosis in Glioma Patients

Mieko E. Fukuda,[1] Yasuo Iwadate,[1] Toshio Machida,[1] Takaki Hiwasa,[2] Yoshinori Nimura,[3] Yuichiro Nagai,[4] Masaki Takiguchi,[2] Hideki Tanzawa,[5] Akira Yamaura,[1] and Naohiko Seki[3]

Departments of [1]Neurological Surgery, [2]Biochemistry and Genetics, [3]Functional Genomics, [4]Molecular Pathology, and [5]Clinical Molecular Biology, Chiba University Graduate School of Medicine, Chiba, Japan

Abstract

Cathepsin D is an aspartyl protease involved in protein catabolism and tissue remodeling which can be secreted from cancer cells. To identify a potential serum marker for gliomas, we investigated the gene expression levels of cathepsin D in 87 tissue samples and measured the protein concentrations in sera of glioma patients. The tissue samples consisted of 43 glioblastomas, 13 anaplastic astrocytomas, 22 astrocytomas, and 9 normal brain tissues. The results of real-time quantitative reverse transcription-PCR analysis showed that cathepsin D transcript levels became significantly higher as the glioma grade advanced (P = 0.0466, glioblastoma and anaplastic astrocytoma; P = 0.0008, glioblastoma and astrocytoma; P = 0.0271, glioblastoma and normal brain tissue; unpaired t test). Immunohistochemical analysis with anti-cathepsin D antibody revealed dense and spotty staining in the tumor cells with high transcript levels. The low expression of cathepsin D significantly correlated with long survival of the glioma patients. Furthermore, the glioblastoma patients with high gene expression of cathepsin D lived significantly shorter than those with low expression (P = 0.0104, Cox-Mantel log-rank test) and frequently had leptomeningeal dissemination (P = 0.0016, χ^2 test). The multivariate analysis confirmed that the cathepsin D expression level was an independent predictor for short survival (P = 0.0102, Cox proportional hazard regression model). Measurement of the serum cathepsin D concentrations by ELISA showed a significant increase in the patients with high-grade gliomas as compared with the low-grade tumors (P = 0.0081, χ^2 test). These results collectively suggest that cathepsin D could be a potential serum marker for the prediction of aggressive nature of human gliomas (Cancer Res 2005; 65(12): 5190-4)

Introduction

Tumors of glial origin, such as glioblastoma, constitute the majority of primary brain tumors. The patients with glioblastoma have an average life expectancy of less than a year (1). The molecular pathogenesis of glioma includes both gain and loss of proteins responsible for the proliferation signals. Identification of the sets of genes that are differentially expressed between the high-grade gliomas and the low-grade tumors or normal brain tissues is important to understand the molecular basis of these nervous system tumors, to accurately predict the patient prognosis, and to develop novel therapeutic strategies (2). If the gene products can be detected in the patient sera, the clinical significance would be high for the preoperative diagnosis and for monitoring the response to therapy. With such a set of serum markers, patients would be better informed about the likely benefits of aggressive treatments.

The most devastating and therapeutically intractable aspect of glioblastoma is the disease highly invasive nature that prevents complete tumor resection and causes significant neurologic morbidity and mortality (1). The transformed cells release increased levels of proteolytic enzymes that facilitate tumor invasion (3, 4). Among the proteases, cathepsin D is an aspartyl protease that is normally localized within the lysosomes and involved in protein degradation and processing of the precursor proteins (5, 6). For example, it is responsible for specific cleavage and processing of myelin and other brain-associated proteins, conversion of procollagen into collagen, and activation of the inhibitors of cysteine proteases (3–6). It has also been suggested that this enzyme may be involved in several actions that facilitate the tumor progression, such as degradation of the extracellular matrix to promote tumor invasion (7–10). Elevated expression and secretion of cathepsin D have been noticed not only among the patients with breast cancer but also among those with other solid tumors (11), and it frequently correlates with poor prognosis (12, 13).

In the previous studies, we did differential proteomics associated with malignant transformation of human gliomas using the two-dimensional gel electrophoresis and mass spectrometry (2). We could identify the scores of proteins, which are abundantly expressed in the high-grade gliomas and can potentially predict the malignant nature of gliomas as serum markers. Especially, the high expression of cathepsin D is one of the common features in the tissue samples of the high-grade astrocytomas (2). Because cathepsin D has been reportedly secreted from cancer cells, we focused on this molecule as a potential serum marker for the diagnosis of glioma malignancy. We measured the degree of gene expression of cathepsin D in cDNA samples obtained from the glioma tissues, and also measured the serum concentration of cathepsin D in the glioma patients. We analyzed the correlation between the molecular information and the clinical data including the tumor grade, invasive nature, and survival period.

Materials and Methods

Tissue specimens. Seventy-eight gliomas (22 diffuse astrocytomas, 13 anaplastic astrocytomas, 43 glioblastomas) and nine normal brain tissues were examined. All tissue samples were obtained from the patients at the Chiba University Hospital under the protocol approved by the institutional review board, and informed consents were obtained from the patients or their guardians. The histopathologic diagnoses of all specimens were confirmed by two neuropathologists according to the criteria established

Requests for reprints: Yasuo Iwadate, Department of Neurological Surgery, Graduate School of Medicine, Chiba University, 1-8-1, Inohana, Chuo-ku, 260-8670 Chiba, Japan. Phone: 81-43-226-2158; Fax: 81-43-226-2159; E-mail: iwadatey@faculty.chiba-u.jp.

by the WHO. All of the gliomas investigated were obtained at the time of each patient's first surgery. The normal samples were obtained from the overlying cortex during resection of deeply seated benign tumors. A portion of each sample was fixed in 10% formaldehyde and embedded in paraffin and the remaining sample was immediately frozen in liquid nitrogen.

Extraction of mRNA and preparation of cDNA. The mRNAs were extracted from the tumors and normal brain tissues using QIAzol Lysis Reagent and RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo, Japan) according to the instructions of the manufacturer. The electrophoretic purity of all the mRNA samples was confirmed. One microgram of each mRNA was reversely transcribed using the oligo dT primer (TAKARA BIO, Inc., Tokyo, Japan) and Super Script II (Invitrogen Corp., Carlsbad, CA) according to the method described by Yoshikawa et al. (14).

Real-time reverse transcription-PCR. Real-time quantitative reverse transcription-PCR (RT-PCR) was done using the Light Cycler (Roche Diagnostics, Meylan, France), which exploited the ability of SYBR green to fluoresce after hybridization with a double-strand DNA. The amplification was done using 5′-CATTGTGGACACAGGCACTTC-3′ (Qiagen) as the forward primer and 5′-GACACCTTGAGCGTGTAGTCC-3′ (Qiagen) as the reverse primer. The analyses were done in 20 μL glass capillaries using the Light Cycler fast start DNA master SYBR green kit (Roche Diagnostics). Then, 1 mmol/L of each primer and 3 mmol/L of $MgCl_2$ in the total volume of 20 μL were used in each real-time RT-PCR amplification. The real-time RT-PCR cycle started with the initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 10 seconds, annealing at 61°C for 10 seconds, and elongation 72°C for 10 seconds. As an internal quantitative control of the gene expression, the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene expression was determined as reported previously (15). The cathepsin D and GAPDH gene expressions of all cDNA samples were determined by fluorescence from SYBR green using the Light Cycler software Version 3.5 (Roche Diagnostics), and the ratios of cathepsin D and GAPDH gene expressions represented the normalized relative levels of cathepsin D expressions.

Immunohistochemical analysis. For immunohistochemical analyses of gliomas, paraffin-embedded samples were sliced and mounted on microscopic slides. Rabbit polyclonal anti-cathepsin D antibody (1:200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) was used as the primary antibody. Heat-induced epitope was formed with microwave in 10 mmol/L citric acid buffer at pH 7.2. The samples were incubated with the antibody overnight in the same buffer followed by incubation with the biotinylated secondary antibody (1:500 dilution, DAKO, Tokyo, Japan). The bound antibodies were visualized by the avidin biotinylated peroxidase complex methods and diaminobenzidine tetrachloride (Santa Cruz Biotechnology).

ELISA of patient sera. We collected serum samples from 20 patients diagnosed with various-grade gliomas. They were 12 preoperative patients, and 8 postoperative and preirradiation patients with apparent tumor on magnetic resonance imaging. Of the 20 patients who underwent ELISA, 6 were included among the original 87 patients. None of them was receiving steroid therapy at the time of blood sampling. All of the blood samples were allowed to clot at 4°C for no more than 3 hours, and were then centrifuged for 5 minutes at 1,000 rpm. The serum (upper phase) was aliquoted and stored at −80°C until use. ELISA plates (96-well) were coated with 20 μg/mL antihuman cathepsin D antibody (GT, Minneapolis, MN), and were filled overnight with 50 μL of patients' sera diluted 1:25. The plates were exposed to biotinylated antihuman cathepsin D antibody diluted at 1:500, and then to peroxidase-conjugated avidin at 1:1,000. The plates were developed with *o*-phenylene-diamine (Sigma-Aldrich, St. Louis, MO) and were read at absorbance of 490 nm.

