TABLE 1: Patient profile and parameters of BNCT in 20 patients with malignant meningiomas*

Case No.	Histology	Age (yrs), Sex	Treatment Prior to BNCT (no. of times)	Tumor Size (mm)	Tumor Depth (mm)	BPA PET (T/N)	First BNCT Dose (Gy-Eq)		No. of BNCTs
							Max	Min	
1	papillary	29, F	SRS (3), resection (2)	52	39	5.0	93.9	39.7	3
2	anaplastic	48, F	EBRT, SRS, resection (5)	31	59	2.8	73.2	44.2	3
3	anaplastic	60, F	SRS, resection (5)	50	49	none	49.0	32.0	1
4	papillary	67, M	EBRT, resection (2)	42	70	5.0	71.8	22.1	2
5	anaplastic	77, F	SRS (3), resection (4)	24	70	4.5	86	37.2	1
6	atypical	72, F	SRS, resection (2)	66	69	2.0	65.6	19.0	2
7	sarcoma	57, F	SRS (2), resection (4)	47	92	2.7	48.3	14.3	2
8	rhabdoid	26, F	EBRT, resection (3)	24	66	3.1	75.8	18.8	1
9	anaplastic	62, M	EBRT, resection (3)	70	66	4.4	111.5	50.7	1
10	anaplastic	56, F	SRS, resection (2)	25	74	3.9	77.3	26.0	1
11	anaplastic	38, M	EBRT, resection (3)	27	68	3.9	88.9	28.8	1
12	anaplastic	67, F	SRS, resection (4)	24	54	3.5	58.0	22.1	1
13	anaplastic	65, F	EBRT, SRS (2), resection (4)	34	64	3.6	50.2	24.2	1
14	atypical	75, F	SRS (3), resection (3)	80 + 40	29	4.0	72.9	42.3	1
15	anaplastic	79, M	SRT, resection (5)	88	26	3.7	61.0	57.2	1
16	atypical	68, M	SRS (2), resection (2)	52	45	2.7	67.0	52.0	1
17	anaplastic	49, F	SRS, resection (6)	61	74	4.0	68.5	15.0	1
18	anaplastic	50, M	SRS (4), resection (3)	43	52	4.4	100.0	59.0	1
19	anaplastic	63, F	SRS (2), SRT, resection (4)	94	76	4.0	69.5	31.0	2
20	atypical	41, M	SRS (4), resection (5)	48	60	3.0	80.2	32.0	1

* T/N = tumor-to-normal-brain ratio.

consecutive images obtained in each patient's follow-up evaluation. Then, changes in these values over time were graphed and investigated for trends among our 20 patients with high-grade meningiomas undergoing BNCT.

Survival Analysis

Patient survival was defined in 2 ways: the number of months survived after diagnosis of high-grade meningioma and the number after the application of BNCT.

Results

Absorbed Dose in Tumor Tissue

The duration of irradiation was planned to not exceed 15 Gy-Eq in normal brain tissue. The absorbed dose to the tumor tissue was dependent on both the boron concentration in the tumor tissue and the neutron irradiation time, which varied in each case. Therefore, the absorbed dose to the tumor tissue in our protocol was not uniform from case to case. The mean maximum and minimum absorbed doses in our series were 73.4 Gy-Eq (95% CI 65.6–81.3 Gy-Eq) and 33.3 Gy-Eq (95% CI 26.9–39.9 Gy-Eq), respectively (Table 1). These absorbed doses were administered not in fractionation but in 1-time irradiation in BNCT.

Volume Reduction of the Treated Mass During Follow-Up

As we reported previously, all of the initial case series (Cases 1–7) showed volume reduction of the mass during the observation.[23,32] In Fig. 1 we show representative volume reductions of the treated masses from more recent cases (Cases 14, 17, 18, and 20). All other treated tumors also showed definitive shrinkage during follow-up; this tendency toward volume reduction is illustrated in Fig. 2. Although all tumors showed a trend of gradual reduction in volume, a transient increase after BNCT was observed in some cases, usually within a month of treatment, before decreasing; this pattern was called pseudoprogression. The mean tumor size prior to BNCT was 52.2 cm^3, ranging from 4.3 cm^3 to 109 cm^3. A mean volume reduction of 64.5% was achieved within only 2 months of BNCT (Fig. 2).

Treatment Failure

Among the 20 cases of high-grade meningioma treated by BNCT, 6 cases demonstrated systemic metastasis, including lung, vertebral bone, clavicle, liver, and lymph node metastasis (Table 2). Four of these 6 patients died from these metastatic lesions and not the original intracranial lesions. A typical systemic metastasis is depicted in Fig. 3 (Case 13, A–D; Case 7, E–H). Figures 3A and B show good local control of the anaplastic meningioma over the first 3 years, compared with the patient's repeated local tumor recurrence every few months prior to BNCT, even with repetitive SRS treatment. This patient died due to lung metastasis (Fig. 3D). Prior to BNCT, the patient had had a metastatic lesion in the left clavicle, which was controlled by EBRT for 3 years during the observation

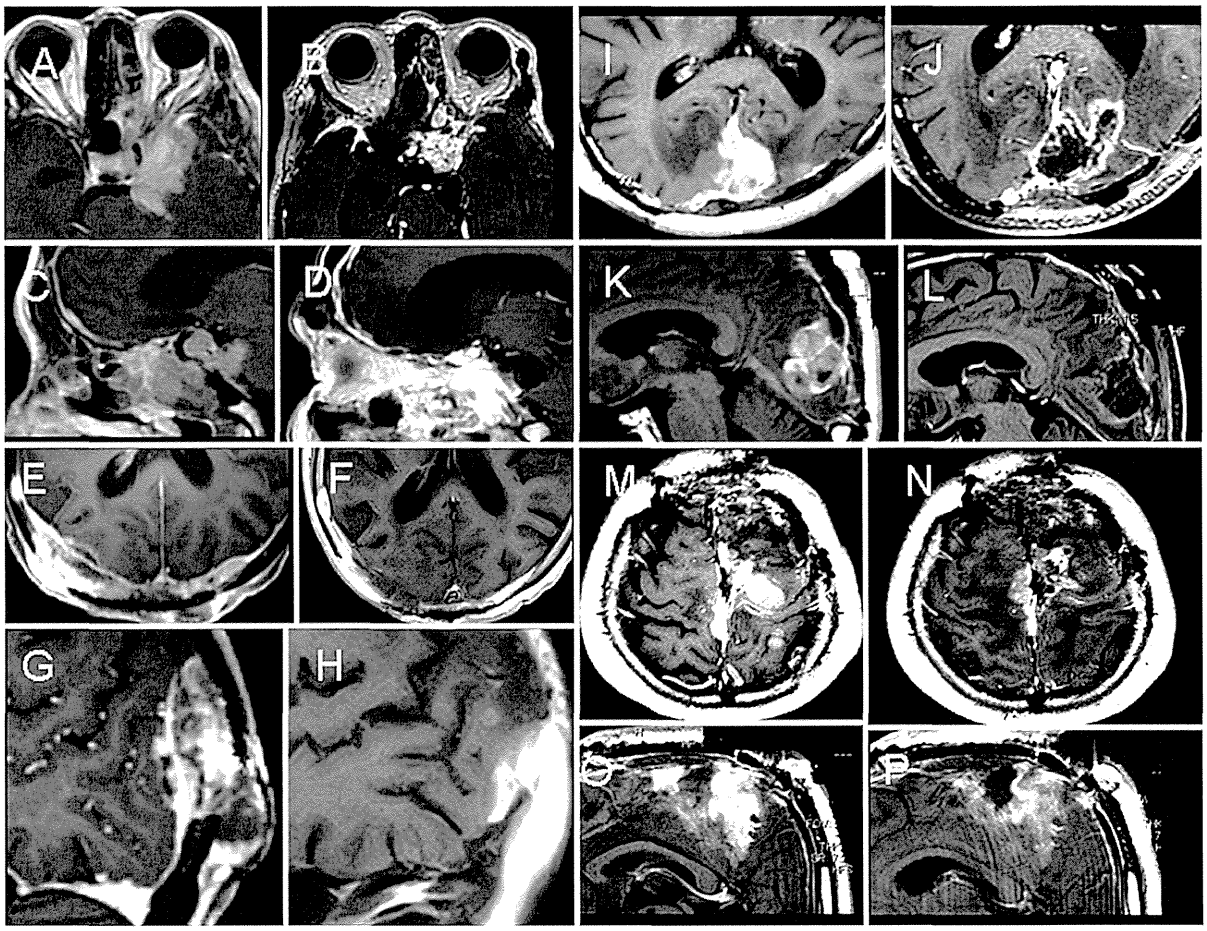

FIG. 1. Representative tumor shrinkage after BNCT demonstrated on axial (A, B, E, F, I, J, M and N) and sagittal (C, D, G, H, K, L, O, and P) Gd-enhanced MR images. Each case shows marked tumor volume reduction after BNCT. **A–D:** Case 17. Images of an anaplastic meningioma before (A and C) and 10 months after BNCT (B and D). **E–H:** Case 14. Images of an atypical meningioma before (E and G) and 6 months after BNCT (F and H). **I–L:** Case 18. Images of an anaplastic meningioma before (I and K) and 10 months after BNCT (J and L). **M–P:** Case 20. Images of an atypical meningioma before (M and O) and 4 months after BNCT (N and P).

period. Also in this case, we experienced tumor recurrence out of the field of neutron irradiation (Fig. 3C).

In Case 7, in which anaplastic meningioma developed into sarcoma, there was continuous tumor shrinkage during the 7 months of follow-up (Fig. 3E and F). This patient developed liver metastasis (Fig. 3G), and died due to dyspnea from the lung metastasis (Fig. 3H). Unfortunately we had no chance to verify that the histological diagnosis of the metastatic lesions was the same as that of the original high-grade meningioma.

