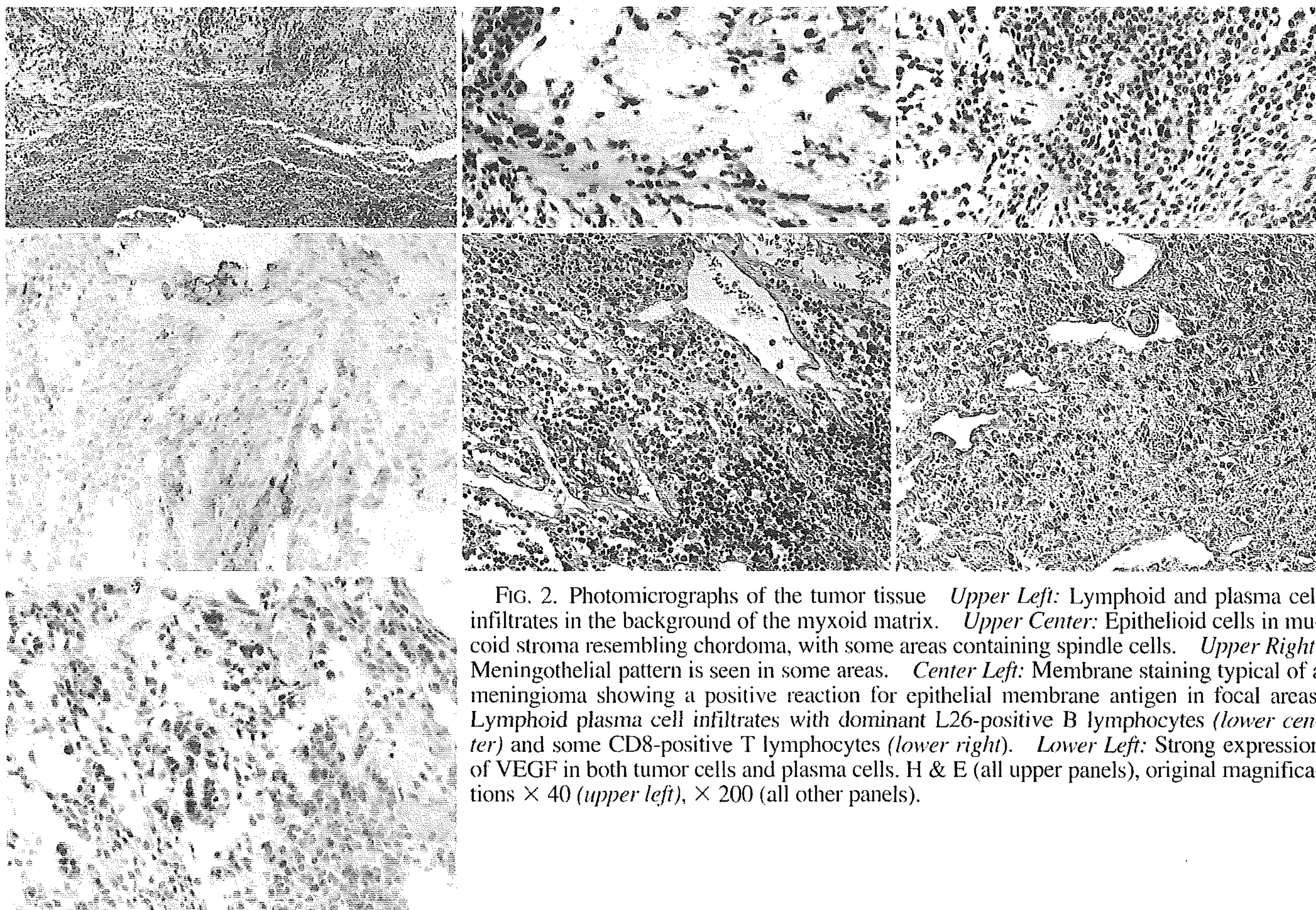

FIG. 2. Photomicrographs of the tumor tissue *Upper Left:* Lymphoid and plasma cell infiltrates in the background of the myxoid matrix. *Upper Center:* Epithelioid cells in mucoid stroma resembling chordoma, with some areas containing spindle cells. *Upper Right:* Meningothelial pattern is seen in some areas. *Center Left:* Membrane staining typical of a meningioma showing a positive reaction for epithelial membrane antigen in focal areas. Lymphoid plasma cell infiltrates with dominant L26-positive B lymphocytes *(lower center)* and some CD8-positive T lymphocytes *(lower right)*. *Lower Left:* Strong expression of VEGF in both tumor cells and plasma cells. H & E (all upper panels), original magnifications × 40 *(upper left)*, × 200 (all other panels).

72°C for 1 minute. A final extension was added at 72°C for 7 minutes. Primers for IL-1β, IL-4, IL-6, TNFα, IFNλ, and β-actin were purchased from Sigma Genomycs (Tokyo, Japan). The PCR products were electrophoresed on 2% agarose gels, visualized by ethidium bromide staining, and documented by photography. Overexpression of IL-1β mRNA and extreme overexpression of IL-6 mRNA were confirmed (Fig. 3). As a control study, an ordinary case of meningothelial meningioma with no systemic effects was also examined in the same manner; in that case there was no detected expression of cytokine mRNA, including IL-1β and IL-6, as in the present case.

Discussion

Chordoid meningioma is a rare subtype of meningioma, which is classified as World Health Organization Grade II and comprises approximately 0.5% or less of all meningiomas.[5,13] Since the first description of an intraventricular meningioma, given by Shaw[24] in 1854, 532 intraventricular meningiomas have been reported to date in the world literature.[19] Nevertheless, there have been no reported cases of chordoid meningioma in the ventricular system.

Interestingly, remittent fever was the only clinical symptom in the present case, and surgical removal of the mass resulted in complete remission. Fever is one of the most frequent clinical signs encountered in human diseases, especially during infections, but is uncommon in cases of brain tumor. The febrile response is thought to be mediated by endogenous mediators, generically called "endogenous pyrogens," which include TNFα, IL-1, IL-6, and the IFNs.[18,20,22] In our case, the RT-PCR assay confirmed the overproduction of proinflammatory cytokine mRNA, including that of IL-6 and IL-1β, within the mass, when compared with an ordinary case of meningothelial meningioma. In the clinical setting, cases of meningioma presenting with inflammatory syndrome are rarely encountered, although some investigators have revealed a certain level of cytokine production in meningiomas.[17] Thus, the combination of chronic overproduction of IL-6 and IL-1β might affect distinct, but partially overlapping, neuronal arrays in the preoptic area of the anterior hypothalamus, and therefore directly or indirectly mediate the host defense response including fever.

Since the first report of Castleman syndrome by Castleman and associates[2] in 1956, many subsequent reports of the same disease in different anatomical locations and possible causative mechanisms for this disease have been published. Interleukin-6, IL-1, and TNFβ are considered to be causative factors of Castleman syndrome,[8,26,27] but no existing evidence supports this hypothesis in the case of chordoid meningioma.

Interleukin-6 is a cytokine that is well known as one of the causative factors of the hypergammaglobulinemia associated with Castleman syndrome.[10] Interestingly, the hypergammaglobulinemia in our case consisted of IgG, IgA, and remarkably IgE, although in most reported cases IgG is pre-

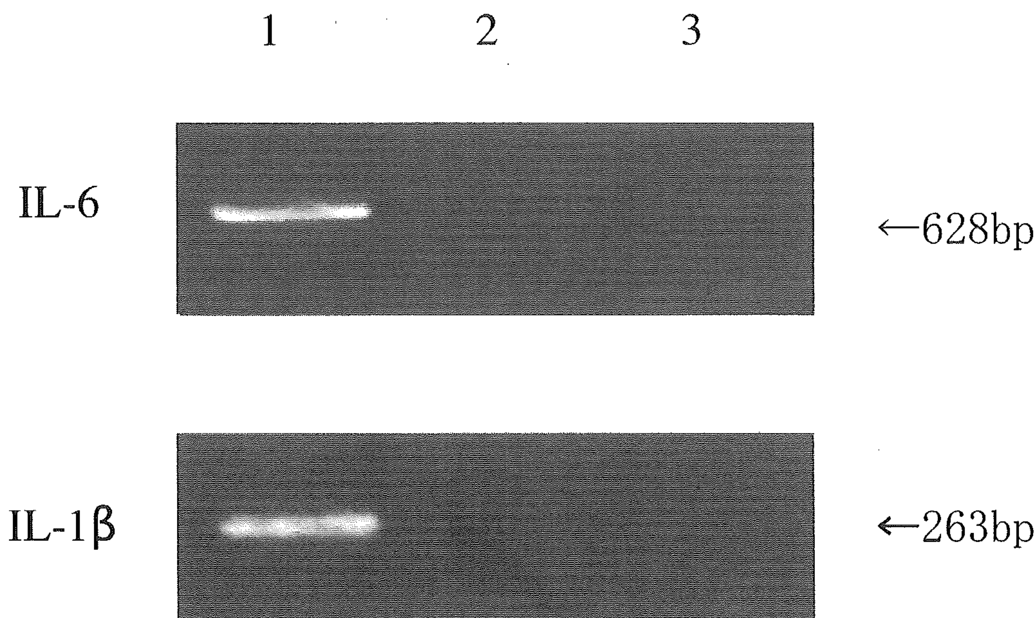

FIG. 3. Results of an RT-PCR assay showing high levels of IL-6 and IL-1β mRNA expression. The RT-PCR products were analyzed on a 2% agarose gel and detected using ethidium bromide staining. Lane 1 = RT positive; Lane 2 = RT negative; Lane 3 = water.

dominant. The IgE production requires cytokines, in particular IL-4, and some investigations have revealed an obligatory role of endogenous IL-6 in IL-4–dependent human IgE synthesis.[25] A balance between Th1 and Th2 is also considered to be critical in the regulation of IgE synthesis.[15] In this case, the overexpression of IL-6 mRNA as Th2 cytokine was confirmed compared with that of IFNλ as the Th1 cytokine, but IL-4 expression could not be demonstrated. It is not possible to draw a definitive conclusion about the dominant levels of IgE in this patient, and a cytokine class switch from Th1 to Th2 profiles, controlled by a variety of environmental or genetic factors, may be associated with an elevated level of IgE. Furthermore, IL-6 may act on B lymphocytes and accelerate the production of IgE as well as IgG.

More recently, VEGF has also been considered to be involved in the pathogenesis of this disease, especially in the marked vascular proliferation of lymph nodes in the interfollicular space.[21] Vascular endothelial growth factor is a specific mitogen for vascular endothelial cells; it therefore has a central role in angiogenesis and can also accelerate the permeability of the vessels.[11] Moreover, its expression is considered to be induced by IL-6.[4] The results of our cytokine study, performed using RT-PCR and immunohistochemical analysis for VEGF, indicate that VEGF-accelerated vasopermeability induced by IL-6 may play a role, at least in part, in lymphoplasmacyte infiltration, which is one of the histopathological characteristics of a chordoid meningioma, although the mechanism for dominancy of T- or B-lymphocytes within the tumor remains unclear.

Conclusions

Chordoid meningioma must be included in the preoperative differential diagnosis of central nervous system neoplasms featuring anemia, hypergammmaglobulinemia, fever of unknown origin, or other systemic illnesses. The clinical outcome of a chordoid meningioma featuring Castleman syndrome is favorable following surgical removal of the tumor, as reported previously. The pathogenesis of Castleman syndrome in the case of chordoid meningioma remains unclear, but a complex cytokine network including IL-6, IL-1β, and VEGF may contribute to the clinicopathological features of chordoid meningioma when associated with this disease.

Acknowledgment

We thank Dr. Y. Nakazato, Department of Pathology, Gunma University Graduate School of Medical Sciences (Maebashi, Japan) for help in the pathological diagnosis.