Statistical analysis. The survival periods of the patients with glioblastoma were calculated and the date of the initial surgery was set as zero. The Kaplan-Meier method was used to estimate the survival rates, and the Cox-Mantel log-rank test was applied to compare the survival differences among the patients using StatView software (SAS Institute, Inc., Cary, NC). We analyzed the correlation between the classification of cathepsin D expression into higher ratio or lower ratio and the patient's survival period. The other potential prognostic variables were age, sex, extent of surgery, and perioperative Karnofsky performance status score. Magnetic resonance images of all patients were obtained with and without Gd enhancement to assess the infiltrative or disseminated areas during their survival. After the surgery, the patients were treated with the conventional radiotherapy and chemotherapy. The multivariate analysis was done with the commercially available software by using the Cox proportional hazard regression model (SPSS, Inc., Chicago, IL).

Results

Differential expression of the cathepsin D gene in astrocytic tumors. The relative cathepsin D and GAPDH gene expression levels in the normal brain tissues, diffuse astrocytomas, anaplastic astrocytomas, and glioblastomas are shown in Fig. 1. The expression level was lowest in the normal brain tissues (0.036 ± 0.16, n = 9), and it increased as the histologic grading advanced. The value was 0.041 ± 0.02 for diffuse astrocytomas (n = 22), 0.139 ± 0.149 for anaplastic astrocytomas (n = 13), and 0.448 ± 0.538 for glioblastomas (n = 43). The unpaired t test indicated significant differences between diffuse astrocytoma and anaplastic astrocytoma (P = 0.0047), and between anaplastic astrocytoma and glioblastoma (P = 0.0466). There was no significant difference between the normal brain tissues and diffuse astrocytomas (P = 0.5356). These observations strongly suggest that the degree of relative cathepsin D gene expressions positively correlated with the progress of histologic grade in astrocytic tumors.

Immunohistochemical staining for cathepsin D. In addition to assessing the gene expression of cathepsin D, the level of gene product was assessed immunohistochemically. Although weak and reticular staining for antihuman cathepsin D antibody was observed in all grades of astrocytomas and some neurons in the normal brain, dense and spotty staining was observed only in the tumors with high cathepsin D expression ratios (Fig. 2). In contrast, glioblastomas with low cathepsin D expression ratios had large tumor areas never stained with the antibody. This result suggests that the cathepsin D expression level measured with real-time RT-PCR directly correlated with the protein production in the glioma tissues.

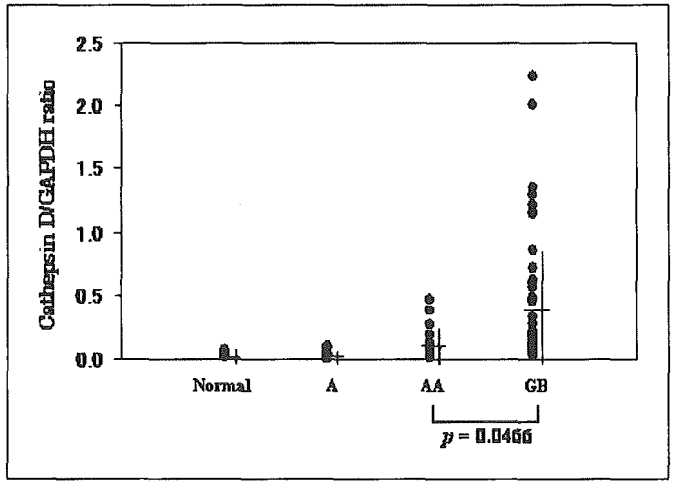


Figure 1. Real-time RT-PCR analysis for the expression of *cathepsin D* in various grades of astrocytic tumors and normal brain tissues. A, AA, and GB indicate diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma, respectively. There are statistically significant differences between anaplastic astrocytoma and glioblastoma (P = 0.0466), diffuse astrocytoma and glioblastoma (P = 0.0008), diffuse astrocytoma and anaplastic astrocytoma (P = 0.0047), and normal brain tissue and glioblastoma (P = 0.027). *Bars*, mean; *columns*, SD.

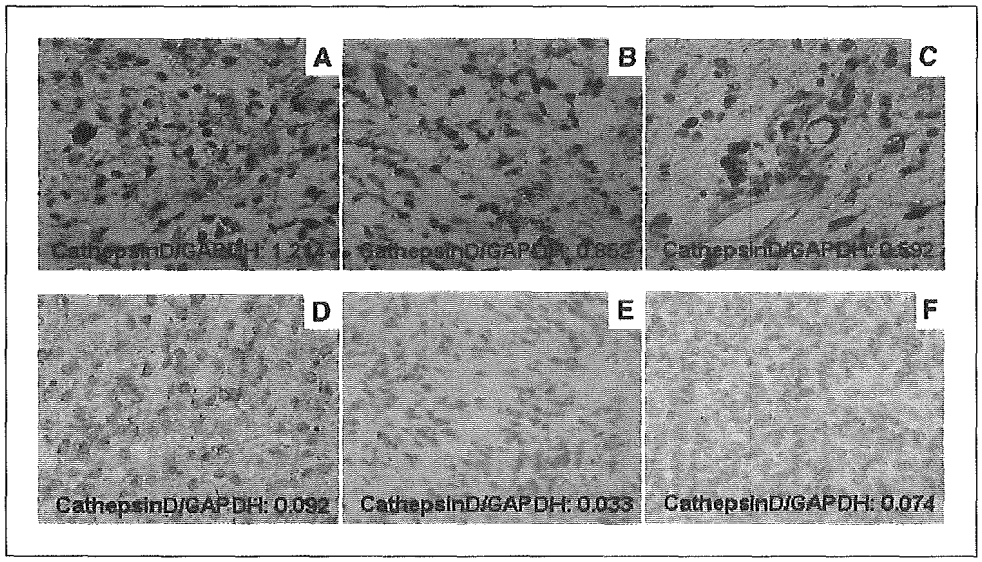


Figure 2. Immunohistochemical analysis for antihuman cathepsin D antibody. Paraffin-embedded sections of representative glioblastomas were stained with the antibody against human cathepsin D. The photographs on the top row (*A-C*) are glioblastomas that have high levels of cathepsin D gene expression with dense and spotty staining for cathepsin D. In contrast, glioblastomas in the bottom row, which had low levels of cathepsin D expression ratio, showed only weak and reticular staining for the cathepsin D antibody (*D*) or large tumor areas that were not stained with the antibody (*E* and *F*).

Correlation between cathepsin D gene expression and survival period. All glioma samples were analyzed for the correlation of cathepsin D/GAPDH ratio with patients survival (observation) periods. It indicated that all the tumors from patients with long survival (>2 years) had low levels of cathepsin D/GAPDH ratio. In contrast, the tumors from patients with shorter survival (<2 years) had a wide range of cathepsin D/GAPDH ratio (Fig. 3*A*).

Because the highest cathepsin D/GAPDH value in anaplastic astrocytomas was 0.465, we compared the overall survival periods of patients with glioblastoma between the two groups [i.e., glioblastomas with cathepsin D/GAPDH expression ratios <0.465 (26 patients) and the tumors with ratios ≧0.465 (17 patients)]. The patients of the former group lived significantly longer than those of the other group (Fig. 3*B*, P = 0.0104, Cox-Mantel log-rank test). There was no significant difference between the two groups in the potential prognostic factors such as age, performance status, extent of surgical resection, and dose of radiotherapy. To confirm the prognostic value of the cathepsin D expression level, we did multivariate analysis for survival on the 43 glioblastoma patients (Table 1). We found that the cathepsin D expression level was an independent factor for poor prognosis (P = 0.0102). The age was the only other significant independent predictor for overall survival (P = 0.0472).

Correlation between cathepsin D expression and leptomeningeal dissemination. To explore the biological mechanisms by which the high expression level of cathepsin D could affect the survival times of the patients with glioblastoma, the infiltrative areas of glioblastomas were examined with magnetic resonance imagings. Because cathepsin D takes part in degradation of the extracellular matrix and is strongly expressed by invading glioblastoma cells at the infiltrative margin (16), cathepsin D may be associated with the invasive nature of glioblastomas. Among the six patients with very high expression level of cathepsin D (≧1.0), three manifested leptomeningeal disseminations on the Gd-enhanced magnetic resonance imaging. The frequency (50%) was significantly higher than in the other 37 patients (cathepsin D expression level <1.0) in whom only 2 tumors (5.4%) manifested magnetic resonance imaging features suggestive of leptomeningeal dissemination (Fig. 4, χ^2 test, P = 0.0016).