Seven of the 20 BNCT-treated high-grade meningioma cases showed recurrence outside the field of neutron irradiation (Table 2). A representative recurrence outside the field of neutron irradiation is depicted in Fig. 3C; this lesion was discovered incidentally 33 months after BNCT. We did not apply a second BNCT for this recurrent lesion because multiple metastases had already been identified in the lung. One patient (Case 14, atypical meningioma) chose to abandon further treatment because of financial difficulties and died due to the metastatic lesion.

Three of the 20 cases experienced increased intracranial pressure by intractable hydrocephalus due to CSF dissemination (Table 2). This type of hydrocephalus could not be controlled by a shunting operation due to the viscosity of the CSF. Typical images are depicted in Fig. 3I–K (Case 11, anaplastic meningioma).

Only 3 of 20 patients died due to local tumor progression (Table 2). Two of these 3 local tumor progression cases were complicated with symptomatic radiation necrosis.

Survival Analysis After Diagnosis and BNCT

The median follow-up duration was 13 months. Six patients are still alive; at present, the median survival times after BNCT and diagnosis are 14.1 months (95% CI 8.6–40.4 months) and 45.7 months (95% CI 32.4–70.7 months), respectively (Fig. 4). It is rather difficult to compare our results to other reports[28] because our patients were refractory to any existent treatments; therefore our study sample was biased due to multiple treatments with repetitive surgeries and radiotherapies (Table 1). Also, many patients in our study died due to systemic metastasis and recurrence outside of the radiation field, as noted above (Table 2).

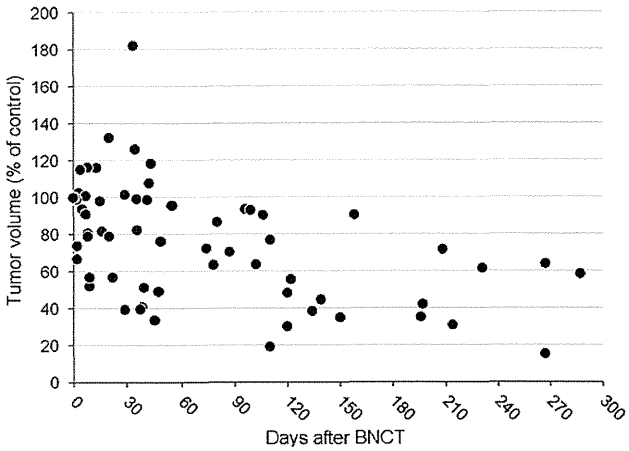

FIG. 2. Scatterplot of the relationship of tumor volume relative to the elapsed time (days) after BNCT. Day 0 means the date of the treatment, and the value (100%) plotted on Day 0 was actually acquired 1–3 weeks before treatment. All the values obtained from each patient from BNCT to relapse were plotted on the same graph.

Discussion

In this report, we present evidence of favorable local control of high-grade meningiomas using BNCT. Only 3 patients died due to local treatment failure, although the follow-up observation periods were rather brief in several recent cases. However, irrespective of good local control of high-grade meningiomas by BNCT, many patients died and the median patient survival time in high-grade meningiomas after BNCT was 14.1 months (Fig. 4). The most prominent pattern of treatment failure in our series was intracranial distant recurrence outside the radiation field, experienced in 7 of 20 cases. The other patterns of treatment failure were systemic metastasis in 6 cases and CSF dissemination in 3 cases. Given that the reported incidence of CSF dissemination in meningioma is only 2%,[5] our series shows a rather high incidence rate. With regard to systemic metastasis in high-grade meningioma, such high incidences have been previously reported in the literature.[5,9] We are not entirely sure of the reason for this high incidence of systemic metastasis. It may be possible that the treatment-refractory nature of our cases resulted in a selection bias for aggressive care and longer survival. Overall, the high incidences of out-of-field recurrence, CSF dissemination, and systemic metastasis might be ascribed to the advanced stage of the patients in our series, as all cases were referred to us after the failure of local tumor control, even using repetitive surgeries and radiotherapies. This selection bias for refractory cases makes it difficult to compare our treatment failure results with the incidence rates reported in the literature. Our patients' advanced tumor stage also makes it difficult to compare the survival data with that of other treatments. All high-grade meningioma cases in this series were recurrent, complicated cases.

TABLE 2: Clinical results and treatment failure patterns after BNCT in 20 patients with malignant high-grade meningiomas*

Case No.	Longevity (mos)		Systemic Metastasis	Dissemination	Symptomatic Radiation Necrosis	Local Recurrence		Cause of Death
	From Diagnosis	From BNCT				Out of Field	In Field	
1	44.2	22.4	yes	no	yes	yes	yes	local progression, radiation necrosis
2	43.2	14.1	yes	no	no	no	no	metastasis
3	32.4	12.9	no	no	yes	yes	no	gastric cancer metastasis
4	70.7	13.1	no	no	no	no	yes	local progression
5	45.7	24.6	no	no	no	no	no	senility
6	64.3	6.4	no	no	yes	no	no	radiation necrosis (DIC)
7	113.4	8.6	yes	no	no	no	no	metastasis
8	30.4	7.3	no	yes	no	no	no	dissemination
9	36.8	9.4	no	yes	yes	no	no	dissemination
10	47.5	44.0	no	no	yes	yes	yes	local progression, radiation necrosis
11	28.3	12.4	no	yes	no	no	no	dissemination
12	56.8	55.6	no	no	no	no	yes	alive
13	59.6	40.4	yes	no	no	yes	no	metastasis (lung, and others)
14	12.3	7.7	no	no	no	yes	no	remote recurrence
15	12.8	8.3	yes	no	no	no	no	metastasis (lung, liver)
16	69.1	15.0	yes	no	no	no	no	alive w/ metastasis (lung)
17	22.1	15.6	no	no	no	no	no	alive
18	68.6	10.7	no	no	no	yes	no	alive
19	32.4	10.4	no	no	no	no	no	alive
20	30.1	8.6	no	no	no	yes	no	alive

* DIC = disseminated intravascular coagulation.

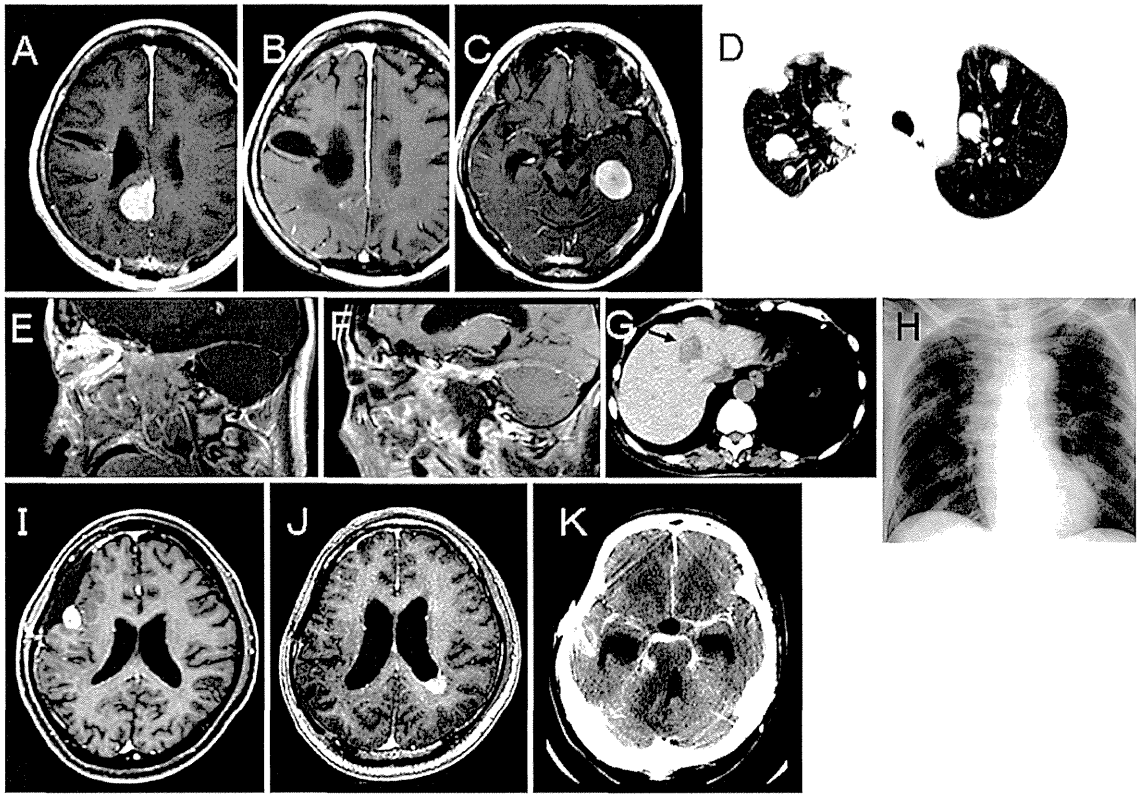

FIG. 3. Typical images of systemic metastasis, intracranial recurrence outside the field of neutron irradiation, and CSF dissemination. **A–D:** Case 13 (anaplastic meningioma). Axial contrast-enhanced MR images obtained prior to BNCT (A) and 33 months after BNCT (B and C), and plain chest CT scan obtained 39 months after BNCT (D). The MRI (C) shows intracranial recurrence out of the field of neutron irradiation. The chest CT scan (D) shows lung metastasis of the meningioma. This patient died due to dyspnea. The patient had already suffered from metastasis of the meningioma at the left clavicle prior to BNCT. **E–H:** Case 7 (sarcoma transformed from anaplastic meningioma). Sagittal contrast-enhanced MR image obtained prior to BNCT (E); sagittal contrast-enhanced MR image (F), plain abdominal axial CT scan (G), and chest radiograph (H) obtained 7 months after BNCT. The original, very large tumor was well controlled by BNCT, but lung and liver metastasis occurred. This patient died due to dyspnea. The *black arrow* (G) shows liver metastasis. **I–K:** Case 11 (anaplastic meningioma). Axial contrast-enhanced MR images obtained prior to BNCT (I) and 7 months after BNCT (J); axial contrast-enhanced CT scan (K) obtained 10 months after BNCT. The original tumor was controlled well, but CSF dissemination with untreatable hydrocephalus occurred.