References

1. Caner H, Acikgoz B, Ozgen T, Colak A, Onol B: Meningiomas of the lateral ventricle. Report on six cases. **Neurosurg Rev 15:** 303–306, 1992
2. Castleman B, Iverson L, Menendez VP: Localized mediastinal lymphnode hyperplasia resembling thymoma. **Cancer 9:** 822–830, 1956
3. Civit T, Taillandier L, Baylac F: Chordoid meningioma. **J Neurosurg 89:**686–687, 1998
4. Cohen T, Nahari D, Cerem LW, Neufeld C, Levi BZ: Interleukin 6 induces the expression of vascular endothelial growth factor. **J Bio Chem 271:**736–741, 1996
5. Couce ME, Aker FV, Scheithauer BW: Chordoid meningioma: a clinicopathologic study of 42 cases. **Am J Surg Pathol 24:** 899–905, 2000
6. Cushing H, Eisenhardt L: **Meningiomas. Their Classification, Regional Behavior, Life History and Surgical End Results.** Springfield, IL: Thomas, 1938
7. Dunn J, Kernahan JW: Observation on the origin of meningioma from the choroid plexus of the lateral ventricle. **Mayo Clin Proc 31:**25–30, 1956

8. Gherardi RK, Bélec L, Fromont G, Divine M, Malapert D, Gaulard P, et al: Elevated levels of interleukin-1 β (IL-1 β) and IL-6 in serum and increased production of IL-1 β mRNA in lymph nodes of patients with polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes (POEMS) syndrome. **Blood 83:**2587–2593, 1994
9. Guidetti B, Delfini R, Gagliardi FM, Vagnozzi R: Meningiomas of the lateral ventricles. Clinical, neuroradiologic, and surgical considerations in 19 cases. **Surg Neurol 24:**364–370, 1985
10. Ishida F, Kitano K, Kobayashi H, Saito H, Kiyosawa K: Elevated IgG4 levels in a case with multicentric Castleman's disease. **Br J Haematol 99:**981–982, 1997
11. Kalkanis SN, Carroll RS, Zhang J, Zamani AA, Black PM: Correlation of vascular endothelial growth factor messenger RNA expression with peritumoral vasogenic cerebral edema in meningiomas. **J Neurosurg 85:**1095–1101, 1996
12. Kepes JJ, Chen WY, Connors MH, Vogel FS: "Chordoid" meningeal tumors in young individuals with peritumoral lymphoplasmacellular infiltrates causing manifestations of the Castleman syndrome. A report of seven cases. **Cancer 62:**391–406, 1988
13. Kleihues P, Burger PC, Scheithauer BW (eds): **Histological Typing of Tumors of Central Nervous Sysem.** New York: Springer-Verlag, 1993
14. Kobata H, Kondo A, Iwasaki K, Kusaka H, Ito H, Sawada S: Chordoid meningioma in a child. Case report. **J Neurosurg 88:** 319–323, 1998
15. Kurasawa K, Iwamoto I: [Mechanism and regulation of IgE production in allergy. **Nippon Rinsho 54:**434–439, 1996 (Jpn)
16. Lee DK, Kim DG, Choe G, Chi JG, Jung HW: Chordoid meningioma with polyclonal gammopathy. Case report. **J Neurosurg 94:** 122–126, 2001
17. Levy EI, Paino JE, Sarin PS, Goldstein AL, Caputy AJ, Wright DC, et al: Enzyme-linked immunosorbent assay quantification of cytokine concentrations in human meningiomas. **Neurosurgery 39:**823–829, 1996
18. Morimoto A, Sakata Y, Watanabe T, Murakami N: Characteristics of fever and acute-phase response induced in rabbits by IL-1 and TNF. **Am J Physiol 256:**R35-R41, 1989
19. Nakamura M, Roser F, Bundschuh O, Vorkapic P, Samii M: Intraventricular meningiomas; a review of 16 cases with reference to the literature. **Surg Neurol 59:**491–504, 2003
20. Netea MG, Kullberg BJ, Van Der Meer JW: Do only circulating pyrogenic cytokines act as mediators in the febrile response? A hypothesis. **Eur J Clin Invest 29:**351–356, 1999
21. Nishi J, Arimura K, Utsunomiya A, Yonezawa S, Kawakami K, Maeno N, et al: Expression of vascular endothelial growth factor in sera and lymph node of the plasma cell type of Castleman's disease. **Br J Haematol 104:**482–485, 1999
22. Rittierodt M, Tschernig T, Samii M, Walter GF, Stan AC: Evidence of recurrent atypical meningioma with rhabdoid transformation and expression of pyrogenic cytokines in a child presenting with a marked acute-phase response: case report and review of the literature. **J Neuroimmunol 120:**129–137, 2001
23. Rohringer M, Sutherland GR, Louw DF, Sima AA: Incidence and clinicopathological features of meningioma. **J Neurosurg 71:** 665–672, 1989
24. Shaw A: Fibrous tumor in the lateral ventricle of the brain, boney deposits in the arachnoid membrane of the right hemisphere. **Trans Path Soc Lond 5:**18–21, 1854
25. Vercelli D, Jabara HH, Arai K, Yokota K, Geha RS: Endogenous interleukin 6 plays an obligatory role in interleukin 4-dependent human IgE synthesis. **Eur J Immunol 19:**1419–1424, 1989
26. Winter SS, Howard TA, Ritchey AK, Keller FG, Ware RE: Elevated levels of tumor necrosis factor-beta, gamma-interferon, and IL-6 mRNA in Castleman's disease. **Med Pediatr Oncol 26:** 48–53, 1996
27. Yoshizaki K, Matsuda T, Nishimoto N, Kuritani T, Taeho L, Aozasa K, et al: Pathogenic significance of interleukin-6 (IL-6/BSF-2) in Castleman's disease. **Blood 74:**1360–1367, 1989

Manuscript received March 26, 2004.

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IFN-β Down-Regulates the Expression of DNA Repair Gene *MGMT* and Sensitizes Resistant Glioma Cells to Temozolomide

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Abstract

Alkylating agents, such as temozolomide, are among the most effective cytotoxic agents used for malignant gliomas, but responses remain very poor. The DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) plays an important role in cellular resistance to alkylating agents. IFN-β can act as a drug sensitizer, enhancing toxicity against a variety of neoplasias, and is widely used in combination with other antitumor agents such as nitrosoureas. Here, we show that IFN-β sensitizes glioma cells that harbor the unmethylated MGMT promoter and are resistant to temozolomide. By means of oligonucleotide microarray and RNA interference, we reveal that the sensitizing effect of IFN-β was possibly due to attenuation of MGMT expression via induction of the protein p53. Our study suggests that clinical efficacy of temozolomide might be improved by combination with IFN-β using appropriate doses and schedules of administration. (Cancer Res 2005; 65(17): 7573-9)

Introduction

Gliomas are the most common primary tumors of the central nervous system; they account for 30% of adult primary brain tumors. The prognosis for patients with the advanced glioma, glioblastoma multiforme, is very poor; the mean survival period is 8 to 10 months (1). Alkylating agents are among the most effective cytotoxic agents used for treating malignant gliomas, including glioblastoma multiforme, but responses remain very poor. The most frequent site of alkylation in DNA is the O^6 position of guanine, which forms cross-links between adjacent strands of DNA, leading to cell death. A cellular DNA-repair protein, namely O^6-methylguanine-DNA methyltransferase (MGMT) protein, reverses alkylation at the O^6 position of guanine, thereby inhibiting the lethal cross-linking and bringing about resistance to alkylating agents (2, 3). A number of studies have suggested that MGMT deficiency is closely related to the sensitivity of brain tumors to alkylating agents (4–6). Furthermore, because MGMT protein loss may be a result of promoter hypermethylation, it was reported that methylation of the MGMT promoter in gliomas is a useful predictor of the responsiveness to alkylating agents (7). Temozolomide is a novel alkylating agent that has been currently approved for use in treatment of anaplastic astrocytoma in Europe and the United States. A phase II clinical trial has been organized in Japan. This drug is of significance because it can be administered orally; it readily crosses the blood-brain barrier and has minimal side effects (8). Before considering the treatment of malignant gliomas with temozolomide, a major obstacle may be the resistance pathway that occurs due to the actions of MGMT. Hence, efficient attenuation of the function of MGMT, which is expressed in ~70% of gliomas (9), either by direct interaction with protein or by indirect means such as the transcriptional control, is required. It could be advantageous if MGMT depletion can be accomplished by a drug that also has antitumor activity, and, therefore, synergistic effects with the alkylating agent may occur. Type I IFNs, including IFN-α and IFN-β, a family of cytokines that elicit pleiotropic biological effects, are widely used either alone or in combination with other antitumor agents such as nitrosoureas in the treatment of malignant gliomas. Among the multiple functions of type I IFNs against human neoplasias, type I IFNs, particularly IFN-β, can act as a drug sensitizer enhancing toxicity against a variety of neoplasias when given in combination with nitrosoureas (10). Therefore, it is of interest to examine whether IFN-β can enhance chemosensitivity of malignant gliomas against temozolomide, the new alkylkating agent; to evaluate the mechanism; and to provide an experimental basis for the rational clinical use of such combinations.

Materials and Methods

Cell lines and reagents. Human glioma cell lines (T98, AO2, SKMG1, U251nu/nu, U251SP, and U251MG) derived from the Memorial Sloan-Kettering Cancer Institute (New York, NY) were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air in Eagle's medium (Nissui, Tokyo, Japan). Medium was supplemented with 10% fetal bovine serum (FBS), 5 mmol/L L-glutamine, 2 mmol/L nonessential amino acids, and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin). Human IFN-β and temozolomide were kindly supplied by Toray, Co., Ltd. (Kamakura, Japan), and the Schering-Plough Research Institute (Kenilworth, NJ), respectively. Temozolomide readily discomposes in aqueous solution into DMSO.

RNA interference experiments. The target sequence in p53 and the control nonsilencing sequence are CGGCAUGAACCGGAGGCCCAU and AATTCTCCGAACGTGTCACGT, respectively. These synthetic sense and antisense oligonucleotides were obtained from Qiagen (Hilden, Germany). For the annealing of small interfering RNA (siRNA) oligonucleotides, sense and antisense oligonucleotides were incubated in siRNA Suspension Buffer (Qiagen) for 1 minute at 90°C, followed by 60 minutes at 37°C. siRNA oligonucleotides were mixed with Oligofectamine reagent (Invitrogen, Carlsbad, CA) in Opti-MEM (Life Technologies, Gaithersburg, MD). Cultured cells were washed with medium without serum and added to the siRNA-Oligofectamine mixture, of which the final concentration was 200 nmol/L. Medium with 10% FBS was added 4 hours later.

Determination of cell growth. To compare chemosensitivity of glioma cell lines to temozolomide, the agent (final concentration of 0-1,000 μmol/L) was added to the culture medium at 24 hours after aliquots of 2×10^4 cells/well were placed in triplicate wells. Incubation was continued for 72 hours, and the number of viable cells was counted by trypan blue exclusion method. The number was expressed as a percentage of untreated control. The statistical significance of difference was determined by ANOVA using

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doi:10.1158/0008-5472.CAN-05-0036

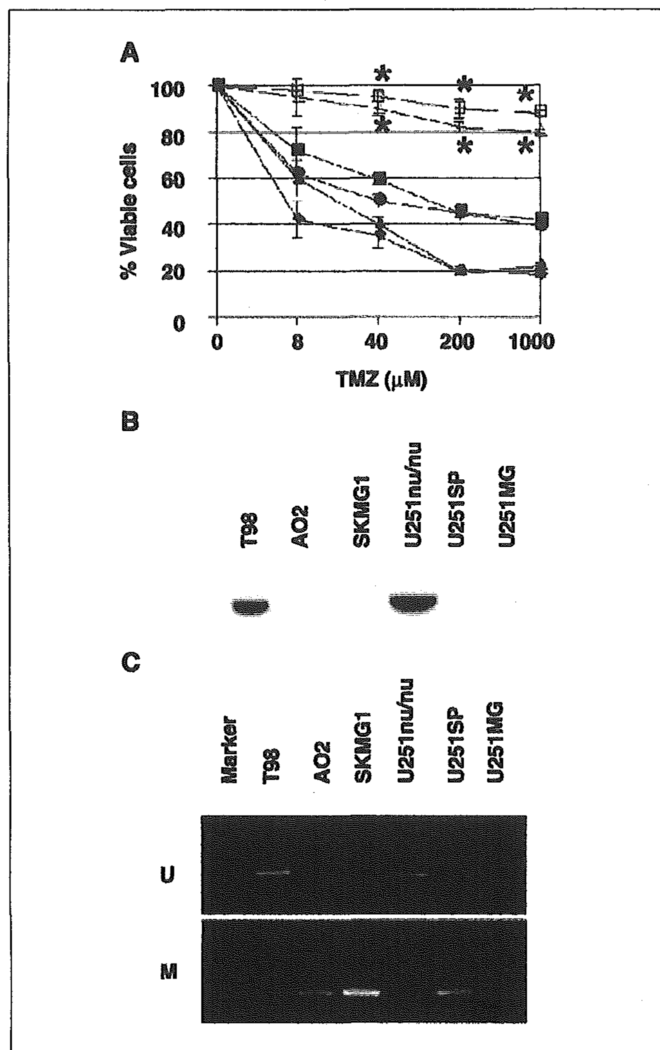

Figure 1. Comparisons among human glioma cell lines. *A*, the antitumor effects of temozolomide against six human glioma cell lines: T98 (□), AO2 (▲), SKMG1 (●), U251nu/nu (△), U251SP (■), and U251MG (♦). At 72 hours after temozolomide (final concentration of 0-1,000 μmol/L) was added to the culture medium, the number of viable cells was counted. The number was expressed as a percentage of untreated control. *$P < 0.05$ versus AO2, SKMG1, U251SP, and U251MG at each dose. *B*, Western blot analysis of MGMT in human glioma cell lines. The cell lysate was subjected to Western blotting with anti-MGMT antibody. *C*, methylation-specific PCR analysis of human glioma cell lines. *U* and *M*, reactions for unmethylated and methylated sequences, respectively.

Bonferroni's correction for the multiple comparisons used. For sensitizing assay of IFN-β to temozolomide, IFN-β (100 IU/mL) and temozolomide (100 μmol/L) were added at 24 and 48 hours after cell inoculation, respectively. The number of viable cells was counted at 96 hours. To examine the effect of siRNA for p53 on the growth of T98 cells treated with IFN-β and temozolomide, either siRNA for p53 or nonsilencing siRNA was transfected as described above at 24 hours after aliquots of 4×10^4 cells/well were placed in triplicate wells. IFN-β (100 IU/mL) and temozolomide (100 μmol/L) were added at 28 and 48 hours, respectively. The number of viable cells was counted at 96 hours.