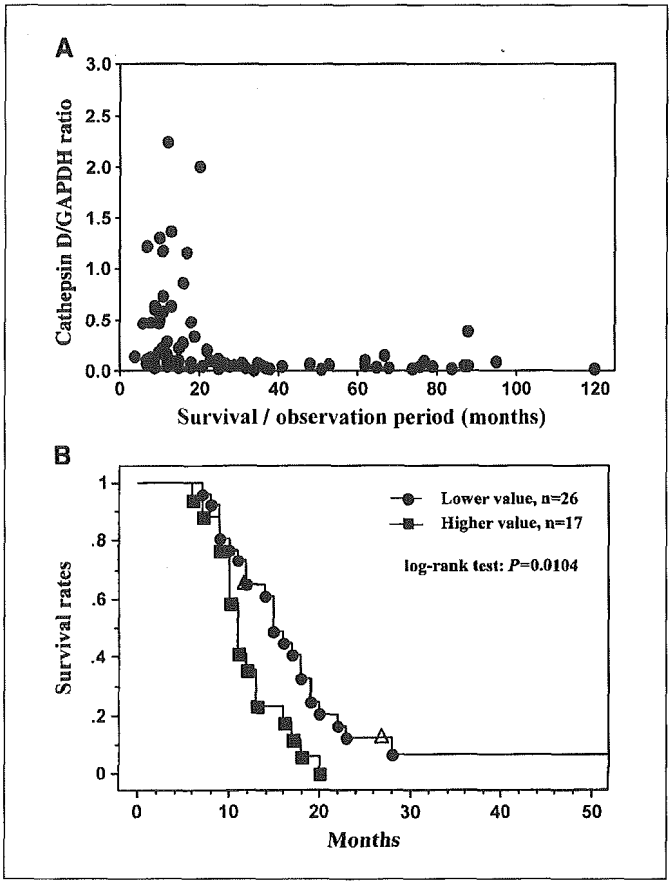


Figure 3. *A*, scatter plot of the relation between survival period and cathepsin D/GAPDH ratio. All the tumors from the patients with long survival (>2 years) had low levels of cathepsin D/GAPDH ratio. In contrast, the gliomas from short-survived patients (<2 years) had a wide range of cathepsin D/GAPDH ratio. Among the patients with long survival (>2 years), several cases were still alive and their observation periods were used. *B*, Kaplan-Meier survival curves of the patients with glioblastoma as divided by the real-time RT-PCR expression levels of cathepsin D. The patients with a gene expression level ≧0.465 (the highest value in anaplastic astrocytomas) and those with low expression tumors (<0.465) are compared. The difference between the two survival curves was statistically significant (log-rank test, P = 0.0104).

Table 1. Multivariate analysis by the Cox proportional hazard regression model in 43 glioblastoma patients

Variable	*P*
Age	0.0472
Sex	0.6901 (ns)*
KPS† score	0.3255 (ns)
Extent of surgery	0.0706 (ns)
Expression level of cathepsin D	0.0102

*ns, not significant.
† KPS, Karnofsky performance status.

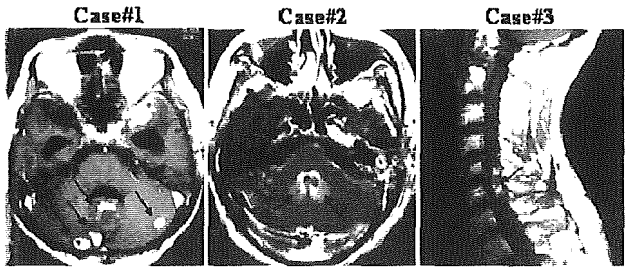
Cathepsin D concentrations in the patient sera. On the basis of the aforementioned data, cathepsin D seems to be a promising candidate serum marker for diagnosis of the biological aggressiveness in gliomas. To test whether cathepsin D can be used as a serum marker correlating with the presence and grade of glioma, we measured the serum levels of cathepsin D in 20 patients with gliomas by ELISA (Fig. 5). Comparison of the serum cathepsin D levels was made between 9 patients with low-grade gliomas and 11 patients with high-grade gliomas. The mean values ± SD were 16.2 ± 32.3 ng/mL for the low-grade tumors and 242.0 ± 329.1 ng/mL for the high-grade tumors. When a cutoff was set at 100 ng/mL, the positive ratio was significantly higher in the high-grade tumors than in the low-grade tumors (χ^2 test, P = 0.0081). Although the tumor tissues from the 20 patients were not necessarily available for analysis of the gene expression, all tumors from the patients with high serum concentrations expressed high cathepsin D transcripts.

Discussion

In this study, we showed that the transcript level of cathepsin D positively correlated with the histologic grade in the astrocytic tumors, and it could be used as a predictive marker for poor prognosis in the glioma patients. Furthermore, the serum levels of cathepsin D protein could be detected in the glioma patients, indicating that cathepsin D was secreted from a subset of gliomas. The serum protein level was significantly higher in the patients with high-grade gliomas as compared with the low-grade gliomas. These data suggest that the serum cathepsin D level may be a potential indicator of the biological aggressiveness of intracerebral gliomas. With its disease specificity and response to treatment demonstrated in further analysis, cathepsin D can be considered as a serum marker of tumor burden and recurrence in human gliomas.

Cathepsin D is an aspartic endopeptidase which is normally involved in the intralysosomal and intraendosomal cellular degradation of proteins and in the processing of precursor proteins (3–5). The coding gene is located on chromosome 11p15, and is a housekeeping gene ubiquitously present in all mammalian cells. Some authors have reported that overexpression of cathepsin D in the carcinoma cells of the breast and some other organs is associated with a higher risk of relapse and metastasis, and consequently with a shortened survival period (11, 12). For brain tumors, there are a few reports indicating the correlation between cathepsin D expression and the biological features of the tumor cells (16–18). Immunochemical studies of tissue extracts from glioblastomas showed increased levels of cathepsin D as compared with the other grade gliomas and normal brain tissues. In a recent study, cathepsin D antibody was shown to inhibit the invasion of a glioblastoma cell line (U251) in a dose-dependent manner (17). In an experimental mouse model of the human glioblastoma, an association between the cathepsin D immunoreactivity and the aggressive proliferative activity of U87 cells was noticed as compared with the less aggressive U373 cell line (18). These data are in accordance with our result.

Although the mechanisms by which overexpressed cathepsin D contributes to tumor relapse and metastasis including leptomeningeal dissemination are not fully understood, three pathways could be involved in this process. First, it has been suggested that cathepsin D acts as an autocrine mitogen by increasing the cell growth and decreasing the contact inhibition (7–10). Cathepsin D can be secreted from the tumor cells as a proenzyme (6, 8). At a neutral pH, the secreted pro-cathepsin D interacts with different



Leptomeningeal dissemination[a]	Cathepsin D ≧1.0[b]	Cathepsin D <1.0
−	3 (50)[c]	35 (95)
+	3 (50)	2 (5)

(χ^2 test: p = 0.0016)

[a] Diagnosed by gadolinium-enhanced MRI
[b] The normalized ratios of cathepsin D/GAPDH
[c] Percentage with raw numbers in parenthesis

Figure 4. Correlation between very high expression of cathepsin D (cathepsin D/GAPDH ratio ≧ 1.0) and leptomeningeal dissemination in 43 glioblastoma patients. The Gd-enhanced magnetic resonance images show three glioblastoma cases with leptomeningeal dissemination. *Black arrows,* disseminated lesions. Among the six patients with very high levels of cathepsin D (≧1.0), three patients (50%) manifested leptomeningeal dissemination.

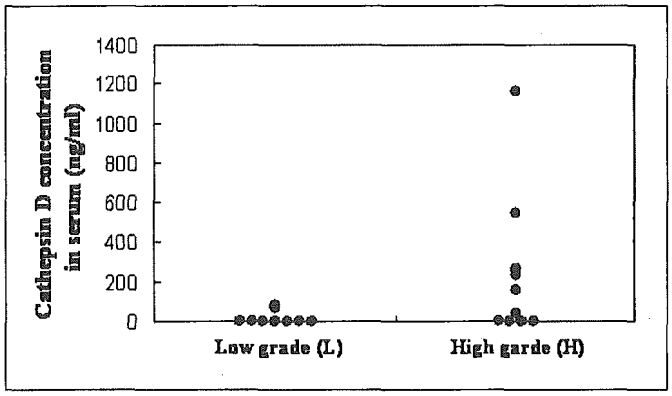


Figure 5. ELISA analysis of sera from 20 glioma patients; 9 low-grade gliomas and 11 high-grade gliomas. There is a significant difference in the seroreactivity to anti-cathepsin D antibody between patients with low-grade gliomas and those with high-grade gliomas ($P = 0.0081$, χ^2 test).

membrane receptors to facilitate cell growth (6, 19). Second, cathepsin D can induce degradation of different components of the extracellular matrix and thereby facilitate tumor invasion and metastasis (20). The secreted pro-cathepsin D requires activation at a low pH to show its proteolytic activity (6, 11, 21). Extracellular pH in the tumor tissues is known to be more acidic than that in the normal tissues. Third, intracellularly, it may either activate the growth factor pathways or inactivate the growth inhibitors. Indeed, cathepsin D is reported to participate in a potent proteolytic cascade to activate pro-cathepsin B, cathepsin B, and the pro-urokinase plasminogen activator (3–6, 22, 23). Cathepsin B has been reported to be functionally important in the process of tumor invasion and angiogenesis during the malignant progression of gliomas (22, 24).