Historically, conventional EBRT was first used to treat high-grade meningiomas, but with unsatisfactory results.[20] In our series, the original high-grade meningiomas were not controlled locally by EBRT alone. Thereafter, SRS was used for the treatment of high-grade meningiomas, as reported in the literature.[13,27,29,31] The gross tumor volume treated by SRS in these studies was relatively small in comparison with that in our series. Additionally, in our series, 9 patients had already received repetitive SRS but experienced recurrence nonetheless. The typical treatment failure pattern of high-grade meningiomas by SRS in our series was marginal recurrence at the SRS fields. Even for these SRS-refractory cases, BNCT was able to provide good local tumor control.

Particle radiotherapies using proton beams[2,6,14,26,30,33] and carbon ion beams[8,30] have been more recently applied

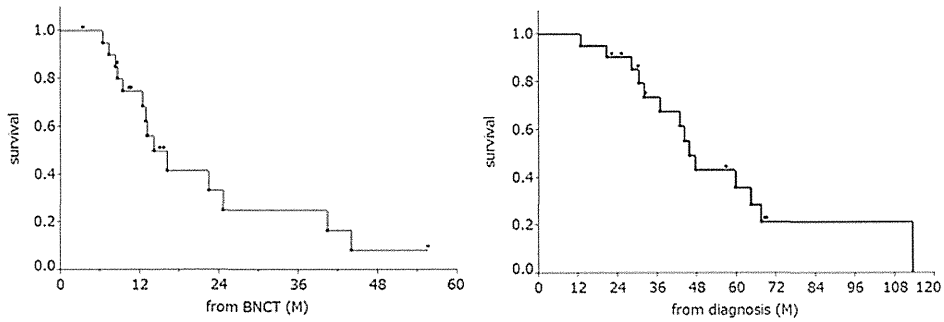

FIG. 4. Graphs of Kaplan-Meyer survival curves after BNCT **(left)** and diagnosis **(right)**. The median survival times after BNCT and diagnosis were 14.1 months (95% CI 8.6–40.4 months) and 45.7 months (95% CI 32.4–70.7 months), respectively.

to cases of high-grade meningioma. Again, it is very difficult to compare our data to the data from particle radiotherapies. First, almost all reported series, including our own, have comprised a limited number of cases. The protocols have varied as well: in some studies, particle therapy was applied just after surgery as the initial radiotherapy, and in others it was applied at recurrence. The applied doses varied and in some trials, particle radiation was followed by fractionated EBRT. In addition, the data on tumor shrinkage after particle irradiation have been scarce. There has been only 1 preliminary report addressing this subject, and the results indicated no prominent early tumor shrinkage using proton and carbon ion beams for the treatment of high-grade meningiomas.[30]

One of the advantages of BNCT is that the radiation field may be planned rather more ambiguously than in SRS and other particle radiotherapies. This merit of BNCT might decrease recurrence in the peri-irradiated field in comparison with other radiation techniques, even with the same absorbed dose as described as "Gy-Eq." Encouragingly, almost all masses in our series responded well, with rapid shrinkage after BNCT (Figs. 2 and 3), as also reported elsewhere.[23] This rapid shrinkage might contribute to the prompt recovery of symptoms in some cases. Our patient in Case 1 became ambulatory 1 week after BNCT, and our patient in Case 7 experienced relief from facial pain within 2 weeks of BNCT, as reported previously.[23,32]

In BNCT, most potent antitumor effects are caused by particles, and we applied 33.3 Gy-Eq and 73.4 Gy-Eq for tumor tissue as minimum and maximum 1-time tumor doses, respectively (Table 1). In the literature on particle radiation, some clinical trials have used proton or carbon particle doses between 18 Gy-Eq and 56 Gy-Eq with fractionation.[8,33] The difference in tumor shrinkage between α and lithium particles and other particles such as carbon and protons may be ascribed to the difference of linear energy transfer. The linear energy transfer of α and lithium particles is higher than that of both protons and carbon particles. It is widely accepted that high linear energy transfer particles have greater biological effects than low linear energy transfer particles;[1,3] of course, there might be other causes. For example, in BNCT a large dose can be delivered at a single time, while other particles are usually applied with fractionation and additional low linear energy transfer EBRT. Because of this difference in protocol, other particles might have less impact on tumor shrinkage.

With respect to adverse effects of BNCT, we experienced 6 cases of symptomatic radiation injury among our 20 cases. One instance was the occurrence of subacute brain swelling after BNCT, as reported previously,[23] while the other 5 cases appeared to show radiation necrosis. Because all cases were introduced to our institute after intensive radiotherapies prior to BNCT, radiation necrosis may have been inevitable, despite the tumor-selective nature of BNCT. Recently, we applied BNCT to a patient with a high-grade meningioma who had never been treated with any radiotherapy, and are now observing this case carefully. Bevacizumab has shown potent effects treating symptomatic radiation necrosis in the brain,[12,18] and we have applied this drug for symptomatic radiation necrosis after BNCT for malignant gliomas.[11] This strategy should be applicable and effective for the treatment of radiation necrosis after BNCT for high-grade meningiomas.

We should emphasize that we found pseudoprogression after BNCT in at least 3 of our 20 high-grade meningioma cases. As we described previously,[22] this phenomenon could itself be an indicator of how promising and intensive the effects of this treatment are.

Conclusions

Boron neutron capture therapy is a new treatment concept and method that has already been used on malignant gliomas, including glioblastomas. Our study suggests that high-grade meningiomas may be an even better candidate for BNCT than those lesions. The meningiomas in our series were somewhat superficial (located on the surface of the brain), except for some specific situations at the skull base, which is advantageous to neutron penetration.

With regard to BPA accumulation, high-grade meningiomas showed a good ratio of tumor to normal brain, even compared with malignant gliomas (Table 1). In addition, judging from the rapid shrinkage of the mass, our assumption about the compound biological effectiveness of BPA for high-grade meningioma—which was assumed to be equal to that of glioblastoma—might have been an underestimation; the real value might be higher than that for glioblastoma. If we can apply BNCT for high-grade meningioma as the initial radiotherapy or at least at the first recurrence, rather than at such advanced stages, more favorable results than those described in our study might be obtained, such as avoiding systemic metastasis or out-of-field recurrence.

Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Miyatake, Kawabata, Ono. Acquisition of data: Miyatake, Kawabata, Hitramatsu. Analysis and interpretation of data: Miyatake, Kawabata, Hitramatsu. Drafting the article: Miyatake, Kawabata. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Miyatake. Study supervision: Miyatake, Kuroiwa, Ono.

References

1. Barendsen GW: RBE-LET relationships for different types of lethal radiation damage in mammalian cells: comparison with DNA dsb and an interpretation of differences in radiosensitivity. **Int J Radiat Biol 66:**433–436, 1994
2. Boskos C, Feuvret L, Noel G, Habrand JL, Pommier P, Alapetite C, et al: Combined proton and photon conformal radiotherapy for intracranial atypical and malignant meningioma. **Int J Radiat Oncol Biol Phys 75:**399–406, 2009
3. Broerse JJ, Barendsen GW, van Kersen GR: Survival of cultured human cells after irradiation with fast neutrons of dif-