Western blot analysis. Cell lysis and immunoblotting were carried out as described (11). Antibodies against the following proteins were purchased: p53 (DO-1: Santa Cruz Biotechnology, Santa Cruz, CA), p21 (EA10: Oncogene Research Products, San Diego CA), MGMT (MT3.1: Neomarkers, Fremont, CA), and β-actin (AC-15: Sigma-Aldrich, St. Louis, MO). Band intensities were quantified by densitometric scanning using the NIH IMAGE program.

Genomic DNA extraction and methylation-specific PCR. Genomic DNA was extracted using the QIAmp DNA Mini kit (Qiagen) following the manufacturer's instructions. DNA methylation patterns in the promoter region of the *MGMT* gene (Genbank accession no. X_61657) were determined by methylation-specific PCR as previously described (12). Primers for either methylated or unmethylated alleles were 5'-TTTCGACGTT-CGTAGGTTTTCGC-3' (sense) and 5'-GCACTCTTCCGAAAACGAAACG-3' (antisense) or 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' (sense) and

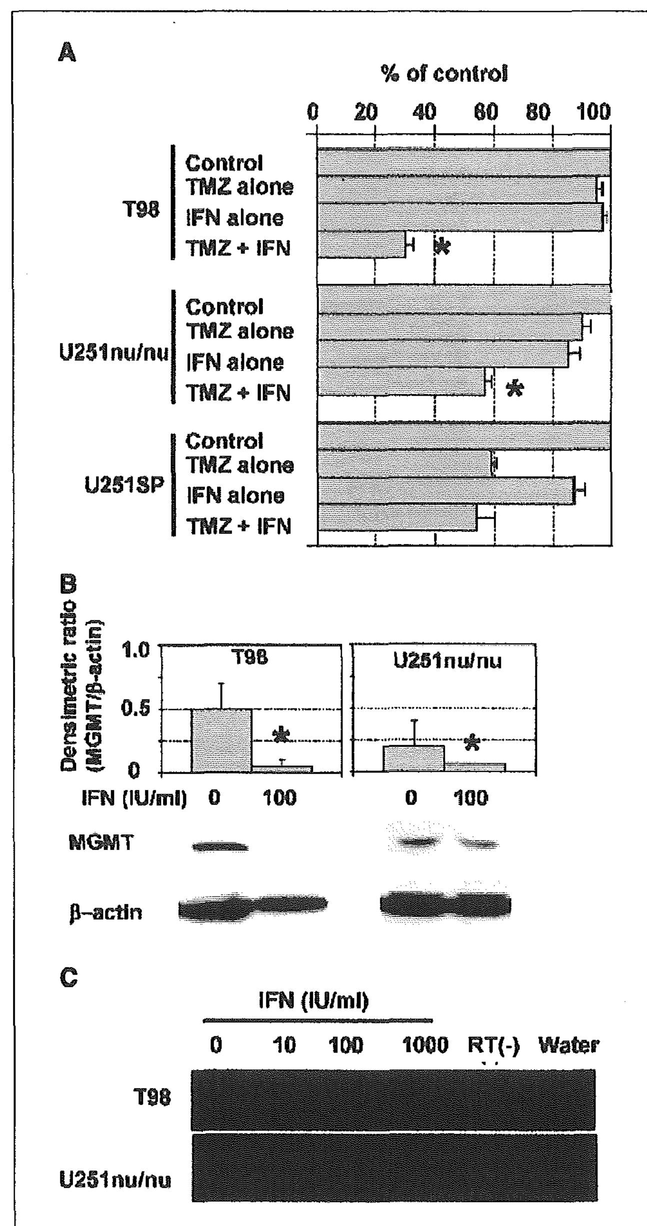

Figure 2. IFN-β sensitizes T98 and U251nu/nu glioma cells to temozolomide and down-regulated MGMT expression. *A*, IFN-β sensitizes the resistant T98 and U251nu/nu cell lines, but not the sensitive U251SP cell line, to temozolomide. The cells were incubated in a culture medium containing IFN-β (100 IU/mL) for 24 hours before the addition of temozolomide (final concentration of 100 μmol/L). Seventy-two hours after, the number of viable cells was counted and expressed as a percentage of untreated control (*$P < 0.05$). *B*, Western blot analysis of MGMT in T98 and U251nu/nu cells treated with IFN-β. Western blotting was done as in Fig. 1*B*. The histogram shows the amount of MGMT relative to that of β-actin. *Columns*, mean from three independent experiments; *bars*, SD (*$P < 0.05$). *C*, RT-PCR analysis for MGMT mRNA. No reverse-transcription control (*RT*−) is also shown.

Table 1.

A. Increase in gene expression in T98 cells by IFN-β

Symbol	Genes	Genbank accession no.	Mean
RBP2	*Retinoblastoma-binding protein 2*	S66431.1	2.012
PKB	*Protein kinase B*	X61037.1	2.024
FGF-9	*Fibroblast growth factor 9*	D14838.1	2.035
Grb14	*Grb14*	L76687.1	2.035
VCAM-1	*Vascular cell adhesion molecule 1 (VCAM-1)*	X53051.1	2.035
IL18	*Interleukin 18 (IFN-γ-inducing factor)*	NM_001562.2	2.047
HOX-11	*Homeobox protein (HOX-11)*	M75952.1	2.055
bcl-w	*Gi|1572492|gb|U59747.1|HSU59747 Human Bcl-w (bcl-w) mRNA, complete cds*	U59747.1	2.074
IGFBP-5	*Insulin-like growth factor binding protein 5*	M62782.1	2.081
Clk2	*Clk2*	L29216.1	2.098
NIK	*Serine/threonine protein kinase*	Y10256.1	2.106
p68K	*p68 kinase*	M35663.1	2.112
IGF-II	*Human insulin-like growth factor II*	M29645.1	2.121
GALNAC4S-6ST	*B-cell RAG-associated protein*	NM_014863.1	2.128
FGF-10	*FGF-10*	AB002097.1	2.129
FRA-2	*Human fra-2*	X16706.1	2.134
FASL	*Fas ligand*	D38122.1	2.146
PDGF	*Platelet-derived growth factor A-chain*	A09204.1	2.158
E1A-F	*E1A-F*	D12765.1	2.163
c-myc	*c-myc*	D89667.1	2.167
hTRIP	*hTRIP (hTRIP)*	U77845.1	2.185
CALM2	*Calmodulin 2 (phosphorylase kinase, δ)*	NM_001743.3	2.207
PUMP-1	*PUMP-1 gene encoding PUMP*	Z11887.1	2.207
Bcl2	*Bcl2, p53-binding protein Bbp/53BP2 (BBP/53BP2)*	U58334.1	2.207
MKK6	*MAP kinase kinase 6*	U39657.1	2.218
JNK3A1	*NK3 α1 protein kinase*	U34820.1	2.242
pS2	*pS2 mRNA induced by estrogen from human breast cancer cell line MCF-7*	X00474.1	2.273
ICAM-2	*ICAM-2, cell adhesion ligand for LFA-1*	X15606.1	2.276
CREM	*Cyclic AMP-responsive element modulator (CREM)*	S68271.1	2.289
Humig	*Humig*	X72755.1	2.289
NFAT1	*Transcription factor NFAT1 isoform C (NFAT1)*	U43342.1	2.289
SFPQ	*Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated; SFPQ)*	NM_005066.1	2.302
N-CoR	*Nuclear receptor corepressor*	AF044209.1	2.333
IGFBP3	*Growth factor-binding protein-3 precursor (IGFBP3)*	M35878.1	2.359
IRF-1	*IFN regulatory factor 1*	X14454.1	2.4
IL-11R	*Interleukin-11 receptor*	Z38102.1	2.417
hsp70	*Heat shock protein 70 (hsp70)*	L12723.1	2.424
bcl-3	*B-cell lymphoma 3-encoded protein (bcl-3)*	M31732.1	2.441
FAS/Apo 1	*FAS/Apo 1 mRNA for FAS soluble protein (clone FAS Exo4Del)*	Z70519.1	2.471
HSP75	*Homo sapiens mitochondrial HSP75*	L15189.1	2.481
ICE-LAP6	*Cysteine protease ICE-LAP6*	U56390.1	2.485
G0S8	*Helix-loop-helix basic phosphoprotein*	L13463.1	2.487
HEK2	*HEK2 mRNA for protein tyrosine kinase receptor*	X75208.1	2.493
ERK2	*40 kDa protein kinase related to rat ERK2*	Z11695.1	2.503
KIAA0347	*KIAA0347*	AB002345.2	2.504
APAF1	*Apoptotic protease-activating factor (APAF1), transcript variant 2*	NM_001160.2	2.513
TRA1	*Tumor rejection antigen (gp96) 1*	NM_003299.1	2.574
TNF	*Tumor necrosis factor superfamily member LIGHT*	AF036581.1	2.582
FGF-5	*Fibroblast growth factor-5 (FGF-5)*	M37825.1	2.616
PPAR	*Peroxisome proliferator-activated receptor*	L02932.1	2.616
TIPM3	*Tissue inhibitor of metalloproteinases-3*	U14394.1	2.628
Mch3	*Mch3 isoform α(Mch3)*	U37448.1	2.698
PTPRZ	*Protein tyrosine phosphatase ζ-polypeptide (PTPRZ)*	M93426.1	2.698
RPGR	*Retinitis pigmentosa GTPase regulator (RPGR)*	NM_000328.1	2.741
ENO3	*Enolase 3, (β, muscle; ENO3), transcript variant 1*	NM_001976.2	2.754

(*Continued on the following page*)

Table 1. (Cont'd)

A. Increase in gene expression in T98 cells by IFN-β

Symbol	Genes	Genbank accession no.	Mean
NFKB1	*Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105; NFKB1)*	NM_003998.2	2.761
HRK	*Activator of apoptosis Hrk (HRK)*	U76376.1	2.774
IFITM3	*IFN-induced transmembrane protein 3 (1-8U; IFITM3)*	NM_021034.1	2.882
IL15RA	*Interleukin-15 receptor αchain precursor (IL15RA)*	U31628.1	2.918
AMPD2	*AMP deaminase 2 (isoform L; AMPD2)*	NM_004037.5	2.989
DNAJA1	*DnaJ (Hsp40) homologue, subfamily A, member 1*	NM_001539.1	3.184
HLA-C	*MHC class I HLA-C allele HLA-4*	M11886.1	3.265
IFN-γ	*IFN-γ*	X13274.1	3.27
p53	*p53 cellular tumor antigen*	M14694.1	3.301
HLA-B	*MHC, class I*	NM_005514.4	3.374
CHED	*cdc2-related protein kinase*	M80629.1	3.686
GADD45	*Gadd45*	S40706.1	3.694
BPAG1	*Bullous pemphigoid antigen 1, 230/240kDa (BPAG1), transcript variant 1e, mRNA*	NM_001723.3	3.793
ISGF-3	*Transcription factor ISGF-3*	M97936.1	3.938
TGFR	*Transforming growth factor-βtype III receptor*	L07594.1	4.341
MC4R	*Melanocortin 4 receptor*	NM_005912.1	10.821

B. Decrease in gene expression in T98 cells by IFN-β

Symbol	Genes	Genbank accession no.	Mean
DR3	*Death receptor 3 (DR3)*	U72763.1	0.5
GST-pi-1	*Anionic glutathione-S-transferase (GST-pi-1)*	X15480.1	0.5
TSHR	*Thyroid stimulatory hormone receptor*	M32215.1	0.5
PRDX6	*Peroxiredoxin 6*	NM_004905.2	0.499
IGFBP6	*Insulin-like growth factor binding protein 6*	M62402.1	0.499
HRR-1	*Farnesol receptor*	U68233.1	0.498
IL8RBA	*Interleukin-8 receptor type A*	U11870.1	0.497
RPS2	*Ribosomal protein S2*	NM_002952.2	0.493
COX6C	*Cytochrome coxidase subunit Vic*	NM_004374.2	0.49
HINT1	*Histidine triad nucleotide-binding protein 1*	NM_005340.2	0.489
E2F-1	*pRB-binding protein*	M96577.1	0.483
SKP1A	*S-phase kinase–associated protein 1A (p19A; SKP1A), transcript variant 1*	NM_006930.2	0.483
HS1	*HS1 protein*	X57347.1	0.478
PPP2CA	*Protein phosphatase 2 (formerly 2A), catalytic subunit, αisoform*	NM_002715.1	0.478
G3PD	*Glyceraldehyde-3-phosphate dehydrogenase*	X01677.1	0.473
VitDR	*Vitamin D receptor*	J03258.1	0.472
SEC61B	*Sec61 βsubunit*	NM_006808.2	0.47
SHC	*SHC*	X68148.1	0.47
COX6A1	*Cytochrome coxidase subunit VIa polypeptide 1*	NM_004373.2	0.46
PIN1	*Peptidyl-prolyl isomerase and essential mitotic regulator*	U49070.1	0.457
IL5Rα	*Interleukin 5 receptor α*	M75914.1	0.443
RAB	*Cellular cofactor*	L42025.1	0.435
CD6	*T-cell glycoprotein CD6*	X60992.1	0.434
NDUFB3	*NADH dehydrogenase (ubiquinone) 1 βsubcomplex, 3, 12 kDa*	NM_002491.1	0.433
TXN	*Thioredoxin*	NM_003329.1	0.431
COX4I1	*Cytochrome coxidase subunit IV isoform 1*	NM_001861.2	0.426
SMRT	*Silencing mediator of retinoid and thyroid hormone action*	U37146.1	0.423
ERK3	*ERK3*	X80692.1	0.422
TCRB	*T-cell receptor βchain*	L07294.1	0.421
LGALS1	*Lectin, galactoside-binding, soluble, 1 (galectin 1)*	NM_002305.2	0.417
SOD1	*Superoxide dismutase 1*	NM_000454.2	0.414
ROX	*ROX protein*	X96401.1	0.41
BAG1	*BCL2-associated athanogene*	NM_004323.2	0.409
DC50	*Hypothetical protein DC50*	NM_031210.3	0.407
FTH1	*Ferritin, heavy polypeptide 1*	NM_002032.1	0.398
XRCC5	*X-ray repair complementing defective repair in Chinese hamster cells 5*	NM_021141.2	0.387