In conclusion, we have shown that measurement of the tissue cathepsin D expression levels can identify a subgroup of gliomas with highly aggressive nature and a high likelihood of leptomeningeal dissemination. Furthermore, this investigation is the first clinical study to show that the serum cathepsin D level correlated with the histologic grade of gliomas, suggesting that the serum cathepsin D level could be a potential indicator for disease aggressiveness in human gliomas.

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Management and Survival of Pineoblastoma: An Analysis of 34 Adults From the Brain Tumor Registry of Japan

John Y. K. LEE, Toshihiko WAKABAYASHI, and Jun YOSHIDA

Department of Neurosurgery, The Nagoya University School of Medicine, Nagoya, Aichi

Abstract

Pineoblastoma is a rare tumor in adults, and factors influencing survival are poorly understood. Data from the Brain Tumor Registry of Japan (BTRJ) was analyzed to examine patient, tumor, and treatment characteristics associated with increased survival in adults with pineoblastomas. All pineoblastoma cases in adults aged 16 years or older were identified in the BTRJ. Data were extracted on demographics, presentation, tumor characteristics, treatments, and outcomes. Kaplan-Meier plots, the log rank method, and p value <0.15 was used to screen variables for inclusion in a multivariate Cox regression estimating survival. In the final Cox multivariate model, all variables with p values <0.05 were considered significant predictors of survival, and all variables with p values 0.05–0.099 were considered trends. The BTRJ contained 34 adults with pineoblastomas diagnosed from 1969–1998. The patients were predominantly male (22 patients), with a median age of 35 years (range 16–66 years). Median survival from diagnosis was 25.7 months, with a median follow up of 20.5 months. Median surgical resection was 75–94%, and five of the 34 patients had gross total resection. Twenty-nine of the 34 patients received cranial irradiation therapy with a median dose of 50 Gy (range 30–70 Gy). In the final multivariate model, cranial irradiation ≥ 40 Gy ($p = 0.014$) and gross total resection ($p = 0.034$) were associated with improved survival. There was a trend towards improved survival for women ($p = 0.099$). Adult pineoblastoma patients have poor survival prognosis. Cranial irradiation therapy using at least 40 Gy and complete surgical resection are associated with improved survival.

Key words: brain tumor, pineoblastoma, radiotherapy, surgery, survival, adult

Introduction

Pineoblastomas are rare, representing less than 0.1% of all primary brain tumors.[6,20] Pineoblastomas usually occur in children, whereas adults account for less than 10% of cases in published series,[19] and there are no adult pineoblastoma clinical series larger than 25 patients.[4,9] The systematic study of pineoblastomas in adults is hindered by their extreme rarity, incomplete staging, and the lack of a patient registry containing data on patient and tumor characteristics, treatments, and outcomes. In most series, treatments include chemotherapeutic and craniospinal irradiation, but the optimal regimen remains unclear.

The Brain Tumor Registry of Japan (BTRJ) is a nationwide registry of patients of all ages with primary or metastatic brain tumors. The BTRJ contains demographic, symptom, imaging, treatment, survival, functional status, and pathologic data.[6,28] An international collaborative project between the University of Pittsburgh and the Nagoya University School of Medicine provided access to the BTRJ. We queried the BTRJ to review the largest series of adults with pineoblastomas presented to date. The BTRJ is a unique source of data as almost all brain tumors in Japan are treated and recorded by the national health care system. The most recent summary report includes a statistical analysis of 81,569 patients with primary and metastatic brain tumors who were registered from 1969 to 1993.[6] The present analysis used an expanded BTRJ dataset collected from 1969–1998. Studies of extremely rare tumors such as adult pineoblastomas can only be accomplished using large databases such as the BTRJ.

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Author's present address: J. Y. K. Lee, M.D., Department of Neurosurgery, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.

Appendix: Performance state classification of functional status

Grade	Description	Karnofsky Performance Status score equivalent
1	Normal: No complaints; No evidence of disease	90–100
2	Normal activity with effort; Some symptoms	80
3	Cares for self; Unable to carry on normal activity	70
4	Requires occasional assistance; Cares for most needs	50–60
5	Disabled; Requires special needs and assistance	40
6	Severely disabled; Hospitalized, death not imminent	20–30
7	Moribund; Fatal processes are rapidly progressing	0–10

We report the presentation, treatment, and survival of 34 adults with pineoblastoma from data deposited in the BTRJ over a 30-year interval.

Methods and Materials

I. Clinical material

We performed a retrospective analysis of the BTRJ, identifying all cases of pineoblastomas in adults 16 years of age or older at the time of original diagnosis. Brain tumors in the BTRJ are classified according to the classification of the Union International Contre Le Cancer in 1965.[38] The registry contains data on sex, date of birth, date of diagnosis, duration of symptoms prior to diagnosis, method of diagnosis (histology, cerebrospinal fluid [CSF] cytology, imaging, autopsy), surgeries (date(s), extent of tumor resection(s), extension, invasion), tumor diameter, functional status (pre- and postoperative performance state [PS]), tumor dissemination, irradiation therapy (type, dose, timing, target(s), use of a radiation sensitizer), chemotherapy (agent(s), timing of administration), date of last follow up, and death (date, cause).[6,28] Surgical tumor resection is coded as 0% (i.e., biopsy or decompressive craniectomy), 1–49%, 50–74%, 75–94%, 95–99%, and 100%. In patients with two surgeries, the value of the more complete tumor resection was used in this analysis. Tumor size was recorded as the geometric mean of two diameters (mean = square root[diameter_1 * diameter_2]). We calculated tumor volume from the diameter by assuming spherical morphology (volume = $4/3 * \Pi *$ (mean diameter/2)^3). Pre- and postoperative functional status were categorized on the PS scale,[6] a seven level classification similar to the Karnofsky Performance Status[22] ranging from 1 = "normal: no complaints; no evidence of disease" to 7 = "moribund; fatal processes are rapidly progressing" (Appendix). Irradiation therapy data included target(s) (cranial or craniospinal) and cranial dose in Gray (Gy). Chemotherapy data detailed agents and the timing of administration.

II. Statistical analysis

Medians, means, and 95% confidence intervals (CIs) were used to describe the central tendencies and dispersions of continuous variables. Median values were used to impute missing values for variables with $\leq 20\%$ missing values for the regression analysis. We did not impute missing values for variables missing $>20\%$ of values, and these variables could not be used in the regression analyses. During the screening process of the regression analyses, nominal variables were recorded as a series of dichotomous indicator variables, and continuous and ordinal variables were dichotomized on median values.

Kaplan-Meier life tables were used to determine the unadjusted survival of the entire cohort. We then built a multivariate Cox proportional hazard model to determine which patient, tumor, and treatment characteristics were independently associated with improved survival. Variables were first screened in a univariate analysis using Kaplan-Meier product-limit estimation and the log-rank test for inclusion in the multivariate model—all variables from the univariate analyses with $p < 0.15$ were candidates for inclusion in a multivariate Cox proportional hazard model. Multivariate Cox proportional hazard modeling allows the estimation of the relative rate of death, or "hazard ratio" (HR) associated with each variable while adjusting for the effects of other variables in the model.[7] In the final Cox multivariate model, p values <0.05 were considered significant, and p values 0.05–0.099 were considered trends. The appropriateness of the proportional hazard assumption for the final model was tested using Grambsch and Therneau's method.[18]

In a secondary analysis, we used multivariate ordinal logistic regression to model the predictors of postoperative functional status. Candidate predictor variables were screened for inclusion in the multivariate model using a univariate ordinal logistic regression. All variables from the univariate regression analyses with $p < 0.15$ were then included in a

stepwise multivariate ordinal logistic regression. In the final multivariate model, p values <0.05 were considered significant, and p values 0.05–0.099 were considered trends.

Table 1 Study population (n = 34)

Mean age (range)	35 (16–66) years
Male	22 (64.7%)
Presenting symptoms	
elevated intracranial pressure	16 (47.1%)
focal signs	9 (26.5%)
subjective complaints	4 (11.8%)
decreased level of consciousness	2 (5.9%)
asymptomatic (incidental)	2 (5.9%)
stupor or coma	1 (2.9%)
Tumor diameter	
median	2 cm
1 cm	2 (5.9%)
2 cm	13 (38.2%)
3 cm	5 (14.7%)
4 cm	5 (14.7%)
5 cm	2 (5.9%)
>8 cm	1 (2.9%)
unknown	6 (17.6%)
Spinal dissemination	
present	6 (17.6%)
absent	24 (70.6%)
unknown	4 (11.8%)
Tumor invasion	
none	22 (64.7%)
arachnoid	4 (11.8%)
dura	1 (2.9%)
unknown	7 (20.6%)

Results

I. Patient characteristics

The BTRJ contains 34 cases of pineoblastoma in adults aged 16 years or older diagnosed between 1969 and 1998 (Table 1). An earlier analysis of the BTRJ identified 76 cases of pineoblastoma in patients of all ages diagnosed from 1969–1993, comprising 0.19% of all primary brain tumors (an analysis of pineoblastoma incidence based on more recent BTRJ data is underway). The cohort of 34 patients contained 22 males (64.7%), with a median age at presentation of 35 years (range 16–66 years). The most common clinical presentations were signs of elevated intracranial pressure (n = 16, 47.1%), focal neurologic signs (n = 9, 26.5%), and subjective neurological complaints (n = 4, 11.8%). Two patients (5.9%) were asymptomatic at the time of diagnosis. The median tumor diameter was 2 cm (range 1–>8 cm), corresponding to a median volume of 4.2 cm^3 (range 0.5–382 cm^3). Only 30 patients had definite data documenting the presence (6 patients) or absence (24 patients) of spinal dissemination. In the remaining four patients, the presence or absence of disseminated disease could not be ascertained from the registry. The majority of patients (n = 22) had no evidence of local tumor invasion noted during surgery.