ferent energies in hypoxic and oxygenated conditions. **Int J Radiat Biol Relat Stud Phys Chem Med 13:**559–572, 1968
4. Chamberlain MC: The role of chemotherapy and targeted therapy in the treatment of intracranial meningioma. **Curr Opin Oncol 24:**666–671, 2012
5. Chamberlain MC, Glantz MJ: Cerebrospinal fluid-disseminated meningioma. **Cancer 103:**1427–1430, 2005
6. Chan AW, Bernstein KD, Adams JA, Parambi RJ, Loeffler JS: Dose escalation with proton radiation therapy for high-grade meningiomas. **Technol Cancer Res Treat 11:**607–614, 2012
7. Coderre JA, Morris GM: The radiation biology of boron neutron capture therapy. **Radiat Res 151:**1–18, 1999
8. Combs SE, Hartmann C, Nikoghosyan A, Jäkel O, Karger CP, Haberer T, et al: Carbon ion radiation therapy for high-risk meningiomas. **Radiother Oncol 95:**54–59, 2010
9. Enam SA, Abdulrauf S, Mehta B, Malik GM, Mahmood A: Metastasis in meningioma. **Acta Neurochir (Wien) 138:**1172–1178, 1996
10. Farr LE, Sweet WH, Robertson JS, Foster CG, Locksley HB, Sutherland DL, et al: Neutron capture therapy with boron in the treatment of glioblastoma multiforme. **Am J Roentgenol Radium Ther Nucl Med 71:**279–293, 1954
11. Furuse M, Kawabata S, Kuroiwa T, Miyatake SI: Repeated treatments with bevacizumab for recurrent radiation necrosis in patients with malignant brain tumors: a report of 2 cases. **J Neurooncol 102:**471–475, 2011
12. Gonzalez J, Kumar AJ, Conrad CA, Levin VA: Effect of bevacizumab on radiation necrosis of the brain. **Int J Radiat Oncol Biol Phys 67:**323–326, 2007
13. Hakim R, Alexander E III, Loeffler JS, Shrieve DC, Wen P, Fallon MP, et al: Results of linear accelerator-based radiosurgery for intracranial meningiomas. **Neurosurgery 42:**446–454, 1998
14. Hug EB, Devries A, Thornton AF, Munzenride JE, Pardo FS, Hedley-Whyte ET, et al: Management of atypical and malignant meningiomas: role of high-dose, 3D-conformal radiation therapy. **J Neurooncol 48:**151–160, 2000
15. Imahori Y, Ueda S, Ohmori Y, Kusuki T, Ono K, Fujii R, et al: Fluorine-18-labeled fluoroboronophenylalanine PET in patients with glioma. **J Nucl Med 39:**325–333, 1998
16. Imahori Y, Ueda S, Ohmori Y, Sakae K, Kusuki T, Kobayashi T, et al: Positron emission tomography-based boron neutron capture therapy using boronophenylalanine for high-grade gliomas: part I. **Clin Cancer Res 4:**1825–1832, 1998
17. Jääskeläinen J, Haltia M, Servo A: Atypical and anaplastic meningiomas: radiology, surgery, radiotherapy, and outcome. **Surg Neurol 25:**233–242, 1986
18. Levin VA, Bidaut L, Hou P, Kumar AJ, Wefel JS, Bekele BN, et al: Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. **Int J Radiat Oncol Biol Phys 79:**1487–1495, 2011 (Erratum in **Int J Radiat Oncol Biol Phys 84:**6, 2012)
19. Locher G: Biological effects and therapeutic possibilities of neutrons. **Am J Roentgenol Radium Ther 36:**1–13, 1936
20. Mahmood A, Caccamo DV, Tomecek FJ, Malik GM: Atypical and malignant meningiomas: a clinicopathological review. **Neurosurgery 33:**955–963, 1993
21. Miyatake S, Kawabata S, Kajimoto Y, Aoki A, Yokoyama K, Yamada M, et al: Modified boron neutron capture therapy for malignant gliomas performed using epithermal neutron and two boron compounds with different accumulation mechanisms: an efficacy study based on findings on neuroimages. **J Neurosurg 103:**1000–1009, 2005
22. Miyatake S, Kawabata S, Nonoguchi N, Yokoyama K, Kuroiwa T, Matsui H, et al: Pseudoprogression in boron neutron capture therapy for malignant gliomas and meningiomas. **Neuro Oncol 11:**430–436, 2009
23. Miyatake S, Tamura Y, Kawabata S, Iida K, Kuroiwa T, Ono K: Boron neutron capture therapy for malignant tumors related to meningiomas. **Neurosurgery 61:**82–91, 2007
24. Morris GM, Coderre JA, Hopewell JW, Micca PL, Fisher C: Boron neutron capture irradiation of the rat spinal cord: effects of variable doses of borocaptate sodium. **Radiother Oncol 39:**253–259, 1996
25. Morris GM, Coderre JA, Micca PL, Fisher CD, Capala J, Hopewell JW: Central nervous system tolerance to boron neutron capture therapy with p-boronophenylalanine. **Br J Cancer 76:**1623–1629, 1997
26. Noël G, Habrand JL, Mammar H, Haie-Meder C, Pontvert D, Dederke S, et al: Highly conformal therapy using proton component in the management of meningiomas. Preliminary experience of the Centre de Protonthérapie d'Orsay. **Strahlenther Onkol 178:**480–485, 2002
27. Ojemann SG, Sneed PK, Larson DA, Gutin PH, Berger MS, Verhey L, et al: Radiosurgery for malignant meningioma: results in 22 patients. **J Neurosurg 93 (Suppl 3):**62–67, 2000
28. Palma L, Celli P, Franco C, Cervoni L, Cantore G: Long-term prognosis for atypical and malignant meningiomas: a study of 71 surgical cases. **J Neurosurg 86:**793–800, 1997
29. Pollock BE, Stafford SL, Link MJ, Garces YI, Foote RL: Stereotactic radiosurgery of World Health Organization grade II and III intracranial meningiomas: treatment results on the basis of a 22-year experience. **Cancer 118:**1048–1054, 2012
30. Rieken S, Habermehl D, Haberer T, Jaekel O, Debus J, Combs SE: Proton and carbon ion radiotherapy for primary brain tumors delivered with active raster scanning at the Heidelberg Ion Therapy Center (HIT): early treatment results and study concepts. **Radiat Oncol 7:**41, 2012
31. Stafford SL, Pollock BE, Foote RL, Link MJ, Gorman DA, Schomberg PJ, et al: Meningioma radiosurgery: tumor control, outcomes, and complications among 190 consecutive patients. **Neurosurgery 49:**1029–1038, 2001
32. Tamura Y, Miyatake S, Nonoguchi N, Miyata S, Yokoyama K, Doi A, et al: Boron neutron capture therapy for recurrent malignant meningioma. Case report. **J Neurosurg 105:**898–903, 2006
33. Weber DC, Schneider R, Goitein G, Koch T, Ares C, Geismar JH, et al: Spot scanning-based proton therapy for intracranial meningioma: long-term results from the Paul Scherrer Institute. **Int J Radiat Oncol Biol Phys 83:**865–871, 2012

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腫瘍細胞選択的粒子線治療「ホウ素中性子捕捉療法」と抗血管新生薬による症候性脳放射線壊死の治療

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Cell-selective Particle Radiation, Boron Neutron Capture Therapy and Treatment of Symptomatic Radiation Necrosis in the Brain by Anti-angiogenic Agent

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Boron neutron capture therapy (BNCT) has been advocated as a novel particle radiation therapy for malignant tumors that targets tumor cells biologically. Since 2002, we have applied this unique radiotherapy for 133 malignant gliomas and malignant meningiomas at our institution. In addition, we recently applied anti-angiogenic agents aggressively for intractable symptomatic radiation necrosis in the brain.

Here is our latest comprehensive data regarding these unique treatments, including those I presented at the 32nd annual meeting of the Japanese Neurosurgical Congress, along with some new findings.

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Key words : bevacizumab, boron neutron capture therapy (BNCT), positron emission tomography (PET), radiation necrosis

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はじめに

Boron neutron capture therapy（以下 BNCT）は原理上腫瘍に対する細胞選択的照射が可能な唯一の放射線治療法である．ホウ素（^{10}B）化合物を投与し，その後，熱中性子もしくは熱外中性子を照射する．ホウ素化合物自体には細胞毒性はなく，また中性子の殺細胞効果もきわめて小さいが，ホウ素同位体^{10}B 原子核は中性子を捕獲し，きわめて線エネルギー付与（粒子が 1 μm 運動する間に周囲に付与するエネルギー：keV/μm）の高いヘリウム原子核（α粒子）とリチウム反跳核をそれぞれ，9 μm と 4 μm という，細胞 1 個に相当する距離に放出し，その細胞を破壊する細胞選択的な粒子線治療ともいえる（Fig. 1）[3)]．すなわち殺細胞効果はホウ素中性子捕獲反応の生じた細胞に限局され，隣接する細胞には影響を及ぼさない．そこで，ホウ素化合物を腫瘍に選択的に集積できれば，腫瘍選択的な細胞破壊が可能となる．

本稿では，まず BNCT 時にその適応決定，線量 simulation に用いる F-BPA-PET を紹介し，次いで悪性神経膠腫に対する治療効果，悪性髄膜腫に対する治療効果，F-BPA-PET による治療効果の判定や放射線壊死，pseudoprogression の鑑別を紹介する．さらには原子炉に代わる新規中性子源として開発してきた小型加速器による治験を紹介する．最後に高線量放射線治療の宿命ともいえ

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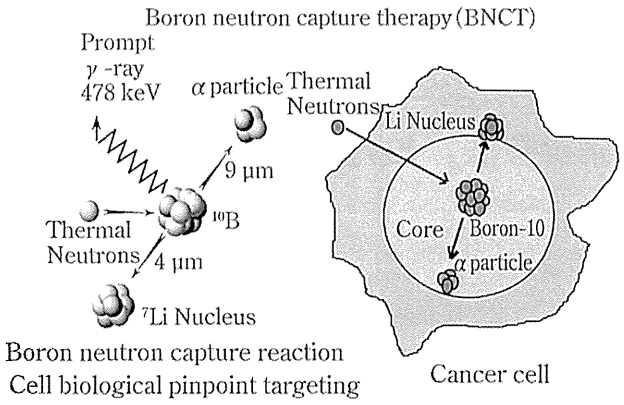

Fig. 1 Principle of BNCT

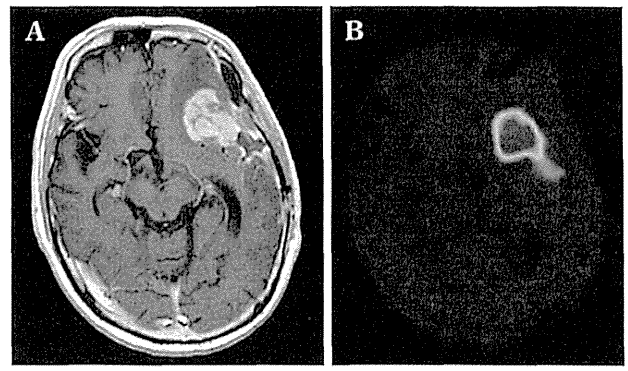

Fig. 2 Typical F-BPA-PET findings in glioblastoma multiforme (GBM)
A: T1-Gd enhanced MRI revealed a left frontal mass. B: F-BPA-PET imaging showed marked BPA accumulation not only in the enhanced area but also in the surrounding brain. The lesion/normal brain ratio of the tracer uptake in this case was 7.1.