(*Continued on the following page*)

Table 1. (Cont'd)

B. Decrease in gene expression in T98 cells by IFN-β

Symbol	Genes	Genbank accession no.	Mean
GSTO1	*Glutathione S-transferase ω1*	NM_004832.1	0.384
TPM2	*Tropomyosin 2 (β)*	NM_003289.1	0.377
CD27L	*CD27 ligand*	L08096.1	0.374
PRDX1	*Peroxiredoxin 1*	NM_002574.2	0.372
MAD-3	*MAD-3 mRNA encoding IkB-like activity*	M69043.1	0.369
CRAF1	*CD40 receptor associated factor 1*	U21092.1	0.365
KIP2	*Cdk-inhibitor p57KIP2*	U22398.1	0.362
RPLP1	*Ribosomal protein, large, P1*	NM_001003.2	0.356
PIG3	*Pig3*	AF010309.1	0.341
GAS	*Growth-arrest-specific protein*	L13720.1	0.33
TMSB10	*Thymosin, β10 (TMSB10)*	NM_021103.2	0.322
ME491	*Melanoma-associated antigen ME491*	X07982.1	0.313
ANXA2	*Annexin A2*	NM_004039.1	0.29
CollagenaseV	*Collagenase type IV*	J03210.1	0.29
S100A2	*S100 calcium-binding protein A2*	NM_005978.2	0.281
calpain	*Calcium-activated neutral protease large subunit*	X04366.1	0.262
FTL	*Ferritin, light polypeptide*	NM_000146.2	0.239

NOTE: p53-related genes are in bold. The mean was based on three replicate arrays analyzed statistically as described in the text.

5′-AACTCCACACACTCTTCCAAAAACAAAACA-3′ (antisense). All PCRs were done with positive controls for methylated alleles and no DNA control. Human placental DNA was treated *in vitro* with excess SssI methyltransferase (New England Biolabs, Beverly, MA), generating DNA completely methylated at CpG sites, served as the positive control for methylated MGMT, and then the PCR conditions were determined. Each PCR product was loaded onto a 3% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Reverse transcriptase-PCR. To investigate MGMT mRNA expression, reverse transcription-PCR (RT-PCR) was done using the Superscript first-strand synthesis system for RT-PCR (Invitrogen) as previously described (13). β-actin–specific PCR products from the same RNA samples were amplified and served as internal controls.

Microarray analysis. T98 cells were incubated with 100 IU/mL of IFN-β for 48 hours, and RNA was isolated. Standard Trizol preparation protocol (Invitrogen) and reagents were used for total RNA isolation. RNA amplification and labeling were done using the Amino Allyl MessageAmp aRNA kit (Ambion, Austin, TX). Briefly, after reverse transcription reactions (2 μg total RNA/sample) were preformed, double-stranded cDNA was transcribed *in vitro* into amino allyl cRNA. The purified and concentrated cRNA (5 μg) was coupled with either Cy3 or Cy5 dyes (Amersham Biosciences). The dye-labeled aRNA was purified from uncoupled dye using Micro Bio-Spin P-30 Tris chromatography columns (Bio-Rad, Hercules, CA) and MicroconYM-30 centrifugal filter devices (Millipore, Billerica, MA). The cRNA was fragmented in fragmentation buffer [40 mmol/L Tris-acetate (pH 8.1), 100 mmol/L potassium acetate, 30 mmol/L magnesium acetate] at 94°C for 15 minutes and purified with Microcon YM-10 (Millipore). Microarrays were preblocked with 1% bovine serum albumin solution. Fragmented cRNA was added to microarrays in hybridization solution and hybridized at 42°C for 16 hours. After this, arrays were washed, scanned at 10 μm pixel size, gridded, and analyzed (GenePix 4000B; Axon Instruments, Union City, CA). Background was subtracted, and the median sum and median ration were calculated. Flagged spots and spots with sum intensity (CH1, CH2) <100 absorbance units were excluded. Data were normalized by trimmed mean at 10% to account for differences in the amounts of RNA labeled or labeling efficiencies.

Chromatin immunoprecipitation assay. The chromatin immunoprecipitation assay was done according to the protocol of the manufacturer (Upstate Biotechnology, Lake Placid, NY) with a slight modification. The specific antibodies used for immunoprecipitations were the anti-p53 antibody and a control antibody. After protein-DNA cross-links in the immunoprecipitates were reversed, the purified DNA was analyzed by PCR (35 cycles; 45 seconds at 95°C, 45 seconds at 55°C, 60 seconds at 72°C) with primers that detect the MGMT promoter sequence [5′-GCTCCAGGGAA-GAGTGTCCTCTGC-TCCCT-3′ (sense) and 5′-GGCCTGTGGTGGGCGAT-GCCGTCCAG-3′ (antisense)] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter (provided with the kit). The PCR products were visualized on an ethidium bromide gel.

Results and Discussion

Chemosensitivity to temozolomide, MGMT expression, and MGMT promoter hypermethylation of human glioma cell lines. First, we compared chemosensitivity of six human glioma cell lines to temozolomide; T98 and U251nu/nu cell lines were significantly resistant, whereas the other four (AO2, SKMG1, U251SP, and U251MG) showed similar degrees of sensitivity in a dose-dependent manner (Fig. 1*A*). MGMT expression was not detected by Western blot in the sensitive cell lines (Fig. 1*B*), and the hypermethylation of MGMT promoter was as assessed by methylation-specific PCR (Fig. 1*C*), but this was not observed in the T98 and U251nu/nu cell lines. Thus, the results showed that hypermethylation of MGMT promoter could prevent expression of this gene and be associated with chemosensitivity of glioma cells to temozolomide, which was consistent with the results of reported studies (7). Because ~70% of gliomas express MGMT (9), it is essential to suppress the expression or function of MGMT in an efficient manner if treatment with the aid of temozolomide is to be considered.

IFN-β sensitizes resistant glioma cells to temozolomide and down-regulates MGMT expression. IFNs are a family of cytokines

that possess pleiotropic biological effects mediated by a number of responsive genes. IFNs were the first human proteins to be recognized as being effective in cancer therapy (14). Although identified and named for their action to interfere with viral replication, IFNs have immunomodulatory, cell differentiative, antiangiogenic, and antiproliferative effects (15, 16). In the previous studies, we showed that IFN-β has multiple functions relevant to antitumor activity: (*a*) cytostatic effect on glioma cells, (*b*) supportive action on the differentiation of CTLs and augmentation of their antitumor immune responses, and (*c*) behavior as a drug or radiation sensitizer enhancing toxicity against gliomas (10).

To examine whether IFN-β sensitizes T98 and U251nu/nu cell lines that are resistant to temozolomide, the cells were incubated in a culture medium containing IFN-β (100 IU/mL) for 24 hours before the addition of temozolomide (final concentration of 100 μmol/L). As shown in Fig. 2*A*, whereas IFN-β or temozolomide alone did not suppress cell growth of both T98 and U251nu/nu cells significantly, a combination of IFN-β and temozolomide markedly inhibits the growth. On the other hand, U251SP cell lines that are sensitive to temozolomide, but are resistant to IFN-β did not show the synergistic cytotoxic effect of temozolomide and IFN-β. Consequently, we hypothesized that IFN-β might enhance chemosensitivity due to reduction of MGMT expression and did Western blotting and RT-PCR for MGMT (Fig. 2*B* and *C*). It was shown that IFN-β decreased both MGMT protein and mRNA levels of both T98 and U251nu/nu cells at 48 hours after treatment. Thus, IFN-β decreases MGMT transcription directly or indirectly and sensitizes resistant glioma cells to temozolomide.

Microarray analysis of IFN-β–regulated genes. Because limited information regarding the transcriptional regulation of IFN-β is available, we introduced microarray technology that enables the simultaneous examination of expression of a large number of genes in an experimental condition. This microarray contains 1,300 functionally well-characterized genes involved in various important cellular processes, including the IFN-related pathway, apoptosis, cell cycle, transcription, and immunology. The microarray experiments were repeated thrice about the T98 cell lines that showed the most drastic change of MGMT expression after IFN-β treatment. Data analysis identified 71 significantly induced genes and 54 repressed genes in several different categories in T98 cells treated with IFN-β compared with the parental cells. The representative results of these comparisons are reported in Table 1. Some of the alterations in gene expression following IFN-β treatment confirm the involvement of pathways known to be active in this process, and this was anticipated from prior evidence. Consistent with a report that the *p53* gene is transcriptionally induced by IFN-β through ISGF3 activation (11), our microarray data showed that the *p53* gene and its downstream genes, including *bcl-2*, *FAS/Apo1*, and *Gadd45*, are up-regulated in glioma cells treated with IFN-β, suggesting that *p53* induction may be a key involved in antitumor action of IFN-β. Thus, we focused on IFN-β–mediated *p53* induction. By Western blot, we confirmed that *p53* expression level in both T98 cells treated with IFN-β was markedly higher than that in the

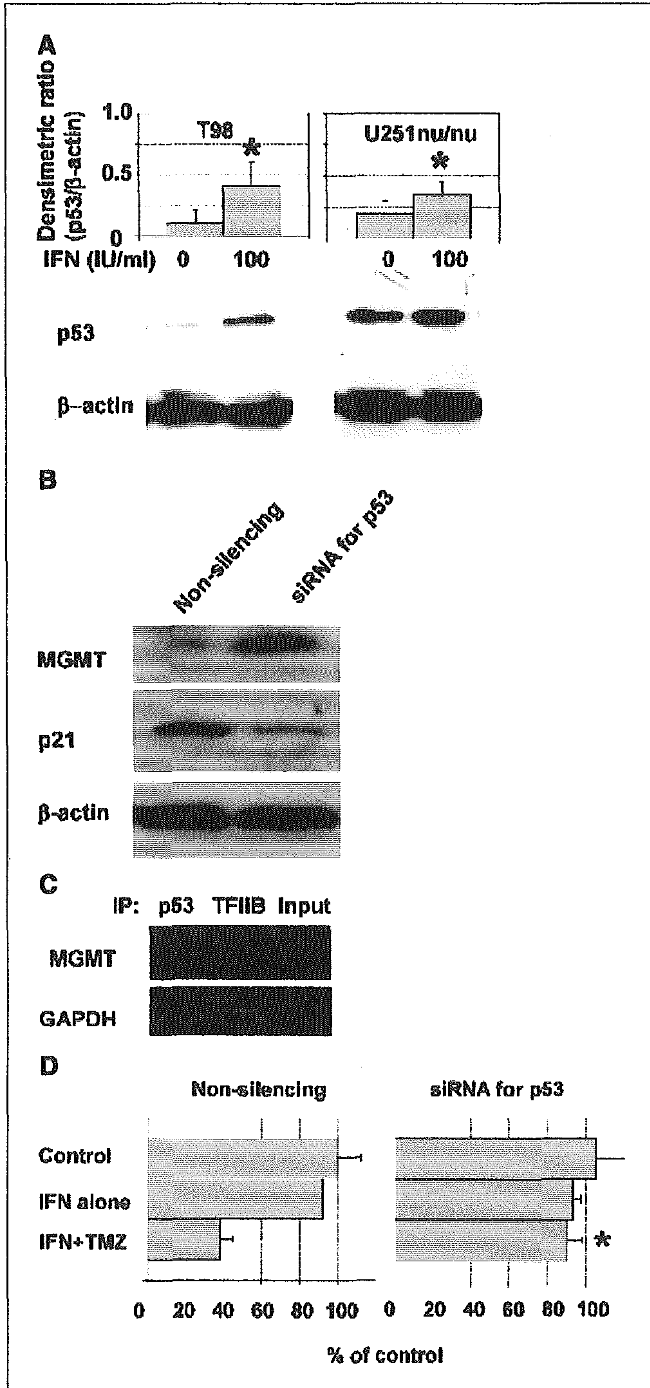

Figure 3. IFN-β down-regulates MGMT expression via induction of p53. *A*, up-regulation of the protein p53 in T98 and U251nu/nu cells by IFN-β. The cell lysate of cells treated with IFN-β (100 IU/mL) was subjected to Western blotting with anti-p53 and anti–β-actin antibodies. The histogram shows the amount of p53 relative to that of β-actin. *Columns*, mean values from three independent experiments; *bars*, SD (* $P < 0.05$). *B*, RNA interference experiments for p53. After p53 was knocked down by the siRNA specific for p53, Western blotting for MGMT, p21, and β-actin was done. The siRNA with nonsilencing sequence was used as a control. The specific knockdown was confirmed by the diminished expression level of p21, a well-known target gene of *p53*. *C*, chromatin immunoprecipitation assay. The protein p53 binding to the MGMT promoter element was examined. *Lane 1*, PCR amplification of MGMT and GAPDH sequences in immunoprecipitated chromatin fragments with anti-p53 antibody. *Lane 2*, result of PCR using immunoprecipitated samples with control antibody against TFIIB, the known transcriptional factor of GAPDH. *Lane 3*, PCR amplification of the total input DNA. *D*, cancellation of the sensitizing effect of IFN-β by p53 siRNA. T98 cells were treated with the siRNA for p53 or the nonsilencing siRNA before the treatments of IFN-β (100 IU/mL) and temozolomide (100 μmol/L). Seventy-two hours after, the number of viable cells was counted. *$P < 0.05$ versus the treatment group with nonsilencing siRNA, IFN-β, and temozolomide.

untreated cells and the similar phenomenon was observed in the U251nu/nu cells as well (Fig. 3*A*).