Only 23 patients had the preoperative PS recorded in the database (Table 2). Fifteen patients had PS grades of 1–2, consistent with the ability to perform normal activities.

Table 2 Performance state (PS) before and after surgery

Preoperative PS	Postoperative PS								Total
	1	2	3	4	5	6	7	Unknown	
1	4	0	2	0	1	0	0	0	7
2	4	3	0	1	0	0	0	0	8
3	1	1	1	1	0	0	0	0	4
4	0	2	1	0	0	0	0	0	3
5	0	0	0	1	0	0	0	0	1
6	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0
Unknown	2	1	1	0	1	2	3	1	11
Total	11	7	5	3	2	2	3	1	34

Twenty-three patients had both pre- and postoperative PS grades recorded in the database. The *shaded boxes* represent patients with identical pre- and postoperative grades (n = 8, 34.8%). Values above and to the right of the *shaded boxes* correspond to patients with a decline in postoperative PS (n = 5, 21.7%). Values below and to the left of the *shaded boxes* correspond to patients with improved postoperative PS grades (n = 10, 43.5%).

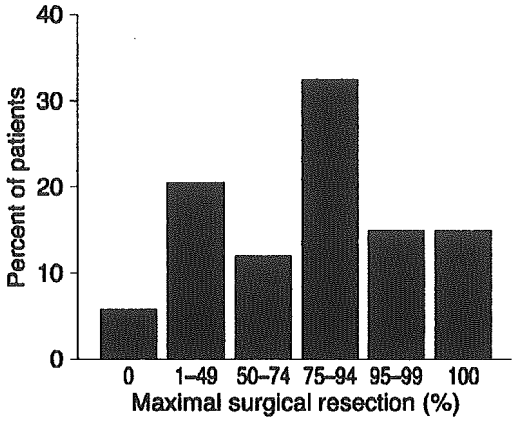

Fig. 1 Maximal extent of surgical resection in one or more procedures (n = 32).

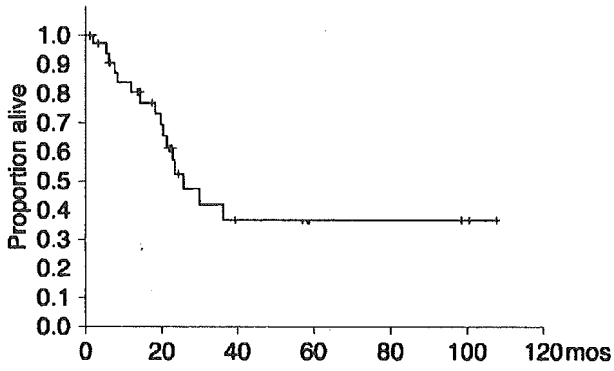

Fig. 2 Kaplan-Meier survival plot illustrating cohort survival after onset of symptoms (n = 34). Tick marks represent censoring at latest follow up. Median survival for the cohort = 25.7 months.

Table 3 Irradiation therapy cranial dose

Irradiation dose (Gy)	n (%)
0	5 (14.7%)
30	1 (2.9%)
40	3 (8.8%)
50	10 (29.4%)
60	9 (26.5%)
70	2 (5.9%)
Unknown	4 (11.8%)
Total	34 (100%)

II. Diagnosis and therapy

Thirty-two of the 34 patients underwent at least one surgical procedure to reduce tumor burden or mass effect, or to establish a diagnosis (Fig. 1). The dates of surgery ranged from April 1970 to January 1997.

Twenty-five of the 34 patients received cranial irradiation, with the tumor receiving a median dose of 50 Gy (range 30–70 Gy) (Table 3). Eleven patients received both cranial and spinal irradiation. Four of the six patients with documented disseminated disease received complete craniospinal radiation.

Ten of the 34 patients received chemotherapy, and nine of these 10 patients received multiple agents. The most common agents were VP-16 (n = 5, 50%), cisplatin (n = 4, 40%), and ACNU (n = 3, 30%). The most popular combination therapy was VP-16 combined with cisplatin (n = 3, 30%). Other agents used included vincristine (n = 2, 20%), tegafur (n = 1, 10%), methotrexate (n = 1, 10%), or bleomycin (n = 1, 10%). No patient received chemotherapy without also receiving radiation therapy. Of the 10 patients receiving chemotherapy, five received only cranial irradiation, and five received craniospinal radiation therapy.

III. Survival

At the time of the last recorded evaluation, 16 patients had died, and median follow up was 20.5 months (range 1–107.5 months). The Kaplan-Meier survival curve for the cohort demonstrates a median survival from time of presentation of 25.7 months (95% CI 16, 35.3 months) (Fig. 2).

Kaplan-Meier univariate analysis was run on the following variables: age, sex, presentation symptoms, date of surgery, maximum tumor resection, tumor size, tumor invasion, extent of intracranial tumor spread, tumor extracranial dissemination, irradiation therapy cranial dose, radiation sensitizers, spinal irradiation, and chemotherapy. Missing data in 11 patients for preoperative PS prevented the inclusion of this variable in the Kaplan-Meier or Cox regression analyses. The Kaplan-Meier univariate analysis identified seven variables that met the criteria of $p < 0.15$ for consideration of inclusion in the multivariate model: sex, maximum tumor resection, irradiation therapy cranial dose, decreased level of consciousness at presentation, focal neurological signs at presentation, extension of tumor beyond the midline, and dural invasion of tumor.

In the multivariate Cox model, two variables were independently associated with improved survival: irradiation therapy cranial dose ≥ 40 Gy (HR 3.8; 95% CI 1.3, 11.2; p = 0.014) and complete surgical resection (HR undefined — no deaths in gross total resection group; Kaplan-Meier p = 0.034) (Table 4). In addition, there was a trend towards improved

Table 4 Variables associated with increased survival in multivariate Cox model

Variable	Hazard ratio (95% CI)	p Value
Irradiation therapy cranial dose ≥ 40 Gy	3.8 (1.3, 11.2)	0.017
Gross total resection*	—	0.034
Female	3.0 (0.81, 11.4)	0.099

*No patient with gross total resection died during follow up, thus for this variable the hazard ratio is undefined and the Cox regression cannot generate a p value. The p value is derived from the Kaplan-Meier log-rank test in the univariate analysis. CI: confidence interval.

survival for females (HR 3.0; 95% CI 0.81, 11.4; p = 0.099).

Figure 3 demonstrates the Kaplan-Meier survival plots for irradiation therapy dose ≥ 40 Gy, gross total resection, and sex. In the final Cox model, Grambsch and Therneau's method showed that there was no significant deviation from the proportional hazards assumption (global test, p = 0.996), and thus a Cox regression can legitimately be used to model survival in this population. Multivariate Cox regression modeling showed no significant independent association between survival and any of the other variables: age, presentation symptoms, date of surgery, tumor size, tumor invasion, extent of intracranial tumor spread, tumor extracranial dissemination, radiation sensitizers, spinal irradiation, or chemotherapy.

IV. PS

The registry did not specify when the postoperative PS was recorded, as PS values in the registry usually indicate the most recent available annual assessment. Twenty-three of the 34 patients had both pre- and postoperative PS grades recorded in the database. Surgery had no impact on the PS grades of eight patients; postoperative grades were improved in 10 patients, but worse in five patients (Table 2).