る，脳放射線壊死に対する抗血管新生薬による治療法とその薬事申請への行程を紹介する．

F-BPA-PET

BNCT における治療用化合物 BPA (boronophenylalanine) を用いた PET 検査を中性子照射に先立って行う．BPA は文字通りホウ素化した phenylalanine であり，腫瘍において亢進したアミノ酸代謝を利用し，腫瘍内に能動的かつ選択的に集積される．フッ素ラベルした BPA をトレーサとして利用することにより，PET により腫瘍内および脳内 BPA 濃度が推測され，治療の適応決定および照射線量が simulation できる．**Fig. 2** に BPA-PET による BPA の取り込みを示す．この症例では左前頭葉部腫瘍は反対側正常脳に比べて，7.1 倍のトレーサの集積を示している．この PET が示す情報は大きく，2 つの情報が存在する．1 つは 7.1 倍という数字は，同一部位に腫瘍細胞と正常細胞が存在すれば（浸潤部領域にそのような situation が想像できる），腫瘍細胞は正常細胞の 7.1 倍の粒子線を吸収することを示す．もう 1 つの情報は，造影 MRI に比較して，その外側にもトレーサの集積を認めることより，造影域より外側に浸潤している細胞にも targeting できていることを物語っている[10]．

悪性神経膠腫に対する BNCT の効果

悪性黒色腫とともに最も初期より BNCT が適応されてきた疾患が悪性神経膠腫であった．われわれは再発悪性神経膠腫に BNCT を適応し，すべての症例で画像上顕著な効果を認めた[7)10)]．また，新規診断膠芽腫にも積極的に BNCT を適応し，化学療法なしに良好な成績を収めている[8)]．この経験をもとに，現在 BNCT 後に追加 X 線外照射および temozolomide を併用した，新規診断膠芽腫に対する多施設共同研究を厚生労働科学研究費のサポートをいただき，展開中である．

初発膠芽腫に対する BNCT の効果および問題点を示す症例を **Fig. 3** として提示する．左側脳室三角部近傍の膠芽腫である．手術による部分摘出の後，BNCT を施行した．BNCT 施行前，施行 8 カ月後の頭部 MRI および脊髄 MRI を A，B，C として提示している．BNCT は良好な局所制御を示しているが，脊髄髄腔内播種をきたし，この症例を亡くしている．われわれの BNCT の経験では，このように局所制御は比較的良好であるが，およそ半分の症例は髄腔内播種で亡くしている．今後の課題と考えている．

すでに放射線治療歴を有する再発悪性神経膠腫に対しても，本治療法は細胞選択性を有するので，積極的な照射を行ってきた．再発神経膠腫に対する RPA 分類を用いて[2)]，BNCT の成績と既存治療法の成績を比較すると，予後不良群でその生存期間中央値を有意に延長している[13)]．しかしながら，たとえば **Fig. 2** に示した症例では，病変と正常脳のトレーサの集積比が 7.1 倍と高値を示すが，逆に考えると正常脳は腫瘍の 1/7.1 の粒子線を被曝する．再発例の場合にはすでに許容線量限界に近い放射線治療が施行されているので，BNCT といえども，脳放射線壊死が問題となる．この点に関しては後述の抗血管新生療法を展開している．

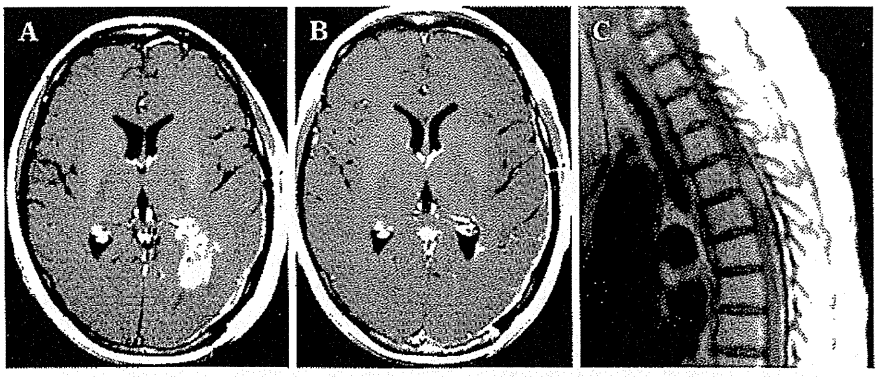

Fig. 3 Periodic Gd-enhanced MRI findings of a GBM case treated by BNCT. A newly diagnosed GBM case in which the left trigonal lesion was treated by BNCT.

A: Brain MRI, prior to BNCT. B: Brain MRI, 8 months after BNCT. C: Spinal MRI, 8 months after BNCT, showing CSF dissemination of the lesion at the spinal cord.

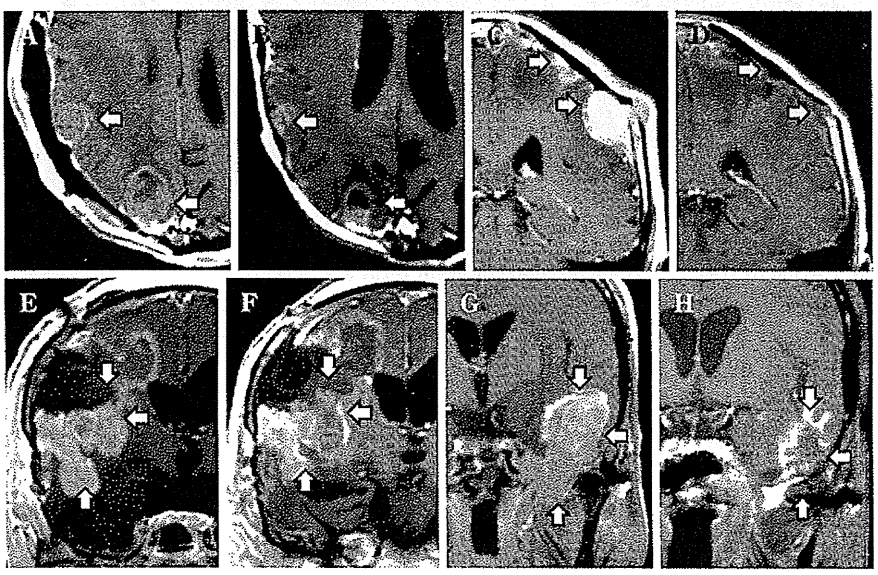

Fig. 4 Typical MRI changes of malignant meningiomas treated by BNCT

A, B: Prior to BNCT and 3 months after BNCT of a anaplastic meningioma.
C, D: Prior to BNCT and 4 months after BNCT of a anaplastic meningioma.
E, F: Prior to BNCT and 5 months after BNCT of an anaplastic meningioma.
G, H: Prior to BNCT and 4 months after BNCT of a rhabdoid meningioma.

高グレード髄膜腫に対する BNCT の効果

高グレード髄膜腫（high grade meningioma：HGM）は手術，定位放射線治療を行っても，その予後は悪い[6)18)]．ことに WHO grade 3 に属する anaplastic meningioma の予後は悪い．われわれは世界に先駆けて，これら HGM に対しても積極的に BNCT を適応してきた[11)19)]．Fig. 4 に anaplastic meningioma 3 例，rhabdoid meningioma 1 例の BNCT 前後の MRI を示す．この 4 例以外でもすべての症例で画像上顕著な腫瘍縮小効果を経験している．われわれの施設に紹介をいただく症例はすべて，複数回の手術，X 線外照射，定位放射線治療等が施行され，それでも制御不能な治療不応症例であるが，良好な局所制御を認めている．2011 年 9 月までに治療を終え，その後 1 年以上の経過を観察しえた 20 例では，局所再発は 4 例で認めたのみであるが，多くの症例を BNCT 後に失っ

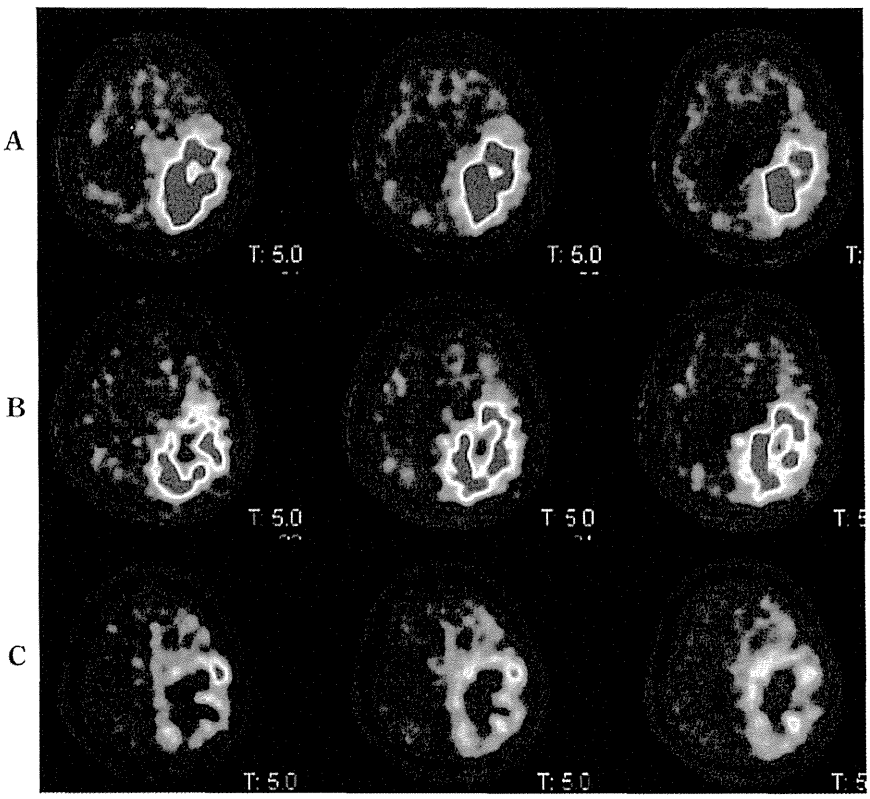

Fig. 5 Periodic change of F-BPA-PET imaging of a recurrent anaplastic astrocytoma treated by BNCT
A：Prior to BNCT, the lesion/normal brain ratio was 7.0.
B：3 months after BNCT, the lesion/normal brain ratio was 3.5.
C：9 months after BNCT, the lesion/normal brain ratio was 2.5.