IFN-β down-regulates MGMT expression via protein p53. For >10 years, p53 has been the focus of intensive research. This has led to a plethora of information regarding p53, its biological roles, and its relevance to cancer (17). p53 is primarily a sequence-specific transcriptional activator. It binds to cognate p53-responsive elements within the genome and activates the transcription of genes residing in the vicinity of these binding sites. The proteins encoded by the p53 target genes, whose number is probably in the hundreds, contribute in multiple ways to the biological effects of p53. The biological outcomes of p53 activity include apoptosis, inhibition of cell cycle progression, senescence, differentiation, and accelerated DNA repair. We first confirmed that siRNA for p53 specifically knocked down the protein p53 function by examining the diminished expression level of *p21*, a well-known target gene of p53 (Fig. 3*B*). As shown in Fig. 3*B*, knockdown of p53 by siRNA increased MGMT expression in T98 cells. Therefore, p53 can down-regulate transcription of MGMT. To further investigate whether protein p53 directly interacts with MGMT promoter and down-regulates transcription of MGMT, we carried out chromatin immunoprecipitation assay. We observed that MGMT promoter, but not GAPDH promoter, coprecipitated with p53. The irrelevant antibody specific to TFIIB, which is a transcription factor of GAPDH, did not coprecipitate with MGMT promoter (Fig. 3*C*). These findings are in agreement with the previous reports showing that p53 reduces the basal activity of the MGMT promoter, and adenoviral vector–mediated overexpression of p53 reduces MGMT expression (18, 19), although some papers suggested that inactivated MGMT may be linked to cytotoxic effect of alkylating agents and cell signaling events, but was independent of p53 status (20). The reasons for these differences are unclear and may be cell type dependent. Moreover, in cell growth experiments, knocking down of p53 before IFN-β treatment nullified the synergistic inhibitory effect of IFN-β and temozolomide on T98 cell growth (Fig. 3*D*). Thus, acting together, IFN-β down-regulates MGMT transcription via induction of the p53 expression and sensitizes resistant glioma cells to temozolomide. In conclusion, this report shows that IFN-β is able to decrease MGMT levels in glioma cells via the inhibition of *MGMT* gene transcription. Moreover, pretreatment of glioma cells with IFN-β markedly enhances chemosensitivity to temozolomide. This suggests that clinical efficacy of temozolomide might be improved by combination with IFN-β using appropriate doses and schedules of administration.

Acknowledgments

Received 1/6/2005; revised 5/7/2005; accepted 6/23/2005.

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The authors especially thank NGK Insulators, Ltd for making the custom made microarray, and thank Ms. Yasuko Yoshida and Mr. Saichi Yamada (NGK Insulators, Ltd) for technical assistance.

References

1. Fine HA, Dear KB, Loeffler JS, Black PM, Canellos GP. Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults. Cancer 1993;71:2585–97.
2. Ludlum DB. DNA alkylation by the haloethylnitrosoureas: nature of modifications produced and their enzymatic repair or removal. Mutat Res 1990;233:117–26.
3. Pegg AE, Dolan ME, Moschel RC. Structure, function, and inhibition of O^6-alkylguanine-DNA alkyltransferase. Prog Nucleic Acid Res Mol Biol 1995;51:167–223.
4. Jaeckle KA, Eyre HJ, Townsend JJ, et al. Correlation of tumor O^6 methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-chloroethylnitrosourea: a Southwest Oncology Group study. J Clin Oncol 1998;16:3310–5.
5. Mineura K, Yanagisawa T, Watanabe K, Kowada M, Yasui N. Human brain tumor *O*(6)-methylguanine-DNA methyltransferase mRNA and its significance as an indicator of selective chloroethylnitrosourea chemotherapy. Int J Cancer 1996;69:420–5.
6. Belanich M, Pastor M, Randall T, et al. Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. Cancer Res 1996;56:783–8.
7. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. N Engl J Med 2000;343:1350–4.
8. Stupp R, Gander M, Leyvraz S, Newlands E. Current and future developments in the use of temozolomide for the treatment of brain tumours. Lancet Oncol 2001;2:552–60.
9. Silber JR, Mueller BA, Ewers TG, Berger MS. Comparison of O^6-methylguanine-DNA methyltransferase activity in brain tumors and adjacent normal brain. Cancer Res 1993;53:3416–20.
10. Yoshida J, Kato K, Wakabayashi T, Enomoto H, Kageyama N. Antitumor activity of interferon-β against malignant glioma in combination with chemotherapeutic agent of nitrosourea (ACNU). In: Cantell K, Schellekens H, editors. The Biology of the Interferon Systems. Boston: Martinus Nijhoff; 1986. p. 339–406.
11. Takaoka A, Hayakawa S, Yanai H, et al. Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. Nature 2003;424:516–23.
12. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 1996;93:9821–6.
13. Esteller M, Toyota M, Sanchez-Cespedes M, et al. Inactivation of the DNA repair gene O^6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-*ras* in colorectal tumorigenesis. Cancer Res 2000;60:2368–71.
14. Chawla-Sarkar M, Lindner DJ, Liu YF, et al. Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. Apoptosis 2003;8:237–49.
15. Borden EC. WB interferons. 5th ed. Toronto: B.C. Decker, Inc.; 2000. p. 815–24.
16. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998;67:431–42.
17. Oren M. Decision making by p53: life, death and cancer. Cell Death Differ 2003;10:431–42.
18. Grombacher T, Eichhorn U, Kaina B. p53 is involved in regulation of the DNA repair gene O^6-methylguanine-DNA methyltransferase (MGMT) by DNA damaging agents. Oncogene 1998;17:845–51.
19. Harris LC, Remack JS, Houghton PJ, Brent TP. Wild-type p53 suppresses transcription of the human *O^6-methylguanine-DNA methyltransferase* gene. Cancer Res 1996;56:2029–32.
20. Yan L, Donze JR, Liu L. Inactivated MGMT by O^6-benzylguanine is associated with prolonged G2/M arrest in cancer cells treated with BCNU. Oncogene 2005;24:2175–83.

J Neurosurg (Pediatrics 3) 102:299–302, 2005

Multicentric atypical teratoid/rhabdoid tumors occurring in the eye and fourth ventricle of an infant

Case report

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✓Atypical teratoid/rhabdoid tumors (AT/RTs) are aggressive malignant tumors found in infants and young children. The tumor is characterized by the presence of a rhabdoid cell component in all cases, but the histological origin is still unclear. Recently, germline mutation of the *hSNF5/INI1* gene has been reported in association with AT/RTs.

The authors report a rare case of an intraocular AT/RT followed by a fourth ventricular tumor. The results of immunohistochemical studies of the surgical specimens revealed the presence of an AT/RT and from this finding the neural origin was inferred. A novel missense mutation of the *hSNF5/INI1* gene was demonstrated by DNA analysis. High-dose chemotherapy with stem cell rescue was effective in treating this patient. The immunohistochemical relationship between rhabdoid cells and the neurogenic zone, which has not been described in AT/RTs, is of great interest in view of the nature of rhabdoid cells.

KEY WORDS • atypical teratoid/rhabdoid tumor • germline mutation • *hSNF5/INI1* • subgranular zone • Musashi1 • nestin • pediatric neurosurgery

ATYPICAL AT/RTs are aggressive malignant tumors found in infants and young children. The prognosis is poor, despite the use of adjuvant therapy, and many patients die within a year following diagnosis.[3,13] The tumor is characterized by the presence of a rhabdoid cell component in all cases, variably mixed with other histological patterns including primitive neuroectodermal, mesenchymal, and epithelial components;[2,6,11,16,17] however, the histological origin is still unclear. Generally, monosomy or deletions from chromosome 22 have been identified in 60 to 90% of the cases.[3,13] The chromatin-remodeling gene *hSNF5/INI1* has recently been reported to act as a rhabdoid tumor suppressor gene,[18] and a germline mutation of *hSNF5/INI1* has been reported in malignant rhabdoid tumors, termed rhabdoid predisposition syndrome.[15]

The locations of AT/RTs vary; most arise in the posterior fossa (52%), followed by the supratentorial (39%), pineal (5%), multifocal (2%), and spinal (2%) areas.[10] Intraocular AT/RTs are rare. We report a case involving a neonate who presented with an intraocular tumor followed by an intramedullary tumor in the fourth ventricle.

Abbreviations used in this paper: AT/RT = atypical teratoid/rhabdoid tumor; MR = magnetic resonance; SGZ = subgranular zone.

Case Report

Patient History. This 5-week-old boy had been the product of a normal pregnancy and delivery. He did not appear to have medical problems and did not have a significant family history, but an abnormal left pupil was observed when the child was 2 weeks of age. An ophthalmological examination revealed a lesion appearing as a white mass behind the lens of his left eye. Ultrasonographic biomicroscopy and MR imaging around the orbit demonstrated an intraocular tumor, as demonstrated in Fig. 1. Because of the diagnostic uncertainty and little hope for useful vision, the patient was followed with observation alone; however, his left eye gradually enlarged and his general condition became worse. Ten months after the initial diagnosis, his left eye was enucleated because a histopathological examination revealed this tumor to be a rhabdoid cell tumor.

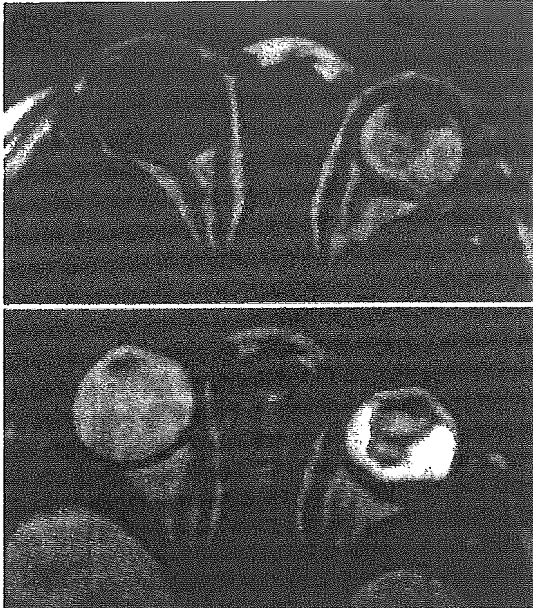

FIG. 1. Preoperative T_1- *(upper)* and T_2-weighted *(lower)* MR images of the left eye demonstrating a cone-shaped structure connecting the lens and posterior pole of the eye.

Operation. An extensive systemic workup demonstrated a fourth ventricular tumor of approximately 1.5 cm in diameter (Fig. 2). The patient immediately underwent a tumor resection via a suboccipital approach. The tumor was rather soft, relatively vascularized, and attached to the floor of the fourth ventricle. Gross-total resection of the tumor was achieved. Immunohistochemical studies of the surgical specimens from the eye and brain were consistent with AT/RT (Fig. 3, Table 1); DNA analysis revealed a germline *hSNF5/INI1* mutation (Fig. 4).

Postoperative Course. The patient received three courses of cisplatin at 90 mg/m^2 and etoposide at 300 mg/m^2, three courses of ifosfamide 9 g/m^2, carboplatin 1.5 g/m^2, and etoposide 450 mg/m^2, followed by two courses of vincristine 1.5 mg/m^2, nimustine 2 mg/m^2, and cisplatin 90 mg/m^2. The patient then underwent myeloablative chemotherapy with thiotepa 560 mg/m^2 and melphalan 130 mg/m^2 with stem cell rescue.[7,8] At last follow up, the boy had been free from recurrence or new occurrence of tumors for 24 months since the operation.