The ordinal logistic univariate regression analysis was run on the following variables: age, sex, presentation symptoms, date of surgery, maximum tumor resection, tumor size, tumor invasion, extent of intracranial tumor spread, tumor extracranial dissemination, irradiation therapy cranial dose, radiation sensitizers, spinal irradiation, and chemotherapy. The univariate ordinal logistic analysis identified three variables that met the criteria of $p < 0.15$ for consideration of inclusion in the multivariate model: maximum tumor resection

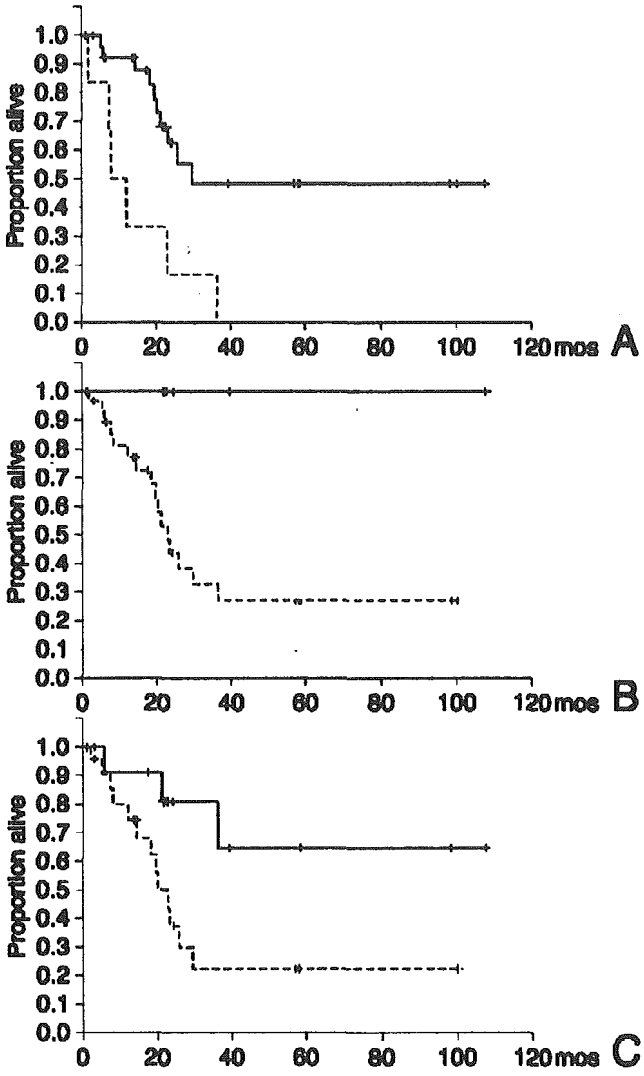

Fig. 3 Kaplan-Meier survival plots comparing cohort subgroup survival after symptom onset using the log-rank test. Tick marks represent censoring at latest follow up. A: Patients who received ≥ 40 Gy of cranial radiation (*solid line*) had significantly longer survival times (median = 29.8 months) compared to patients who received lesser doses (*dashed line*, median = 8.1 months) (p = 0.014). B: Patients who had gross total surgical resection (*solid line*) had significantly longer survival times (median > 107.5 months) compared to patients with subtotal resections (*dashed line*, median = 22.3 months) (p = 0.034). C: There was a trend towards longer survival in women (*solid line*, median > 107.5 months) compared to men (*dashed line*, median = 20.0 months) (p = 0.099).

>50%, irradiation therapy cranial dose ≥40 Gy, and any invasion of tumor noted at surgery. In the multivariate ordinal logistic regression model, only irradiation therapy dose ≥40 Gy was independently associated with better postoperative PS ($p = 0.003$). The analysis showed no significant independent association between postoperative functional status and the following variables: age, sex, presentation symptoms, date of surgery, tumor size, tumor invasion, extent of intracranial tumor spread, tumor extracranial dissemination, radiation sensitizers, spinal irradiation, or chemotherapy.

Discussion

I. Histopathology

Pineoblastomas and pineocytomas are pineal parenchymal tumors that are distinct from germ cell tumors and glial tumors.[3,33] A two-tiered staging system distinguishes pineoblastomas from pineocytomas. The pineocytoma is a well-circumscribed tumor that grossly compresses the surrounding tissue. The cells appear benign and look similar to mature pineal parenchymal cells in sheets or irregular lobules. Identification of pineocytomas is facilitated with the finding of Homer-Wright rosettes and the finding of larger pineocytomatous rosettes. Pineoblastomas, in contrast, are grossly invasive with poorly defined borders and frequent leptomeningeal spread. Histologically, pineoblastoma is very similar to medulloblastoma and has been described as "medulloblastoma pineales," emphasizing its similarity to other primitive neuroectodermal tumors.[20,31] Pineoblastoma is highly cellular, with small poorly differentiated cells in pattern less sheets or aggregates. Mitosis are found along with Homer-Wright rosettes, Flexner-Wintersteiner rosettes, and areas of necrosis.[3,33]

More recently, Schild et al.[33] has divided pineal parenchymal tumors into four types: pineocytomas, intermediate tumors, mixed tumors, and pineoblastomas. The intermediate tumor is described as a transitional form between pineocytomas and pineoblastomas. The mixed tumor shows patterns of both pineocytomas and pineoblastomas. In addition to Schild's grading system, Fauchon et al.[13] have defined another scale emphasizing the number of mitoses and thereby correlating survival with mitotic activity. These grading scales are relatively recent descriptions, and most series of pineal parenchymal tumors have used the two-tiered grading system.[3,20]

Pineoblastomas are rare tumors that are sometimes simply described as supratentorial primitive neuroectodermal tumors (PNETs).[20,31] Pineoblastomas and cerebellar medulloblastomas both share the presence of Homer-Wright rosettes, which are widely accepted to represent abortive attempts at neuroblastic differentiation.[20] In addition, mononucleated and multinucleated tumor giant cells are present in pineoblastomas as in cerebellar medulloblastomas.[3] Hence, the category of PNETs has been said to include both cerebellar medulloblastomas and pineoblastomas.

The BTRJ does not require a centralized review of pathologic tissue for entry into the database, so we could not verify the classification of pineal parenchymal tumors in this series. The pathologic grading scale employed in the BTRJ is derived from Zulch[38] and describes only the two-tiered grades of pineocytoma versus pineoblastoma.

II. Survival

The overall median survival in our series of adults with pineoblastomas was 25.7 months. Chang et al.[4] reported a series of 11 adults with pineoblastomas with a median survival of 30 months. Using a grading scale for pineal parenchymal tumors based on number of mitoses, Fauchon et al.[13] reported a median survival time of 38 months and 16 months for adult and pediatric patients with the higher grade tumors (grades 3 and 4), respectively. Schild et al.[33] reported a 5-year survival rate of 58% for the 21 adult and pediatric patients with mixed, intermediate, or pure pineoblastomas. If the intermediate grade tumors are removed from Schild's or Fauchon's analysis, the survival rates remain poor.

III. Extent of resection

The most striking predictor of survival in our patient population was gross total resection. No patient in whom the surgeon was able to obtain gross total resection died during follow up ranging from 21.7 to 107.5 months (Fig. 3B). The importance of obtaining gross total resection has not been previously described for PNETs in any location or age distribution. No statistical difference between >90% resection versus <90% resection was found in children with pineoblastomas.[21] In children with cerebral PNETs, there was no correlation between extent of resection and survival in those children treated with radiation therapy.[35] However, gross total resection was associated with better local control.[35] Similarly, an adult series of medulloblastomas of the posterior fossa did not demonstrate an improvement in progression free survival with gross total resection, although complete resection was associated with a decreased rate of local recurrence.[14]

The extent of surgical resection may be influenced

by many factors: goal of surgery (biopsy, debulking, complete resection), surgeon's skill and experience, tumor invasion and involvement with adjacent structures, and physician beliefs about the effectiveness of alternative therapies (irradiation therapy, chemotherapy). While our data indicate a survival benefit from complete resection, it is unclear whether the surgeon's goal for every adult case of pineoblastoma should be the complete resection of tumor. Incomplete functional status data did not allow a comprehensive assessment of the impact of complete resection on quality of life. It is conceivable that more aggressive surgery may even reduce quality of life, resulting in a Pyrrhic victory for the surgeon and patient.

IV. Radiotherapy

We found that cranial irradiation therapy doses of at least 40 Gy were associated with improved survival in adults with pineoblastomas. Patients who received ≥ 40 Gy of cranial irradiation had a death rate 3.8 times less than that of patients who received less radiation. Median survival for the higher dose patients (29.8 months) was triple that of patients receiving lesser doses (8.1 months) (Fig. 3A).

In the pediatric pineoblastoma population, the CCG-921 report suggests that radiation therapy has a significant impact on survival with a 3-year survival of 61%.[21] However, the early delayed toxicity of radiation therapy in the pediatric population was devastating — all patients <9 years of age suffered severe neurocognitive deficits. Craniospinal radiation was "somewhat effective" as part of initial therapy in adults with pineoblastomas, but this study was not designed to evaluate the effects of radiation therapy.[4] Nevertheless, the findings in the pediatric populations have been extended to the adult population, and irradiation therapy is generally accepted as beneficial for adults with pineoblastomas. Our study supports this practice, and provides evidence for a threshold dose of >40 Gy for improved adult survival. A similar radiation dose threshold effect has been seen in children with PNETs. Several investigators have demonstrated that children with posterior fossa medulloblastomas treated with doses less than 50 Gy have poorer survival than those receiving greater than 50 Gy.[5,8,25,34] Adults with medulloblastomas of the posterior fossa show a trend towards improved survival with higher radiation doses, but no statistically significant benefit.

V. Sex

This is the first pineoblastoma series to suggest a possible survival difference between males and females. The multivariate model showed only a trend towards improved survival in women ($p = 0.099$), but the death rate in women was 3.0 times lower than that for men, and the difference in median survival was quite striking — 20.0 months in men versus >107.5 months in women (Fig. 3C). One other study of PNETs has shown a similar sex-related survival advantage. Male sex was correlated in the proportional hazards model with decreased survival ($p = 0.06$) studied 47 adults with medulloblastomas.[29] The observed survival advantage of adult females with pineoblastomas may be caused by endocrinological differences between adult females and males. This novel preliminary finding suggests that further investigations focusing on sex-specific differences in biochemistry may prove fruitful. For example, assays of hormone receptors in banked tumor samples may provide useful information, ultimately leading to hormonal strategies in the treatment of adult PNETs, including adult pineoblastomas.