ている．このうちの7例は照射野外再発，6例が全身転移によるものであり，3例が髄腔内播種による難治性水頭症による頭蓋内圧亢進により失っており，この疾患のコントロールの困難さを痛感している（論文印刷中）．これらはいずれも疾患が治療時にはすでに進行していることに起因しており，これを回避するには初期の再発時にBNCTを行う以外に有効な手立てはないと考えている．

F-BPA-PETによる治療効果判定

Fig. 5 に再発神経膠腫（anaplastic astrocytoma）のBNCT前，BNCT 3カ月後，9カ月後のF-BPA-PETを示す．BNCT 3カ月後に本PETを施行した理由は若干の浮腫，造影域の拡大を認めたため，腫瘍のprogressionかpseudoprogressionかの判断を行うために施行したものであり，このPETにより病変/正常脳比の低下を確認してpseudoprogressionと判断し，経過を観察している．9カ月後のPETではさらにこの比が低下を示しており，良好な治療効果を確認している．本症例は前述の再発神経膠腫に対するRPA分類ではクラス3に分類され，再発時治療後の生存期間中央値は文献上，わずか3.8カ月と報告されている．本症例は，本稿準備時すでにBNCT後11カ月が経過しているが再発の徴候は認めていない．われわれは本PETを腫瘍再発と脳放射線壊死の鑑別[9]やpseudoprogressionとtrue progressionの鑑別に用いている[12]．

加速器中性子源によるBNCT

上述のBNCTはすべて原子炉からの中性子を用いたBNCTの成績である．原子炉を用いる限り，またホウ素化合物も医薬品としてのGMPグレードを開発しない限り，BNCTは医療としては認知されない．数年前にわれわれが厚生労働省に本治療を当時の高度先進医療に申請した折から，この点は指摘されており，大きな宿題であった．この要求に応えるため，某製薬メーカーと医療機器開発メーカーの主導により，加速器中性子源とGMPグレードBPAの開発が行われ，著者が治験責任医師とな

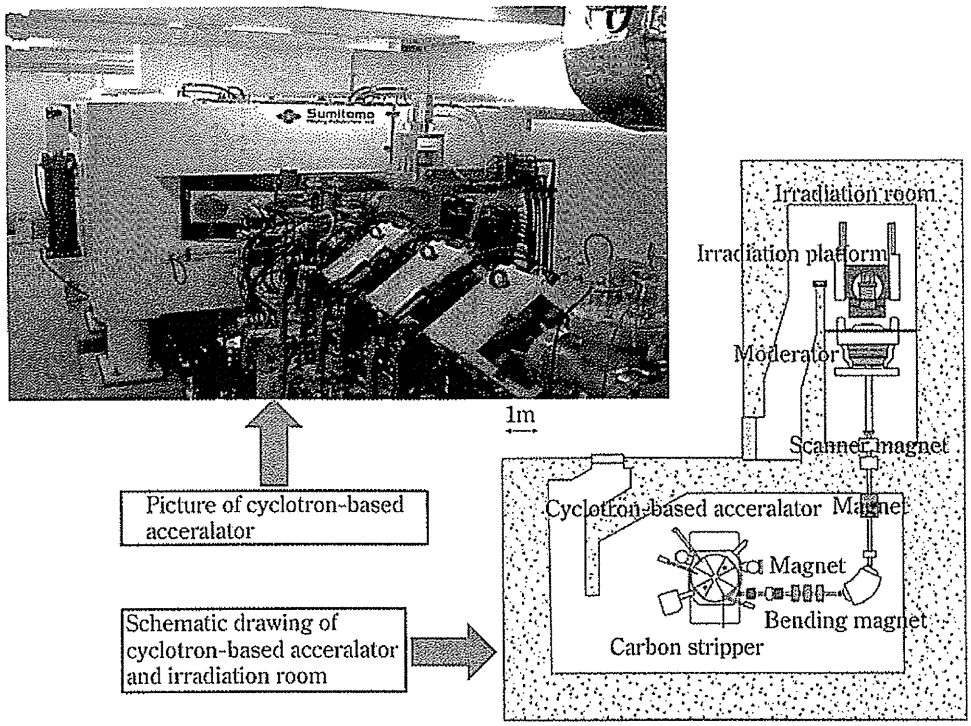

Fig. 6 Photograph of the cyclotron-based accelerator for neutron generation and a schematic drawing of the total irradiation room

り，再発悪性神経膠腫を対象として，第 1 相臨床試験（治験）を開始している．Fig. 6 にサイクロトロン型小型加速器中性子源（実写）とその治療室の見取り図を提示する．原子炉に比してはるかに小型化された加速器の実寸が見て取れる．本治験が成功すれば院内 BNCT が可能となり，薬事承認を目指して臨床試験を遂行中である．

症候性脳放射線壊死に対するベバシズマブの静脈内投与による治療

BNCT や強度変調放射線治療あるいは定位放射線治療等の高線量放射線治療は着実に，頭蓋内悪性腫瘍の治療成績を向上させている[5)8)17)20)]．一方でこれら高線量，高精度放射線治療の適応により，症候性脳放射線壊死が問題となっている．症候性脳放射線壊死に対してはステロイドホルモン等が経験的に投与されてはいるが，有効な治療法は確立されていない．

脳放射線壊死組織の手術摘出標本の検討から，放射線壊死における脳浮腫の原因が壊死巣周囲の脆弱な血管新生であり，さらにその原因が血管内皮増殖因子（vascular endothelial growth factor：VEGF）の過剰産生にあることをわれわれは解明した[16)]．そこでこの知見をもとに，抗 VEGF 抗体製剤であるベバシズマブを症候性脳放射線壊

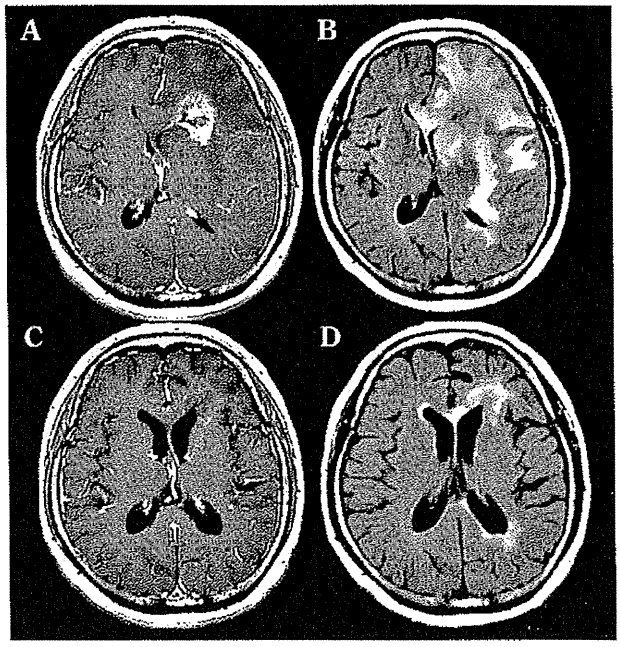

Fig. 7 Case of radiation necrosis successfully treated by bevacizumab. Radiation necrosis was due to repetitive SRSs for a metastatic brain tumor of uterus cancer.
A：Pre-treatment T1-Gd MRI. B：Pre-treatment FLAIR MRI. C：Post-treatment T1-Gd MRI. D：Post-treatment FLAIR MRI. A drastic decrease of Gd-enhancement and brain edema was observed by 6 cycles of bevacizumab treatment.

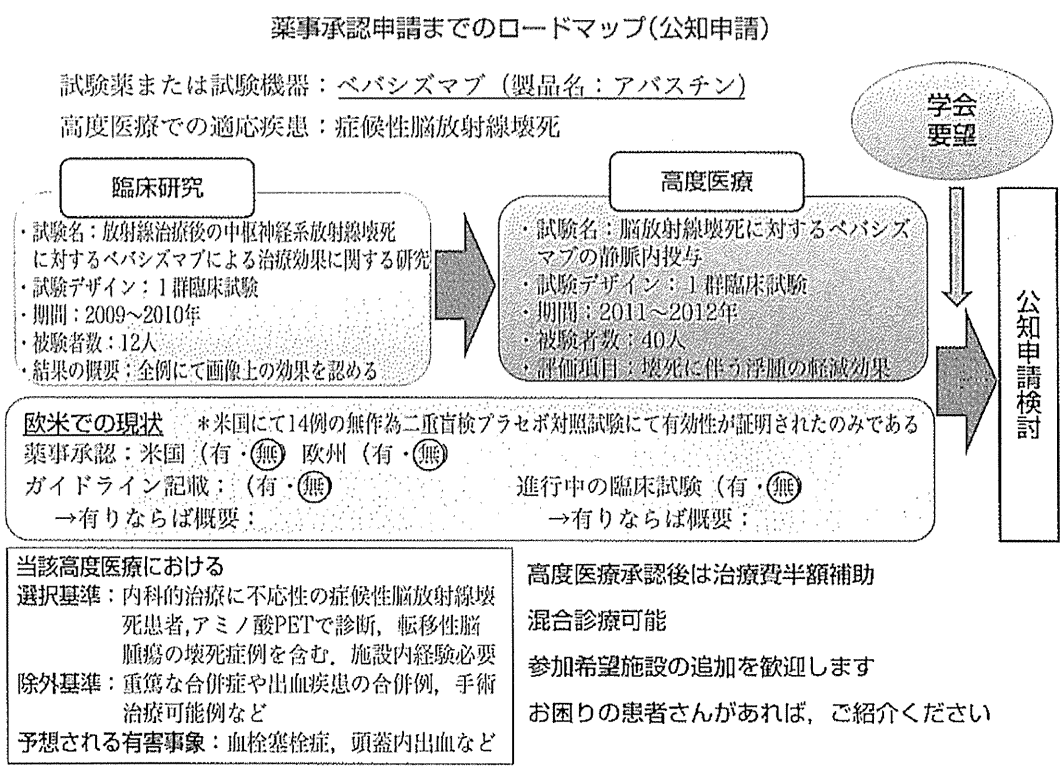

Fig. 8 Roadmap for obtaining permission for on-label use of bevacizumab for symptomatic radiation necrosis in the brain

死の症例に投与したところ，原因となる腫瘍の組織型や用いた放射線の種類を問わず，投与症例全例で顕著な脳浮腫および造影領域の縮小を認めた[4]．ここで問題となるのは放射線治療後に生じる脳浮腫の増悪が，腫瘍再発によるものかそれとも脳放射線壊死によるものかの鑑別を要する点である．われわれは上述の F-BPA-PET により，その鑑別を行っている[4)14)]．