Histopathological Findings. Routine H & E staining of the intraocular tumor revealed that it consisted largely of necrotic tissues and hemorrhage, and that a small amount of tumor tissue remained. Tumor cells were large, pale, polygonal cells with distinct borders, vesicular nuclei, and moderate amounts of eosinophilic cytoplasm (Fig. 3 *upper left*), presenting a typical pattern of a rhabdoid cell tumor. The fourth ventricular tumor was composed mainly of poorly differentiated small and hyperchromatic cells resembling those of primitive neuroectodermal tumors and medulloblastomas, and it included rhabdoid cell components (Fig. 3 *upper right*). Both tumor cells demonstrated diffusely cytoplasmic positivity for cytokeratin AE1/AE3 and CAM-5.2, S100, vimentin, epithelial membrane antigen, and MIC2. Glial fibrillary acidic protein was expressed only in the brain tumor. Desmin, smooth-muscle actin, and HMB45 were not expressed. These results were used to aid in the diagnosis of both tumors as rhabdoid cell tumors. In addition, RNA-binding protein Musashi1 was expressed (Fig. 3 *lower left*) but nestin was not (Fig. 3 *lower right*) in both the intraocular and the fourth ventricular tumors; these proteins have been recently reported as markers for neural precursor cells.[9,14] The immunohistochemical results obtained with the different markers are summarized in Table 1.

Analysis of DNA. With the informed consent of his parents, genomic DNA was extracted from the peripheral blood of the patient and his parents as well as from the fourth ventricular tumor. Exons 1 to 9 of the *hSNF5/INI1* gene were amplified by polymerase chain reaction by using primers and conditions as previously described,[15] and the products of polymerase chain reaction were directly sequenced (Fig. 4). A novel heterozygous missense mutation of the *hSNF5/INI1* gene at codon 40 was identified from the peripheral blood of the patient's DNA. This mutation was considered de novo because no mutation was detected in his parents. In addition, the DNA analysis of the brain tumor showed only a mutated allele, suggesting a loss of het-

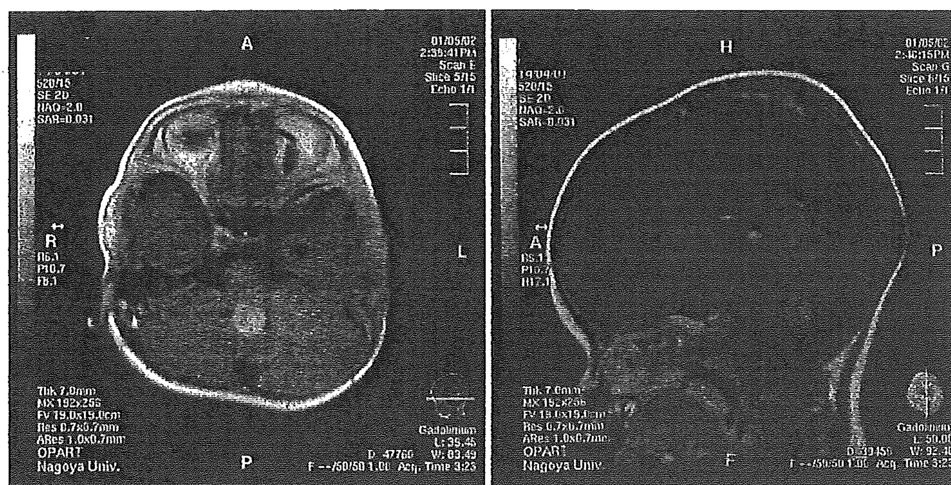

FIG. 2. Preoperative enhanced MR image of the brain demonstrating a fourth ventricular tumor with mass effect.

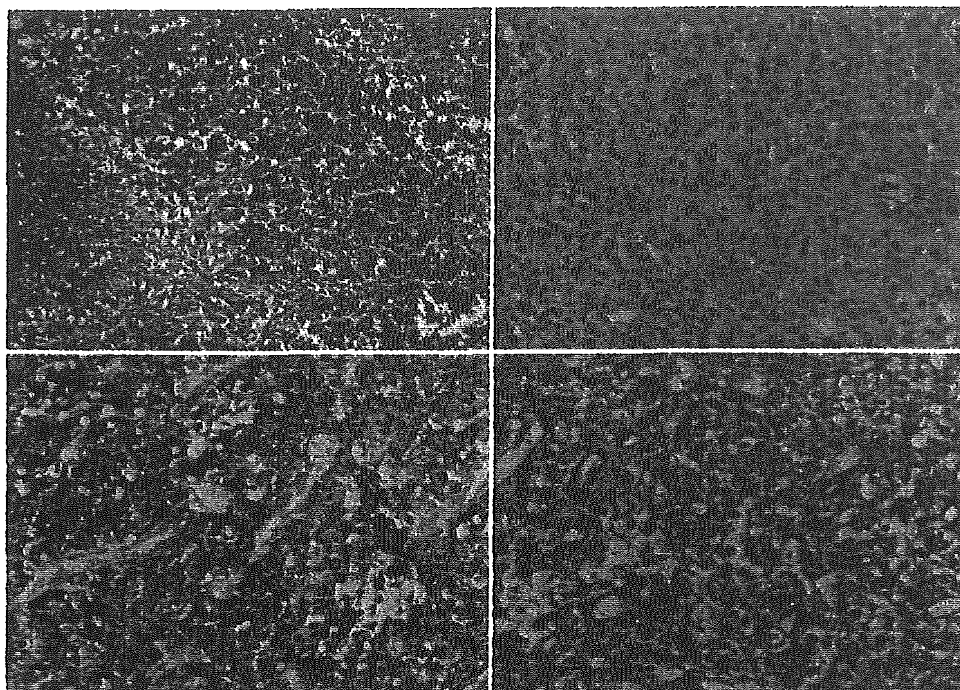

FIG. 3. Immunohistochemical studies demonstrating the intraocular tumor, the tissue of which is composed mainly of hematoma and necrosis, with tumor cells consisting of typical rhabdoid cells (*upper left*). The fourth ventricular tumor with poorly differentiated, small, hyperchromatic cells (*upper right*); the brain tumor with cytoplasmic positivity (*lower left*); the brain tumor specimen with limited positivity in vascular endothelial cells (*lower right*). H & E (*upper left* and *upper right*), Musashi1 (*lower left*), and nestin (*lower right*). Original magnifications × 200.

erozygosity. We could not analyze the intraocular tumor DNA because a limited amount of surgical specimens were available.

Discussion

Intraocular AT/RTs are extremely rare. Until now, there has been only one reported case in the literature of an intraocular malignant rhabdoid tumor that was believed to have metastasized from a renal tumor.[1] The relationship between intraocular and fourth ventricular tumors has not been clearly determined; however, the clinical course of the present case indicates that AT/RTs can possibly arise in the eyeball. Moreover, the germline mutation of *hSNF5/INI1* detected in this patient provides evidence of the multicentric origins of these tumors. The boy was thought to be suffering from a so-called rhabdoid predisposition syndrome, which is most likely to predispose a patient to a variety of cancers.[15]

The phenotypic diversity of AT/RT cells is generally observed in tumor tissues; therefore, the histogenesis of AT/RT remains uncertain. Various cellular origins have been proposed, including neuroectodermal,[16] myogenic,[17] histiocytic,[6] neural,[2] and epithelial.[11] It may be inferred from these reports that an AT/RT has multiphenotypic characteristics, but the true origin of this enigmatic tumor is unknown. Recent studies involving normal neurons have established that neurogenesis of the brain persists in at least two discrete regions, the subventricular zone of the lateral ventricle[5] and the SGZ of the dentate gyrus.[4] Both nestin and the neural RNA-binding protein Musashi1 are expressed in neural stem cells in the subventricular zone.[9,14] In contrast, neuronal progenitor cells in the SGZ present with Musashi1 but not nestin.[16] In this case, Musashi1 was expressed but nestin was not in both the intraocular and the fourth ventricular tumors; these results indicate that these tumors might be derived from SGZ. Another hypothesis is that new peculiar cells are the origin for AT/RTs, although we cannot discuss this issue at present. As far as we are aware, this report provides the first documentation of a relationship between AT/RTs and the SGZ.

High-dose chemotherapy with stem cell rescue was effective in this case; however, the prognosis of AT/RTs is

TABLE 1

*Summary of the immunohistochemical findings of the intraocular and brain tumors**

Agent	Intraocular Tumor	Fourth Ventricular Tumor
cytokeratin		
AE1/AE3	+	+
CAM-5.2	+	+
S100	+	+
vimentin	+	+
EMA	+	+
MIC2	+	+
Musashi1	+	+
GFAP	−	+
SMA	−	−
desmin	−	−
HMB45	−	−
nestin	−	−

* EMA = epithelial membrane antigen; GFAP = glial fibrillary acidic protein; SMA = α-smooth muscle actin; + = positive; − = negative.

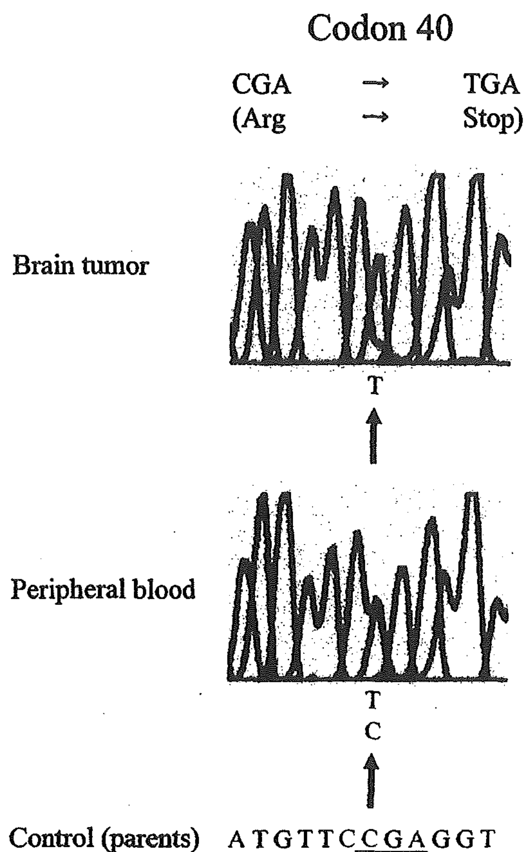

FIG. 4. Analysis of DNA revealing a missense mutation at codon 40 *(arrow)* in the brain tumor and peripheral blood specimens that predicts truncation of the protein. Control sequence obtained from the patient's parents is demonstrated below.

generally very poor. Even after aggressive surgery and chemotherapy, overall survival rates for children, particularly for those younger than 2 years of age, have been extremely poor, with less than 20% of patients surviving less than 12 months from the time of diagnosis.[3,13] A variety of different chemotherapeutic agents have been used, but no single agent or combination of agents have been shown to be most effective. Ronghe, et al.,[12] recently reported the effectiveness of intensified therapy for AT/RTs. More consideration is needed to develop a new therapeutic strategy for these types of tumors.

Conclusions

We report a rare case of an intraocular AT/RT followed by a fourth ventricular tumor. Immunohistochemical studies indicated the neural origin of these tumors. High-dose chemotherapy with stem cell rescue was an effective treatment in this case.