VI. Age

Age was not a statistically significant predictor of survival for adults ≥ 16 years of age in our study. Nevertheless, children with pineoblastomas may have better outcomes than do adults. The 3-year progression-free survival was $61 \pm 13\%$ in a group of 17 children older than 18 months with pineoblastomas who were treated with craniospinal irradiation therapy and chemotherapy.[21] Thus, it appears that children (>18 months to 21 years of age) with pineoblastomas treated with irradiation therapy (≥ 45 Gy) and chemotherapy may fare better than do adults. Unfortunately, these two populations cannot be directly compared because of the many differences between the patient groups. The effect of age on survival may be better studied by analyzing a homogeneous group such as the BTRJ series of both adults and children.

VII. Staging

Medulloblastomas tend to metastasize through the CSF pathways, and the extent of disease dissemination through the craniospinal axis is accepted as an important prognostic factor.[14,29] Similarly, the presence of disseminated disease in patients with pineoblastomas may be a significant predictor of survival.[4] In our series of 34 patients, six patients (17.6%) were categorized as having spinal dissemination. The details and extent of staging of individual patients were not recorded in the BTRJ, and it is unclear whether every patient underwent spinal imaging and CSF cytology. For comparison, five of 11 adults with pineoblastoma had spinal dissemina-

tion.[4] Although the lack of systematic criteria for determining the presence of metastasis or dissemination suggests that our observed incidence of 17.6% is low, the incidence varies considerably within the pediatric literature on PNETs.[10,11,20,32]

Given the presumed similarity of medulloblastomas and pineoblastomas, it is advisable to stage patients with both magnetic resonance imaging and CSF cytology prior to operative resection. If magnetic resonance imaging is not available, computed tomography myelography is an appropriate substitute. Although rare reports of metastases of pineoblastomas to extraneural sites exist, the frequency is so low that further studies are not warranted.[24,30] Multivariate analysis did not find the presence of tumor dissemination to be a significant predictor of survival, but rigorous prospective staging protocols will be needed to determine the importance of staging in adults with pineoblastomas.

VIII. Functional status

Missing functional status data for 11 of the 34 patients precluded including this variable in the survival models. This is unfortunate, as functional status is a strong predictor of survival in many neurological diseases, e.g., ischemic stroke,[27] aneurysmal subarachnoid hemorrhage,[23] malignant gliomas,[36,37] and brain metastases.[1,16] Since functional status is also an important outcome measure, despite the limited number of patients with analyzable data, we searched for predictors of postoperative functional status in the subset of patients with pre- and postoperative PS data. Cranial irradiation therapy dose ≥ 40 Gy was the only factor that improved postoperative functional status as measured by the PS grade. Functional status can be used as a proxy for quality of life. The beneficial effect of irradiation therapy on PS suggests that irradiation therapy may improve both the quality of life and duration of survival.

IX. Chemotherapy

The most controversial decision in the treatment of patients with PNETs is the role of chemotherapy. Considering the biologic similarity of medulloblastomas and pineoblastomas, the effectiveness of chemotherapeutic agents in medulloblastomas has suggested a parallel role of these agents in the treatment of patients with pineoblastomas.[12,15,26] Current agents used for PNETs include cyclophosphamide, cisplatin, carboplatin, vincristine, ifosmide, and etoposide.[2,17] While our study did not show any impact of chemotherapy on survival, the power of our analysis was limited by the small number of patients who received chemotherapy (n = 10, 29.4%) and the many different regimens used in these patients. Future studies should be directed towards the determination of the best chemotherapy regimen.

X. Limitations

This retrospective study of a national centralized registry study has several limitations. The analysis was limited to variables and coding methods contained in the dataset. Some patients had missing values in some data fields, e.g., PS measurement of functional status. Nevertheless, the BTRJ is a unique resource that provides useful data for the study of rare tumors such as pineoblastomas.

XI. Conclusion

Pineoblastomas in adults are rare. A retrospective study of 34 patients identified from 30 years of BTRJ data demonstrates an overall median survival of 25.7 months. Patients with gross total resection and cranial radiation ≥ 40 Gy survive longer than do patients with subtotal resection or lower cranial radiation doses. Females had a trend towards longer survival than males. The role of chemotherapy remains unclear. Given the striking survival benefit of gross total surgical resection and higher radiation doses, the role of radiosurgery in the management of adult pineoblastomas remains to be assessed.

Acknowledgments

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Address reprint requests to: J. Y. K. Lee, M.D., Department of Neurosurgery, University of Pittsburgh, Suite B-400, 200 Lothrop Street, Pittsburgh, PA 15213, U.S.A.
e-mail: leejy2@upmc.edu

Commentary

Pineoblastoma is a malignant tumor that is considered a PNET and radiosensitive. It is rare in adults and therefore, the systematic study concerning the management and survival of the tumor is hindered. In order for us to understand the factors influencing survival, the authors analyzed in detail the data from the Brain Tumor Registry of Japan (BTRJ) to examine patients, tumors and treatment characteristics associated with increased survival in 34 adults with pineoblastomas diagnosed during the 30 years from 1969 to 1998. As the authors mentioned, it is the largest clinical series up to now in the literature.

They found that patients with gross total resection and cranial radiation ≥ 40 Gy survive longer than do patients with subtotal resection or lower cranial radiation doses. Women also had a trend towards longer survival than men (p = 0.099).

This research work is well done and the results have significant value to our clinical practice. As the authors mentioned, due to limitations of the study, some of the factors influencing survival still need to be clarified. If more cases could be involved with more complete data, or an international multi-centered study could be done, the factors influencing survival such as the importance of the tumor seeding through the CSF pathways, the chemotherapy regimen, and the role of radiosurgery in the management of adult pineoblastomas would become more clear.

Shengde BAO, *M.D.*
Department of Neurosurgery
Peking University First Hospital
Beijing, P.R.C.

This is an extremely valuable paper covering a rare tumor about which very little is known. The authors studied 34 adults with pineoblastoma and utilized the Brain Tumor Registry of Japan for initial and follow-up data. They found that the factors associated with improved prognosis for this very deadly tumor were gross total resection at the time of surgery and postoperative radiation therapy with a dose of 40 Gray or more. Unfortunately, only ten of the patients received chemotherapy and nine of these had combination chemotherapy. The data were not powerful enough to determine a significant beneficial effect of chemotherapy, primarily because of the short survivals; the overall follow-up periods were very short as well. The authors correctly recommend that a staging system similar to that utilized for medulloblastoma be utilized for pineoblastoma. They also indicate the importance of assessing the role for radiosurgery in the adjunctive management of these difficult lesions. This will be a widely quoted benchmark paper and the authors are to be congratulated for their careful and helpful review.

Edward R. LAWS, *Jr., M.D., F.A.C.S.*
Department of Neurosurgery
University of Virginia
Charlottesville, Virginia, U.S.A.

The authors described an analysis of 34 adult patients with pineoplastoma from the Brain Tumor Registry of Japan. Pineoblastomas, especially of adults, are extremely rare and the treatment strategy of this disease has not been established. They evaluated the effects of various factors on the survival rate of pineoblastoma patients and concluded that cranial irradiation therapy using over 40 Gy and gross total resection are

associated with improved patient's survival rate. This study provides useful suggestions for the treatment methods of adult pineoblastomas based on an analysis of a large number of cases of the disease.

The Brain Tumor Registry of Japan does not require a centralized review on pathological information and the pathological classification of pineal parenchymal tumor comprises only pineocytoma and pineoblastoma. It must be remembered that pineoblastomas of infants are mostly PNET, whereas pineoblastomas of adults may vary in histology showing mixed type, intermediate type or pure pineoblastoma and the prognoses of patients may vary depending on the histology.

Ryuichi TANAKA, M.D.
Department of Neurosurgery
Brain Research Institute
Niigata University
Niigata, Japan

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Intraventricular chordoid meningioma presenting with Castleman disease due to overproduction of interleukin-6

Case report

TORU ARIMA, M.D., ATSUSHI NATSUME, M.D., HISASHI HATANO, M.D., NORIMOTO NAKAHARA, M.D., MITSUGU FUJITA, M.D., DAI ISHII, M.D., TOSHIHIKO WAKABAYASHI, M.D., MANABU DOYU, M.D., TETSURO NAGASAKA, M.D., AND JUN YOSHIDA, M.D.

Departments of Neurosurgery and Neurology; Division of Pathology, Clinical Laboratory; and Center for Genetic and Regenerative Medicine, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

✓ A rare case of chordoid meningioma in the lateral ventricle observed in an adult is reported. The first clinical manifestation of the disease was a prolonged fever of unknown origin. Abnormalities in the patient's blood chemistry, principally polyclonal hypergammaglobulinemia (immunoglobulin [Ig]G, IgA, and markedly IgE) and an elevated serum level of C-reactive protein, were associated with the disease. The tumor was histologically confirmed to be a chordoid meningioma, and its surgical removal resulted in complete resolution of the patient's symptoms. By combining reverse transcription–polymerase chain reaction and immunohistochemical analysis, it may be shown that cytokine production, including that of interleukin (IL)-6, IL-1β, and vascular endothelial growth factor, plays a role in the pathogenesis of chordoid meningioma associated with Castleman syndrome.