これらの経験をもとに，厚生労働省に高度医療（第3項先進医療）として「症候性脳放射線壊死に対する核医学的診断とベバシズマブの静脈内投与による治療」を申請し，厚生労働科研費のサポートもいただき，2011年4月1日より40症例を登録予定数として全国16施設による多施設共同臨床研究を展開している[14]．本臨床試験ではより多くの施設の参画を期待して，その診断には BPA-PET のほか，Met-PET も利用可としており，その詳細は拙稿をご参照いただきたい[14]．われわれの経験症例を Fig. 7 に示す．

53歳女性．子宮体癌に対して全摘出術を受けた．その直後より脳内に多発性転移巣を認め，複数回の定位放射線治療を受けている．左前頭葉の病変には2度の定位照射が施行され，2度目の照射の2カ月後より，頭痛，失語症を発症し，ステロイドホルモンの投与によっても軽快せず，当科に紹介された．多発性の転移巣はいずれも BPA-PET で活動性を認めず，ことに左前頭葉の病変は病変/正常脳比 2.1 と算出され，われわれの定めた脳放射線壊死の基準値を満たし，ベバシズマブの投与を biweekly，5 mg/kg で行った．数回投与により症状は改善し，6回投与後の MRI では造影域，浮腫とも顕著に軽快している．

本稿準備中の 2103 年 1 月 25 日に予定症例数の 40 例の登録を終え，順調に臨床試験は進行している．臨床試験の成績をもとにした，われわれの描いている薬事承認を目指したロードマップを Fig. 8 に示す．症候性脳放射線壊死は重篤な機能予後および生命予後をきたす疾患ではあるが，母集団となる患者総数はそれほど多くなく，治験は組みにくい．そこで治験というプロセスを踏まずに薬事承認を目指すシステムとして，高度医療に思い至った次第である．本臨床試験にて万人が認めうる優れた成績を治めることができれば，各種学会からの学会要望を添えて，公知申請を行い，厚生労働省に薬事申請を認可していただくという戦略を厚生労働省の担当官との間で構築した．よっておよそ2年後に，日本定位放射線治療学会，日本脳神経外科学会，日本放射線腫瘍学会，日本核医学会からの学会要望がいただけるよう，臨床試

験を行っている次第である[14].

最後に再発悪性神経膠腫に対して，BNCT を行うと，しばしば pseudoprogression に遭遇する[12]．この pseudoprogression は intensive treatment の証として認識されているが[1]，多くの場合無症候であり，ステロイドホルモンの投与で対応可能であることが多い．最近われわれは再発悪性神経膠腫に対しての BNCT 後に symptomatic pseudoprogression となった症例に，ベバシズマブを投与したところ，劇的な改善を経験しており，本治療の新たな展開と考えている[15]．Pseudoprogression と脳放射線壊死との間に明確な線を引くことは難しい．一般には pseudoprogression は脳放射線壊死と比べて，画像上の悪化に比して症状は軽く，かつ治療から発症までの期間が短いことが特徴と理解されている．文献 15 で紹介した症例は治療から画像上の増悪までの期間が短く，PET 上も壊死の診断はできず，symptomatic pseudoprogression と判断した症例である．ベバシズマブの治療効果を考えると，今後再発症例の BNCT 後には本治療を適応すべき症例も増加すると考える．

謝　辞

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文　献

1) Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ: Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. *Lancet Oncol* **9**: 453-461, 2008.
2) Carson KA, Grossman SA, Fisher JD, Shaw EG: Prognostic factors for survival in adult patients with recurrent glioma enrolled onto the new approaches to brain tumor therapy CNS consortium phase I and II clinical trials. *J Clin Oncol* **25**: 2601-2606, 2007.
3) Coderre JA, Morris GM: Review: The radiation biology of boron neutron capture therapy. *Radiation Res* **151**: 1-18, 1999.
4) Furuse M, Kawabata S, Kuroiwa T, Miyatake S: Repeated treatments with bevacizumab for recurrent radiation necrosis in patients with malignant brain tumors: a report of 2 cases. *J Neurooncol* **102**: 471-475, 2011.
5) Iuchi T, Hatano K, Narita Y, Kodama T, Yamaki T, Osato K: Hypofractionated high-dose irradiation for the treatment of malignant astrocytomas using simultaneous integrated boost technique by IMRT. *Int J Radiat Oncol Biol Phys* **64**: 1317-1324, 2006.
6) Jaaskelainen J, Haltia M, Servo A: Atypical and anaplastic meningiomas: radiology, surgery, radiotherapy, and outcome. *Surg Neurol* **25**: 233-242, 1986.
7) Kawabata S, Miyatake S, Kajimoto Y, Kuroda Y, Kuroiwa T, Imahori Y, Kirihata M, Sakurai Y, Kobayashi T, Ono K: The successful treatment of glioblastoma patients with modified boron neutron capture therapy. Report of two cases. *J Neurooncol* **65**: 159-165, 2003.
8) Kawabata S, Miyatake S, Kuroiwa T, Yokoyama K, Doi A, Iida K, Miyata S, Nonoguchi N, Michiue H, Takahashi M, Inomata T, Imahori Y, Kirihata M, Sakurai Y, Maruhashi A, Kumada H, Ono K: Boron neutron capture therapy for newly diagnosed glioblastoma. *J Radiat Res (Tokyo)* **50**: 51-60, 2009.
9) Miyashita M, Miyatake S, Imahori Y, Yokoyama K, Kawabata S, Kajimoto Y, Shibata MA, Otsuki Y, Kirihata M, Ono K, Kuroiwa T: Evaluation of fluoride-labeled boronophenylalanine-PET imaging for the study of radiation effects in patients with glioblastomas. *J Neurooncol* **89**: 239-246, 2008.
10) Miyatake S, Kawabata S, Kajimoto Y, Aoki A, Yokoyama K, Yamada M, Kuroiwa T, Tsuji M, Imahori Y, Kirihata M, Sakurai Y, Masunaga S, Nagata K, Maruhashi A, Ono K: Modified boron neutron capture therapy for malignant gliomas performed using epithermal neutron and two boron compounds with different accumulation mechanisms: an efficacy study based on findings on neuroimages. *J Neurosurg* **103**: 1000-1009, 2005.
11) Miyatake S, Tamura Y, Kawabata S, Iida K, Kuroiwa T, Ono K: Boron neutron capture therapy for malignant tumors related to meningiomas. *Neurosurgery* **61**: 82-91, 2007.
12) Miyatake S, Kawabata S, Nonoguchi N, Yokoyama K, Kuroiwa T, Matsui H, Ono K: Pseudoprogression in boron neutron capture therapy for malignant gliomas and meningiomas. *Neuro Oncol* **11**: 430-436, 2009.
13) Miyatake S, Kawabata S, Yokoyama K, Kuroiwa T, Michiue H, Sakurai Y, Kumada H, Suzuki M, Maruhashi A, Kirihata M, Ono K: Survival benefit of Boron neutron capture therapy for recurrent malignant gliomas. *J Neurooncol* **91**: 199-206, 2009.
14) 宮武伸一，古瀬元雅：脳放射線壊死の成因と治療—高度医療（第 3 項先進医療）の申請—．脳外誌　**21**: 472-479, 2012.
15) Miyatake S, Furuse M, Kawabata S, Maruyama T, Kumabe T, Kuroiwa T, Ono K: Bevacizumab treatment of symptomatic pseudoprogression after boron neutron capture therapy for recurrent malignant gliomas. Report of 2 cases. *Neuro Oncol* **15**: 650-655, 2013.
16) Nonoguchi N, Miyatake SI, Fukumoto M, Furuse M, Hiramatsu R, Kawabata S, Kuroiwa T, Tsuji M, Fukumoto M, Ono K: The distribution of vascular endothelial growth factor-producing cells in clinical radiation necrosis of the brain: pathological consideration of their potential roles. *J Neurooncol* **105**: 423-431, 2011.
17) Ohguri T, Imada H, Kohshi K, Kakeda S, Ohnari N, Morioka T, Nakano K, Konda N, Korogi Y: Effect of prophylactic hyperbaric oxygen treatment for radiation-induced brain injury after stereotactic radiosurgery of brain metastases. *Int J Radiat Oncol Biol Phys* **67**: 248-255, 2007.
18) Palma L, Celli P, Franco C, Cervoni L, Cantore G: Long-term prognosis for atypical and malignant meningiomas: a study of 71 surgical cases. *J Neurosurg* **86**: 793-800, 1997.

19) Tamura Y, Miyatake S, Nonoguchi N, Miyata S, Yokoyama K, Doi A, Kuroiwa T, Asada M, Tanabe H, Ono K: Boron neutron capture therapy for recurrent malignant meningioma. Case report. *J Neurosurg* 105: 898-903, 2006.

20) Tanaka M, Ino Y, Nakagawa K, Tago M, Todo T: High-dose conformal radiotherapy for supratentorial malignant glioma: a historical comparison. *Lancet Oncol* 6: 953-960, 2005.

要　旨

腫瘍細胞選択的粒子線治療「ホウ素中性子捕捉療法」と
抗血管新生薬による症候性脳放射線壊死の治療

宮武　伸一

悪性腫瘍に対する新規放射線（粒子線）治療法として，ホウ素中性子捕捉療法（boron neutron capture therapy：BNCT）が提唱されている．われわれは2002年より本治療法をのべ133例に及ぶ悪性神経膠腫と悪性髄膜腫に適応してきた．また最近，症候性脳放射線壊死に対する抗血管新生療法を積極的に展開している．本論文では，第32回日本脳神経外科コングレス総会「グリオーマ 新しい時代の到来」において発表した上記内容に若干の加筆を行い，ここに発表した．

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ORIGINAL ARTICLE

IDH1 mutation as a potential novel biomarker for distinguishing pseudoprogression from true progression in patients with glioblastoma treated with temozolomide and radiotherapy

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Abstract The purpose of this study was to distinguish pseudoprogression (PP) from early true progression in patients with glioblastoma (GBM) based on the presence of a mutation in isocitrate dehydrogenase 1 (IDH1). We retrospectively surveyed 32 patients with GBM or GBM with oligodendroglioma component (GBMO) who underwent biopsy or maximal tumor resection followed by concurrent radiotherapy and temozolomide (TMZ). We then selected patients with early radiological progression in magnetic resonance imaging within 6 months after concurrent radiotherapy and TMZ treatment. DNA was extracted from their tumor blocks. The IDH1 mutation was analyzed in the genomic region by direct sequencing as a biomarker for PP. Twenty-eight patients were diagnosed with GBM and four with GBMO. Eleven patients were discovered to have early radiological progression. PP was detected in two patients (6.3 %) diagnosed with GBMO and one patient with GBM. Both of the GBMO patients with PP had the IDH1 mutation, the one GBM patient with PP and the other eight patients with early true progression with wild type. The sensitivity and specificity of the IDH1 mutation for detecting PP were 66.7 and 100 %, respectively. This study suggests the IDH1 mutation may become a novel molecular biomarker for PP. Analyzing the IDH1 mutation, in the case of recognizing early radiological progression, may enable distinction of PP from early true progression, and we could determine the need for second-look surgery.