References

1. Akhtar M, Ali MA, Sackey K, Bakry M, Johnson T: Malignant rhabdoid tumor of the kidney presenting as intraocular metastasis. **Pediatr Hematol Oncol 8:**33–43, 1991
2. Bonnin JM, Rubinstein LJ, Palmer NF, Beckwith JB: The association of embryonal tumors originating in the kidney and in the brain. A report of seven cases. **Cancer 54:**2137–2146, 1984
3. Burger PC, Yu IT, Tihan T, Friedman HS, Strother DR, Kepner JL, et al: Atypical teratoid/rhabdoid tumor of the central nervous system: a highly malignant tumor of infancy and childhood frequently mistaken for medulloblastoma: a Pediatric Oncology Group study. **Am J Surg Pathol 22:**1083–1092, 1998
4. Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J: Multipotent progenitor cells in the adult dentate gyrus. **J Neurobiol 36:**249–266, 1998
5. Garcia-Verdugo JM, Doetsch F, Wichterle H, Lim DA, Alvarez-Buylla A: Architecture and cell types of the adult subventricular zone: in search of the stem cells. **J Neurobiol 36:** 234–248, 1998
6. Gonzalez-Crussi F, Goldschmidt RA, Hsueh W, Trujillo YP: Infantile sarcoma with intracytoplasmic filamentous inclusions: distinctive tumor of possible histiocytic origin. **Cancer 49:** 2365–2375, 1982
7. Guruangan S, Dunkel IJ, Goldman S, Garvin JH, Rosenblum M, Boyett JM, et al: Myeloablative chemotherapy with autologous bone marrow rescue in young children with recurrent malignant brain tumors. **J Clin Oncol 16:**2486–2493, 1998
8. Heideman RL, Packer RJ, Reaman GH, Allen JC, Lange B, Horowitz ME, et al: A phase II evaluation of thiotepa in pediatric central nervous system malignancies. **Cancer 72:** 271–275, 1993
9. Kaneko Y, Sakakibara S, Imai T, Suzuki A, Nakamura Y, Sawamoto K, et al: Musashi1: an evolutionally conserved marker for CNS progenitor cells including neural stem cells. **Dev Neurosci 22:**139–153, 2000
10. Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC, et al: The WHO classification of tumors of the nervous system. **J Neuropathol Exp Neurol 61:** 215–229, 2002
11. Parham DM, Peiper SC, Robicheaux G, Ribeiro RC, Douglass EC: Malignant rhabdoid tumor of the liver. Evidence for epithelial differentiation. **Arch Pathol Lab Med 112:**61–64, 1988
12. Ronghe MD, Moss TH, Lowis SP: Treatment of CNS malignant rhabdoid tumors. **Pediatr Blood Cancer 42:**254–260, 2004
13. Rorke LB, Packer R, Biegel J: Central nervous system atypical teratoid/rhabdoid tumors of infancy and childhood. **J Neurooncol 24:**21–28, 1995
14. Sakakibara S, Imai T, Hamaguchi K, Okabe M, Aruga J, Nakajima K, et al: Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. **Dev Biol 176:**230–242, 1996
15. Sevenet N, Sheridan E, Amram D, Schneider P, Handgretinger R, Delattre O: Constitutional mutations of the *hSNF5/INI1* gene predispose to a variety of cancers. **Am J Hum Genet 65:** 1342–1348, 1999
16. Suzuki A, Ohta S, Shimada M: Gene expression of malignant rhabdoid tumor cell lines by reverse transcriptase-polymerase chain reaction. **Diagn Mol Pathol 6:**326–332, 1997
17. Tsokos M, Kouraklis G, Chandra RS, Bhagavan BS, Triche TJ: Malignant rhabdoid tumor of the kidney and soft tissues. Evidence for a diverse morphological and immunocytochemical phenotype. **Arch Pathol Lab Med 113:**115–120, 1989
18. Vujanic GM, Sandstedt B, Harms D, Boccon-Gibod L, Delemarre JF: Rhabdoid tumor of the kidney: a clinicopathological study of 22 patients from the International Society of Paediatric Oncology (SIOP) nephroblastoma file. **Histopathology 28:** 333–340, 1996

Manuscript received April 26, 2004.
Accepted in final form October 21, 2004.

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● 特 集 ● 悪性脳腫瘍治療の新しい展開

化学療法の新展開

渋井壮一郎*

〔*Jpn J Cancer Chemother* 32(4): 442-447, *April,* 2005〕

Recent Advances in Chemotherapy for Malignant Brain Tumors: Soichiro Shibui (*Neurosurgery Division, National Cancer Center Hospital*)

Summary

Most malignant brain tumors are resistant to the chemotherapeutic agents because of the existence of several mechanisms or substances such as the blood-brain barrier, genes and proteins. Recently many studies have been started to overcome the chemoresistance. Especially recent advances in the field of molecular biology have contributed to examination of the chemosensitivities of tumor cells. Trials for the individualization of the treatment, so-called Taylor-made therapy, is one of these challenges. Loss of chromosome 1 p and 19 q is considered to be closely related to chemosensitivity in anaplastic oligodendrogliomas. This is one of the breakthroughs in the field of chemotherapy for malignant brain tumors. O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme which reduces the cytocidal effect of nitrosourea. In order to overcome the chemoresistance, drugs except nitrosourea or some drugs which reduce the MGMT activity are used for tumors expressing MGMT. New technology targeting growth factor receptor such as EGFR or VEGFR is also applied to cancer chemotherapy. On the other hand, multi-institutional cooperative studies have been started to obtain evidence in cancer treatment. Phase II study for a small number of patients is not sufficient to demonstrate the efficacy of the treatment and to establish the standard therapy. Multi-institutional randomized controlled study by JCOG Brain Tumor Study Group is the first trial for the treatment of malignant astrocytomas under well-established quality control and quality assurance systems. It can be a model of clinical trials for malignant brain tumors in Japan. Key words: Malignant glioma, Randomized controlled study, Molecular target therapy, Taylor-made chemotherapy, Drug resistance, Corresponding author: Dr. Soichiro Shibui, Neurosurgery Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

要旨 悪性脳腫瘍は一部を除いて，化学療法に抵抗性を示す疾患である。血液脳関門の存在による薬物到達性の問題，各種耐性機構の存在などがその原因とされているが，近年それらを克服し効果を高めようという工夫がなされてきた。分子生物学的手法を用いて薬剤感受性の有無の検索，さらに耐性機構の克服が徐々に進みつつあり，個々の腫瘍の特質に合わせた個別化治療（テーラーメード治療）も試みられている。退形成性乏突起膠腫における染色体 1 p，19 q 欠失と薬剤感受性の関係は，脳腫瘍の化学療法の歴史のなかでも極めて意義深いものである。薬剤耐性面では，悪性神経膠腫に対して最も広く用いられている nitrosourea 系薬剤に対する耐性機構として O^6-methylguanine-DNA methyltransferase (MGMT) の存在が知られており，MGMT が高値の腫瘍では nitrosourea 系薬剤以外を第一選択として用いたり，MGMT 活性を低下させる工夫もなされている。このような治療の個別化の試みが進む一方，多施設共同試験によるエビデンスの蓄積の動きもある。ある治療法の有効性を確認し，標準治療を作り上げていくためには，少数の第II相試験では不十分であり，大規模な第III相臨床試験が必要である。JCOG 脳腫瘍研究グループによる多施設共同試験は，国内初の JCOG 管理下の脳腫瘍に対する臨床試験であり，今後の臨床試験の方向性を示すものとして期待できる。

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はじめに

悪性神経膠腫は，従来より化学療法に抵抗性を示す疾患とされ，他臓器の悪性腫瘍と比較しても極めて予後不良といえる。脳腫瘍全国集計調査報告によれば，退形成性星細胞腫（anaplastic astrocytoma: AA）の5年生存率は23%，膠芽腫（glioblastoma: GBM）に至ってはいまだ7%にすぎない[1]。悪性脳腫瘍に対する化学療法の動向としては二つの方向性があるといえる。一つは個々の腫瘍の特性を調べ，感受性の高い薬剤を使用したり，耐性機構を克服していくという個別化治療（テーラーメード治療）であり，もう一つはエビデンスに基づいた治療法の確立のための大規模な無作為化臨床試験（第III相試験）の実施である。個別化治療と大規模試験という一見相反するコンセプトであるが，両者は相補的な役割を演じて，化学療法の発展に寄与している。

I．悪性神経膠腫治療のエビデンス

臨床試験は，対象となっている薬剤の安全性や薬物動態を調べる第I相試験，有効性を比較的少数の症例を対象として調べる第II相試験，そして従来の標準治療との比較を多数の症例を対象に無作為に割り振って行う第III相試験に大別される。ここでいう標準治療とは，科学的証拠（エビデンス）に裏付けされ，現時点で最も有効で有害事象の少ない治療のことであるが，希少疾患の一つである悪性神経膠腫に対しては，国内では今まで大規模な臨床試験が十分に行われているとはいえず，標準治療といえる治療法が存在しないというのが現状である。一方，欧米では1970年代後半より無作為化試験が行われ，エビデンスの蓄積が行われてきた。

悪性神経膠腫の治療法としては，手術・放射線・化学療法が三本柱になっているが，手術摘出度についての無作為化比較試験は困難であり，retrospective な検索により，全摘に近いほど生存率が高いことが知られている[1,2]。Anderson らは，glioblastoma 108例に対して術後の放射線の有無での比較試験を行った結果，手術単独では1年生存率が0%であったが，45 Gyの照射で19%に上昇したと報告し，術後の放射線治療の有効性を報告している[3]。さらに，Walker らは悪性神経膠腫467例に対する術後補助療法として，化学療法の効果を調べる目的で，「BCNU＋全脳照射60 Gy」，「MeCCNU（semustine）＋全脳照射60 Gy」，「放射線治療（全脳照射60 Gy）単独」，「MeCCNU単独」の4群での比較試験を行い，化学療法単独に対して他の放射線照射を含む治療法が生存において有意に優っていることを報告した[4]。その他，Chang ら，Green らの報告でも「BCNU＋全脳照射60 Gy」群が生存で最も優っており，これらの結果からBCNU＋同時併用放射線照射が米国での標準治療とされるようになった[5,6]。

放射線の総線量に関しては，Walker らの比較試験で，照射線量を45，50，55，60 Gyと増量するにつれて，生存期間中央値が13.5，28，36，42週と延長し，前述のAnderson らの45 Gy照射群での28週と比較しても明らかに延長しているため，60 Gyが術後放射線照射の標準線量と考えられている[3,7]。

一方，国内における無作為化試験については，十分な体制が整っていないこともあり，欧米に比べ立ち遅れているといわざるを得ない。BCNUが欧米で用いられているのに対し，わが国では国内で開発されたACNUが用いられているが，十分なエビデンスの下での治療とはいえない。すなわち高倉らの報告によれば，星細胞腫grade 3・4に対し，術後局所照射50～60 GyにACNU 100

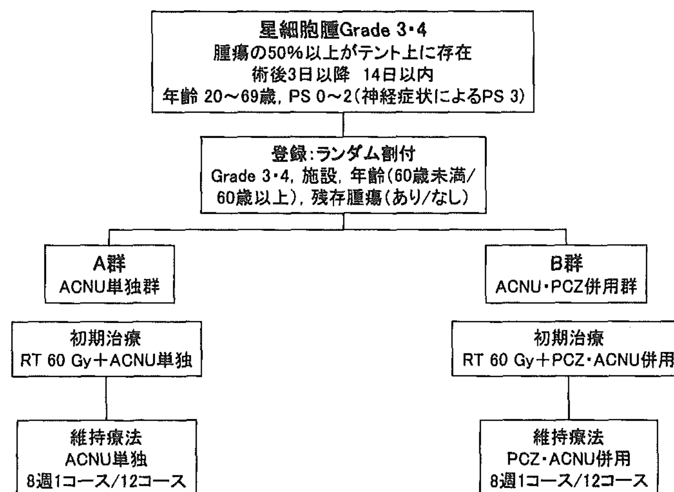

図1 JCOG脳腫瘍研究グループによる多施設共同試験プロトコールシェーマ

mg/m²の1〜2回の同時併用の有無で比較試験を行ったところ，奏効割合（CT上の計測にて腫瘍が50%以上縮小した割合）が前者では13.5%であったのに対し，後者は47.5%と有意に向上したが，生存に関しては各群40例程度の症例の集積ということもあり，ACNU併用群におけるAAの3年生存割合は59.0%，GBMで16.3%と，放射線単独群の3年生存割合48.9，0%と比べ有意に良好とはならなかった。しかし，ACNU併用群が点推定値では生存で上回っていることと，同様の薬剤であるBCNUが米国での標準となっていることから，現在わが国においては，局所照射60 Gy＋ACNU同時併用療法を第一選択として用いている施設が多い[8]。

II．JCOG脳腫瘍研究グループによるランダム化試験

これに対し，国内においての標準治療を確立するための新しい動きもみられるようになった。日本臨床腫瘍研究グループ（Japan Clinical Oncology Group: JCOG）内に設立されたJCOG脳腫瘍研究グループでは，国内での現時点での標準治療ともいうべきACNU＋放射線治療に対し，procarbazine＋ACNU＋放射線治療の2群において第II/III相試験を開始した。これは，JCOGによるデータの品質管理（quality control: QC）および品質保証（quality assurance: QA）の下での臨床試験ということで，完遂された後には日本から世界に発信されるエビデンスとなることが期待されている[9]。

ここでの標準治療に対する新治療は，procarbazineを先行投与した後のACNU投与である（図1）。ProcarbazineはO^6-methyguanineを形成し，その結果，ACNUに対する耐性機構の主体をなしているとされているO^6-methylguanine-DNA methyltransferaseが消費され，ACNUの効果を高めることが期待される[10]。臨床的にも，再発glioblastomaに対し，BUNUおよびvincristineに先行してprocarbazineを投与する方法で29%の奏効率があったとする報告もあり，今回の臨床研究の治療成績の結果が待たれる[11]。

III．新薬の開発

従来より，悪性神経膠腫は化学療法に抵抗性を示し，手術・放射線治療に比べると，化学療法の予後への影響は小さいと考えられてきた。血液脳関門の存在，腫瘍への血流分布の問題，mdr-1，glutathione-S-transferase-π（GST-π），O^6-methylguanine-DNA methyltransferase（MGMT）など薬剤耐性遺伝子や蛋白の存在などがその要因と考えられてきた。このような状況のなかで，耐性を克服し，少しでも奏効率が高く，有害事象の少ない薬剤の開発が続けられている。

表1 癌治療における分子標的薬

血管新生阻害剤・転移抑制剤
MMP阻害剤，VEGF阻害剤，抗VEGF抗体，PDGF阻害剤，angiostatin，エンドスタチン，Thalidomide
シグナル伝達阻害剤
ハーセプチン，イレッサ，グルベック，ファルネシルトランスフェラーゼ阻害剤
細胞周期調節剤
サイクリン依存キナーゼ阻害剤
proteinkinase C（PKC）阻害剤
遺伝子治療
ワクチン，免疫療法