KEY WORDS • **intraventricular tumor** • **chordoid meningioma** • **Castleman syndrome** • **interleukin-6** • **interleukin-1β** • **vascular endothelial growth factor**

THE primary occurrence of meningiomas in the ventricular system without dural attachment is extremely rare, with an incidence of 0.5 to 5% among all intracranial meningiomas.[6,23] Histopathologically, the fibroblastic (fibrous) subtype is predominant among intraventricular meningiomas, and both the meningothelial and transitional forms have also been encountered at this site.[1,7,9] Nevertheless, no case of intraventricular meningioma has been reported to date.

Chordoid meningioma is a rare meningioma variant named by Kepes and colleagues[12] in 1988, and has been listed in the latest classification of the World Health Organization. Several cases of this variant have been reported in the world literature. Chordoid meningioma is sometimes associated with Castleman syndrome,[3,12,14,16] which includes hematological abnormalities such as hypochromic or microcytic anemia and dysgammaglobulinemia with bone marrow plasmacytosis or other symptoms, but the pathogenesis of this disease is still unknown. Although some cytokines or tissue growth factors have been considered to play a critical role in the pathogenesis of Castleman syndrome,[8,21,26,27] the mechanism of this syndrome in conjunction with chordoid meningioma has not been elucidated. In this report we describe a rare case of intraventricular chordoid meningioma in which the patient presented with a prolonged fever and hypergammaglobulinemia, especially an elevated level of IgE.

Abbreviations used in this paper: CSF = cerebrospinal fluid; CT = computerized tomography; IFN = interferon; Ig = immunoglobulin; IL = interleukin; MR = magnetic resonance; mRNA = messenger RNA; PCR = polymerase chain reaction; RT = reverse transcription; Th1 = T helper cell Type 1; Th2 = T helper cell Type 2; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

Case Report

History. This 37-year-old woman had suffered from a remittent fever of unknown origin for a few months. Several examinations failed to detect the origin of the fever; however, MR imaging incidentally revealed a mass lesion in the anterior horn of the left lateral ventricle. The patient's medical and family histories were unremarkable, including the investigation of atopic or allergic inflammation.

Examination. On admission, the woman's body temperature was 38.2°C. Her neck did not display any meningeal signs and no lymph nodes were palpable in the anterior, posterior, and supraclavicular regions. There were no abnormalities in somatic or genital development and her menstruation cycle was uneventful. Hepatosplenomegaly was not detected in the abdomen by an echo scanner.

The neurological examination revealed no deficits, and

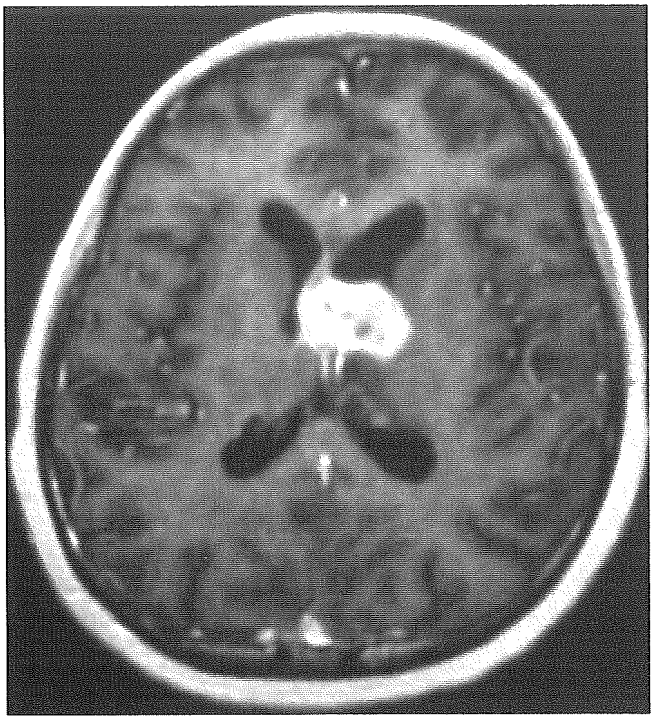

FIG. 1. Axial T_1-weighted Gd-enhanced MR image demonstrating a heterogeneously well-enhancing lesion approximately 2 cm in diameter, which is located in the anterior horn of the left lateral ventricle. The mass is attached to the colloid plexus at the foramen of Monro.

no evidence of a choked disc was found during the fundoscopic examination. Laboratory findings did not include anemia, leukocytosis, or thrombocytosis. Total plasma proteins were 7.5 g/dl (albumin, 3.5 g/dl; globulin, 4.2 g/dl), and an additional workup demonstrated that this represented a polyclonal gammopathy with high serum levels of Igs: IgG, 1740 mg/dl (normal range 870–1700 mg/dl); IgA, 579 mg/dl (normal range 110–410 mg/dl); and especially IgE, 3100 IU/ml, which was elevated approximately eightfold from the normal range. A high serum level of C-reactive protein (4.2 mg/dl) was also demonstrated in the hematological examination. Serological investigations for parasitic diseases, viruses, and an extensive search for an autoimmune disease were all nondiagnostic.

In the CSF study, elevated cell numbers (115 leukocytes, of which 88% were monocytes) and a high protein concentration, 1.16 g/L (normal range 0.15–0.45 g/L), with a normal glucose level were demonstrated. The CSF cultures were negative for virus, bacteria, and fungus, and the CSF cytology was negative for malignant cells.

Computerized tomography scans and MR images obtained at hospital admission displayed a 2-cm mass lesion located in the anterior horn of the left lateral ventricle, which enhanced homogeneously after injection of contrast medium. Sequential contrast-enhanced T_1-weighted MR images obtained 1.5 months after a previous examination demonstrated tumor growth with a central necrotic change, which was displayed heterogeneously (Fig. 1). Digital subtraction angiography did not demonstrate any marked staining of the tumor.

Operation. The patient underwent tumor resection via a left frontal craniotomy with the aid of a navigational system. The mass was found to be attached to the colloid plexus near the foramen of Monro. The tumor was rather soft and relatively vascularized. Gross-total resection was achieved by removing the lesion in a piecemeal fashion. Although temporary insertion of a ventricular drain was necessary for the control of hydrocephalus postoperatively, it was removed without any difficulty 1 week later.

Postoperative Course. After the operation, complete resolution of the prolonged fever and normalization of the C-reactive protein level were obtained within 1 week. Furthermore, all serum levels of γ-globulin, including IgE, had normalized by several months postoperatively. During the following year, all laboratory findings returned to normal and the patient experienced no fever. No recurrence of the tumor has been demonstrated on MR images.

Pathological Findings. Histopathological examination of the surgical specimen revealed that the tumor cells were arranged such that epithelial cells were in the background of a myxoid matrix with prominent infiltration of lymphoid and plasma cells (Fig. 2 *upper left*) into brain tissue around the tumor; some of the tumor cells appeared to be vacuolated. The tumor cells appeared spindly or multipolar, and formed clusters and rows in patterns that resembled a chordoma (Fig. 2 *upper center*). Furthermore, a meningothelial pattern was detected in a small portion of cells throughout the tumor (Fig. 2 *upper right*). Some nuclear pleomorphism and focal necrosis were noted. Mitoses were noted in the most active areas, but invasion of the brain parenchyma was absent. Immunohistochemically typical of meningiomas, the tumor cells exhibited membrane staining for epithelial membrane antigen in focal areas (Fig. 2 *center left*) as well as diffuse cytoplasmic staining for vimentin. Vascular endothelial growth factor was also strongly expressed in the tumor cells and in plasma cells (Fig. 2 *lower center*). None of the tumor cells expressed glial fibrillary acidic protein, cytokeratin, or S100 protein. The Ki-67 proliferative index of the tumor was 9.2%. A striking feature was the presence of several dense lymphoid and plasma cell infiltrates within the lesion. The B lymphocytes (CD20-positive and L26 positive cells) predominated within the infiltrates (Fig. 2 *lower right*), although T lymphocytes (CD3-positive cells) were also present (Fig. 2 *lower left*). The T lymphocytes were predominantly CD8-positive rather than CD4-positive T lymphocytes. A few CD56-positive natural killer cells and moderate infiltrates of plasma cells were also revealed within the tumor. Polyclonality of the plasma cells was confirmed by immunostaining for κ and λ Ig light chains. All these findings were consistent with the presence of a chordoid meningioma.

The RT-PCR Assay

Total RNA was isolated from fresh-frozen sections of the tumor by using an RNeasy Mini Kit (QIAGEN, Tokyo, Japan). Reverse transcription reactions were performed using 2 μg of total RNA and Superscript II Reverse Transcriptase (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's protocol. As a positive control for each sample, β-actin complementary DNA was also amplified. The PCR was performed using Taq DNA polymerase (Life Technologies, Inc., Tokyo, Japan). Samples were incubated at 94°C for 5 minutes, followed by 40 cycles at 94°C for 30 seconds, and