（国立がんセンターHPより）

1．新しいアルキル化剤

1）Temozolomide

temozolomideは，現在最も期待されている悪性神経膠腫に対する治療薬の一種である。経口アルキル化剤で投与方法が簡便であり，悪心・嘔吐，骨髄抑制などの副作用も比較的軽微でありながら，悪性神経膠腫，特にAAに有効であるという報告が多く，現在，多くの臨床研究が進んでいる。国内でもAA再発例に対し第II相試験が進行中であり，早ければ平成17年度中にも厚生労働省の認可を受ける可能性がでている。MD Anderson Cancer Centerでの第II相試験では，28日ごとに最大4コース200 mg/m²のtemozolomideを5日間連続経口投与した後，放射線治療を行ったところ，22例のAAで奏効率は34%，median survival 23.5か月，36例のGBMについてはそれぞれ45%，13.2か月という結果を得ている[12]。また，EORTC Brain & RT Groups and Clinical NCIC Clinical Trial Groupは，GBMに対し放射線単独治療とtemozolomide併用放射線治療との第III相試験を行い，前者のmedian survival 12か月に対し，後者は15か月と生存期間の有意な延長をみている[13]。しかしながら，いずれもこれまでの多くの報告と比べ飛躍的な改善とはいえず，現在までのところ，AAに対する若干の生存期間の延長と経口という利便性において優っているといえる程度である。

2）Gliadel

gliadelはBCNUを包埋したポリマーで，手術時に腫瘍摘出腔に投与することにより徐放性にBCNUが放出される。手術時にGliadel注入後，放射線治療を行うという方法での臨床研究が進められており，現在までの報告では，GBMのmedian survivalは13か月前後とされ，placebo群に比べ2か月程度の生存延長がみられている[14,15]。

2．分子標的治療薬

分子生物学の発展により，個々の腫瘍の生物学的特性

が詳細にわかるようになってきた。細胞障害性をもつ薬剤のなかから，臨床研究でのスクリーニングによって選択された抗腫瘍薬と異なり，分子レベルでの標的の機能を修飾することで治療を行うのが分子標的治療である。主な分子標的を表1に示すが，それぞれが関連性をもっているため，ある標的分子を修飾することにより，その下流の複数の分子に影響が現れることもある。悪性神経膠腫の治療においては，比較的発現の高いといわれている epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR) などが標的の候補とされている（表1)。新しい治療法として期待がもたれるが，悪性神経膠腫に対しては，現在までのところ画期的な治療効果はみられていない。

1) Thalidomide

GBM は血管新生を伴って増大する腫瘍であるため，Thalidomide のように VEGF 受容体に作用し血管新生を抑制する薬剤には抗腫瘍効果が期待できる。Thalidomide 単剤あるいは BCNU や temozolomide などのアルキル化剤との併用での臨床研究が始まっており，若干の生存延長が報告されている[16,17]。

2) Imatinib mesylate (Gleevec)

imatinib mesylate (STI 571, Gleevec) は Bcr-Abl 蛋白および platelet-derived growth factor (PDGF) 受容体を含むチロシンキナーゼレセプターに作用し，癌細胞の増殖抑制作用をもつ。すでに慢性骨髄性白血病に有効であるという報告があり，脳腫瘍に対しても臨床研究が始まっている[18]。

3) Gefitinib (Iressa)

gefitinib (Iressa) は epidermal growth factor receptor (EGFR) に作用し，細胞増殖抑制作用を発揮し，一部の肺癌においてその効果が証明されているが，EGFR 発現率の高い悪性神経膠腫に対しても効果が期待されている。Rich らは，再発 GBM に対して event-free survival 8.1週, overall survival 39.4週で，副作用は grade 1または2の皮膚反応あるいは下痢であったと報告している[19]。

3. 毒　素

弱毒化した緑膿菌毒素 PE 38 を腫瘍に直接あるいは腫瘍周辺に陽圧で注入する (convection-enhanced delivery system) ことにより，深部にまで毒素を浸透させて腫瘍効果を高めようとする試みも行われている。interleukin-13 (IL-13) 受容体は神経膠腫に高率に発現しているが，正常脳にはほとんど発現していないといわれている。これと PE 38 を結合させた IL 13-PE 38 を陽圧で注入する臨床研究が始まっている[20]。同様に Transferrin とジフテリア毒素を結合させた Transferrin-CRM 107 も臨床応用が開始された[21]。

IV. 個別化治療，テーラーメード治療

従来の化学療法は，それまでに蓄積されたエビデンスに基づき，最も治療効果の期待できる薬剤を使用するという形をとってきた。それに対し，最近では，同一の病理学的診断の腫瘍でもそれぞれ薬剤に対する感受性が異なるという観点から，個々の患者ごとに最も適した治療法を選択するという手法もとられるようになってきた。これが個別化治療であり，テーラーメード治療とも呼ばれる治療法である。薬剤感受性を調べる手法として，従来は腫瘍細胞を培養系に移してのコロニー形成能試験や MTT assay などが主体であったが，近年では，分子生物学的手法の発達により，遺伝子解析に基づいた治療法の選択がなされるようになった。悪性神経膠腫の化学療法においては，耐性の克服という点を主体にテーラーメード治療が工夫されている。化学療法剤に対する耐性機構として代表的なものは，multidrug resistance (MDR)-1の遺伝子産物である p-glycoprotein であり，細胞外に薬剤を排出する働きにより，adriamycin, vincristine, cyclophosphamide, methotrexate などに同時に耐性を示す[22]。multidrug resistance-associated protein (MRP)-1 も同様に，etoposide や adriamycin に耐性を示すといわれている[23]。その他，悪性神経膠腫に最も広く使われている nitrosourea 系薬剤に対する耐性に関与しているのが O^6-methylguanine-DNA methyltransferase (MGMT) であり，その耐性を克服する試みが実際に臨床的に開始されている[24,25]。

1. MGMT による nitrosourea 系薬剤耐性の克服

nitrosourea 系抗癌剤に対する MGMT による薬剤耐性機構を克服するため，MGMT の mRNA 発現や MGMT 蛋白自体の検出が行われ，MGMT の発現の強い腫瘍に対しては，nitrosourea 系以外のプラチナ系薬剤などを第一選択として用いるという工夫が始まっている。しかしながら，国内で脳腫瘍に対して保険適応となっている薬剤が余りにも少ないため，実際の運用が困難であるだけでなく，MGMT 測定だけでは積極的に他の薬剤に感受性が高いという証拠になり得ていない。前述の procarbazine による MGMT 低下作用を利用した治療は，その意味でテーラーメード治療の一つとなり得る。腫瘍細胞での MGMT を測定し，高値を示す例に対しては，ACNU 投与前に procarbazine による前処置をすることで耐性を克服できる可能性がある[9]。

2. 染色体 1p, 19q 欠失を示す乏突起膠腫の治療

分子生物学的知見が脳腫瘍の治療に直接的に役立っている唯一の事象である。星細胞腫に比べ，乏突起膠腫は

化学療法に感受性の高い腫瘍と考えられてきた。特に染色体1番の短腕(1p)および19番長腕(19q)の欠失を認める症例においてprocarbazine, CCNU, vincristineによる化学療法(PCV療法)が極めて有効であることが示されている[26,27]。手術検体において染色体欠失を調べ，1p，19q欠失のある例については，積極的にPCV療法を行っていくのが望ましい。国内ではCCNUの代わりにACNUを用いるPAV療法が代替療法として用いられている。

おわりに

悪性神経膠腫は化学療法に抵抗性を示し，化学療法併用による有益性は余り重要視されていなかった。しかしながら，従来からのエビデンスによれば，nitrosourea系薬剤が一定の効果を示し，現在では，放射線との併用が標準治療となっている。さらに有効で有害事象の少ない治療法を開発するため，しっかりとしたquality control/quality assuranceの下での大規模臨床試験は必須であるとともに，一方では個々の腫瘍の特殊性を考慮したテーラーメード治療を開発し，悪性神経膠腫の治療成績の向上を図ることは極めて重要である。

文献

1) Committee of Brain Tumor Registry of Japan: Report of Brain Tumor Registry of Japan (1969-1996) 11TH ed. *Neurol Med Chir* **40**(suppl), 2003.
2) Winger MJ, Macdonald DR and Cairncross JG: Supratentorial anaplastic gliomas in adults: The prognositic importance of extent of resection and prior low-grade glioma. *J Neurosurg* **71**: 487-493, 1989.
3) Anderson AP: Postoperative irradiation of glioblastoma. *Acta Radiol* **17**: 475-484, 1978.
4) Walker MD, Green SB, Byar DP, *et al*: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med* **303**: 1323-1329, 1980.
5) Chang CH, Harton J, Schoenfeld D, *et al*: Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. *Cancer* **52**: 997-1007, 1983.
6) Green SB, Byar DP, Walker MD, *et al*: Comparisons of carmustine, procarbazine and high-dose methylprednisolone as additions surgery and radiotherapy for the treatment of malignant gliomas. *Cancer Treat Rep* **67**: 121-133, 1983.
7) Walker MD, Strike TA and Sheline GE: An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys* **5**: 1725-1731, 1979.
8) Takakura K, Abe H, Tanaka R, *et al*: Effects of ACNU and radiotherapy on malignant glioma. *J Neurosurg* **64**: 53-57, 1986.
9) Shibui S: A randomized controlled trial on malignant brain tumors. The activities of Japan Clinical Oncology Group (JCOG)-Brain Tumor Study Group (BTSG). *Neurol Med Chir* **44**: 220-221, 2004.
10) Valanvanis C, Souliotis VL and Kyrtopoulos SA: Differential effects of procarbazine and methylnitorosourea on the accumulation of O^6-methylguanine and the deplection and recovery of O^6-alkylguanine-DNA alkyltransferase in rat tissues. *Carcinogenesis* **15**: 1681-1688, 1994.
11) Brandes AA, Turazzi S, Basso U, *et al*: A multidrug combination designed for reversing resistance to BCNU in glioblastoma multiform. *Cancer* **82**: 355-361, 1998.
12) Gilbert MR, Friedman HS, Kuttesch JF, *et al*: A phase II study of temozolomide in patients with newly diagnosed supratentorial malignant glioma before radiation therapy. *Neurooncol* **4**: 261-267, 2002.
13) Stupp R, Mason WP, Van Den Bent MJ, *et al*: Concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) for newly diagnosed glioblastoma multiforme (GBM). Consecutive results of a randomized phase III trial by EORTC Brain & RT Groups and NCIC Clinical Trial Group. *J Clin Oncol*, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). Vol 22, No 14 S (July 15 Supplement), 2004: 2
14) Westphal M, Hilt DC, Bortey E, *et al*: A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neurooncol* **5**: 79-88, 2003.
15) Kleinberg LR, Weingart J, Burger P, *et al*: Clinical course and pathologic findings after Gliadel and radiotherapy for newly diagnosed malignant glioma: implications for patient management. *Cancer Invest* **22**: 1-9, 2004.
16) Morabito A, Fanelli M, Carillio G, *et al*: Thalidomide prolongs disease stabilization after conventional therapy in patients with recurrent glioblastoma. *Oncol Rep* **11**: 93-95, 2004.
17) Fine HA, Wen PY, Maher EA, *et al*: Phase II trial of thalidomide and Carmustine for patients with recurrent high-grade gliomas. *I Clin Oncol* **21**: 2299-2304, 2003.
18) Kilic T, Alberta JA, Zdunek PR, *et al*: Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class. *Cancer Res* **60**: 5143-5150, 2000.
19) Rich JN, Reardon DA, Peery T, *et al*: Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* **22**: 133-142, 2004.
20) Kunwar S: Convection enhanced delivery of IL13-PE38QQR for treatment of recurrent malignant glioma: presentation of unterim findings from ongoing phase 1 studies. *Acta Neurochir Suppl* **88**: 105-111, 2003.
21) Weaver M and Laske DW: Transferrin receptor ligand-targeted toxin conjugate (Tf-CRM107) for therapy of malignant gliomas. *J Neurooncol* **65**: 3-13, 2003.
22) Fojo AT, Ueda K, Slamon DJ, *et al*: Expression of a multidrug resistance gene in human tumors and tissues. *Proc Natl Acad Sci* **84**: 265-269, 1987.
23) Grant CE, Valdimarsson G, Hipfner DR, *et al*: Overexpression of multidrug resistance-associated protein (MPR) increases resistance to natural product drug. *Cancer Res* **54**: 357-361, 1994.
24) Pegg AE: Mammalian O^6-alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therpeutic agents. *Cancer Res* **50**: 6119-6129. 1990.

25) Silber JR, Bobola MS, Ghatan S, *et al*: O^6-methylguanine-DNA methyltransferase activity in adult gliomas: relation to patient and tumor characteristics. *Cancer Res* **58**: 1068-1073, 1998.
26) Cairncross JG, Ueki K, Zlatescu MC, *et al*: Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst* **90**: 1473-1479, 1998.
27) Ino Y, Betensky RB, Zlatescu MC, *et al*: Molecular subtypes of anaplastic oligodendroglioma: Implications for patient managament at diagnosis. *Clin Cancer Res* **7**: 839-845, 2001.