Keywords Biomarker · Concurrent radiotherapy and temozolomide · Glioblastoma · IDH1 mutation · Pseudoprogression

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Introduction

Glioblastoma (GBM) is one of the most malignant brain tumors, with patients having a median life expectancy of 13–17 months after diagnosis despite standard treatment of maximal tumor resection and concurrent radiotherapy (RT) and temozolomide (TMZ) followed by six courses of maintenance TMZ [1]. Contrast enhancement on magnetic resonance imaging (MRI) is a standard tool for assessing response to treatment. Recently, there have been increasing numbers of reports of pseudoprogression (PP), which is difficult to distinguish from early true progression (TP) based on contrast enhancement on MRI [2]. There are few effective biomarkers of PP, so many patients may undergo an unnecessary and potentially harmful second surgery. Recently, genome-wide mutational analysis revealed somatic mutations of cytosolic NADP+-dependent isocitrate dehydrogenase 1 (IDH1) in approximately 12 % of GBMs [3]. The IDH1 mutation is associated with a favorable prognosis in adult patients with GBM [3, 4]. Pseudoprogression also indicates a good prognosis [5]. We investigated the incidence of PP according to the IDH1 mutation.

Materials and methods

Patients

Between December 2005 and November 2010, a total of 32 patients with GBM or GBM with oligodendroglioma component (GBMO) who underwent biopsy or maximal tumor resection and treatment with 60 Gy of brain-localized RT concurrent with continuous TMZ (75 mg/m^2/day) followed by maintenance TMZ (150–200 mg/m^2/day for 5 days, every 28 days) were reviewed. All histological slides were re-evaluated by two neuropathologists blinded to the clinical background and outcome of patients, and classified according to the 2007 WHO classification. GBMOs are defined based on histological specimens that identify tumor parts with features of oligodendroglial differentiation within typical histological findings of GBM. The pathological diagnosis did not refer to genetic information.

Clinical, radiographic and pathological records were reviewed. All patients underwent MRI exams (T2, T1, T1 with gadolinium, diffusion-weighted images and fluid attenuated inversion recovery) before and within 48 h after surgery, within 7 days, and every 2 or 3 months after concurrent chemoradiation therapy. Patients with early radiological progression (RP), within 6 months after concurrent treatment, were defined according to MacDonald's criteria on MRI. Pseudoprogression was defined as radiological improvement or stability of lesions without further treatment other than adjuvant TMZ.

IDH1 sequencing and MGMT promotor methylation status assessment

Tumor DNA was extracted from fixed paraffin-embedded tissues using the TAKARA DEXPAT kit (Takara) as follows. After review of hematoxylin-and-eosin-stained slides to confirm normal and neoplastic tissues, then determination was made of where sufficient invasive GBM was present. DNA was also extracted from tumor specimens, quickly frozen and stored at −80 °C using AllPrep DNA/RNA Mini Kit (Qiagen). The genomic region spanning wild-type R132 of IDH1 was analyzed by direct sequencing using the following primers: IDH1 forward 5-TGC AAAATCACATTATTGCC and IDH1 reverse 5-AATGG CTTCTCTGAAGACCG. The PCR products were purified using the LaboPass PCR Purification Kit (COSMO GENETECH). All sequence reactions were carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The reactions were carried out in an automated DNA analyzer (3730xl DNA Analyzer; Applied Biosystems). O6-Methylguanine-DNA methyltransferase (MGMT) promotor methylation status was also evaluated with the methylation-specific polymerase chain reaction (MSP), which was performed as previously described [6].

Statistical analysis

Sensitivity and specificity for detecting PP with IDH1 mutation, pathological diagnosis of GBMO or MGMT promotor methylation status were assessed. Significance of correlations between the parameters was assessed using Fisher's exact test, and values of $P < 0.05$ were considered statistically significant.

Results

Eleven (34.4 %) of 32 patients developed early RP. In these 11 patients with RP, 8 developed true progressions (TP), whereas three patients were classified as having PP (Table 1). Two of 11 patients had an IDH1 mutation, and both mutations were of the R132H type. Two PP patients had an IDH1 mutation, the other patient had wild-type IDH1, and the MGMT promotor methylation status could be determined for two out of three PP patients. Eight patients with early TP had progressive disease, and died or developed terminal disease during the investigation. All early TP patients had wild-type IDH1, but MGMT promotor methylation statuses were various: three patients were methylated, and five were unmethylated (Tables 2, 3). Within early RP group, the sensitivity and specificity of IDH1 mutation for detecting PP were 67 and 100 %, respectively; meanwhile, those of MGMT promotor methylation status were 67 and 63 %, respectively.

Histopathologically, two PP patients were diagnosed with GBMO and one patient with GBM. Meanwhile, seven early TP patients were diagnosed with GBM, and one was diagnosed with GBMO (Table 4). The sensitivity of GBMO was 67 %, but the specificity was 88 %. Using Fisher's exact test, there were no significant associations between the IDH1 mutation and PP ($P = 0.055$) mutations, MGMT methylation status and PP ($P = 0.424$), or GBMO and PP ($P = 0.156$).

Illustrative case

A 44-year-old woman presented with mild hemiparesis on the left side. MRI with contrast media demonstrated a heterogeneously enhanced mass in the right parietal region (Fig. 1a). Surgical resection was performed, and the enhanced lesion was totally removed (Fig. 1b). The tumor was diagnosed as a GBM with an oligodendroglioma component (Fig. 2a, b). Concurrent TMZ and radiotherapy was performed, followed by 5 consecutive days of TMZ

Table 1 Cases developing early radiological progression

Case	Pathological diagnosis	Course	IDH1	MGMT promoter methylation status
1	GBM	True progression	Wild type	Methylated
2	GBM	Pseudoprogression	Wild type	Methylated
3	GBM	True progression	Wild type	Unmethylated
4	GBM	True progression	Wild type	Unmethylated
5	GBM	True progression	Wild type	Unmethylated
6[a]	GBMO	Pseudoprogression	Mutation	Methylated
7	GBM	True progression	Wild type	Methylated
8	GBM	True progression	Wild type	Unmethylated
9	GBMO	True progression	Wild type	Unmethylated
10	GBMO	Pseudoprogression	Mutation	Unmethylated
11	GBM	True progression	Wild type	Methylated

IDH1 isocitrate dehydrogenase 1, *GBM* glioblastoma multiforme, *GBMO* glioblastoma with oligodendroglioma component

[a] Illustrative case

Table 2 Relevance between IDH1 mutation and pseudoprogression

IDH1 mutation	PP	TP
Mutation	2	0
Wild type	1	8
Sensitivity	67 %	
Specificity	100 %	
Fisher's exact test	$P = 0.055$	

Table 3 Relevance between MGMT methylation status and pseudoprogression

MGMT	PP	TP
Methylation	2	3
Unmethylation	1	5
Sensitivity	67 %	
Specificity	63 %	
Fisher's exact test	$P = 0.424$	

Table 4 Relevance between pathological diagnosis and pseudoprogression

Pathological diagnosis	PP	TP
GBMO	2	1
GBM	1	7
Sensitivity	67 %	
Specificity	88 %	
Fisher's exact test	$P = 0.156$	

IDH1 isocitrate dehydrogenase 1, *GBM* glioblastoma multiforme, *GBMO* glioblastoma with oligodendroglioma component, *MGMT* O6-methylguanine-DNA methyltransferase

administered every 28 days. Early radiological progression was found 2 months after the concurrent treatment (Fig. 1c) without clinical deterioration. Spontaneous improvement of the enhancing lesion was observed without further treatment other than adjuvant TMZ after another 2 months (Fig. 1d). By immunohistochemistry, the tumor cells exhibited diffuse IDH1-R132H mutation (Dianova, Hamburg, Germany; monoclonal, Clone H09, diluted 1:50) (Fig. 2c). Her lesion was revealed to have an IDH1 mutation also by direct sequencing (Fig. 2d). This patient has remained free of relapse for more than 3 years.

Discussion

Pseudoprogression was first described by Hoffman et al. [7] in a group of malignant glioma patients treated with RT and carmustine. This phenomenon has been increasingly reported since the standard therapy of concurrent radiation and TMZ was developed [2, 8–11] but the incidence rates of PP vary (5.5–46.7 %) among reports [5, 9, 11–13]. Pseudoprogression is defined as the spontaneous improvement of enhancing lesions without further treatment other than adjuvant TMZ. Some studies have demonstrated that PP is associated with favorable prognosis [5, 8, 13, 14]. Hence, meaningful biomarkers are desired so that TMZ can be used more effectively and in order to avoid unnecessary second-look surgeries for PP. The MGMT promoter methylation status can predict the incidence and outcome of PP [8]. Brandes et al. found that its sensitivity for PP was 66 % and specificity 89 %. Kan et al. reported overexpression of p53 as a potential biomarker for predicting the development of PP. Its sensitivity and specificity were 87.5 and 70 %, respectively [13]. Methylated MGMT

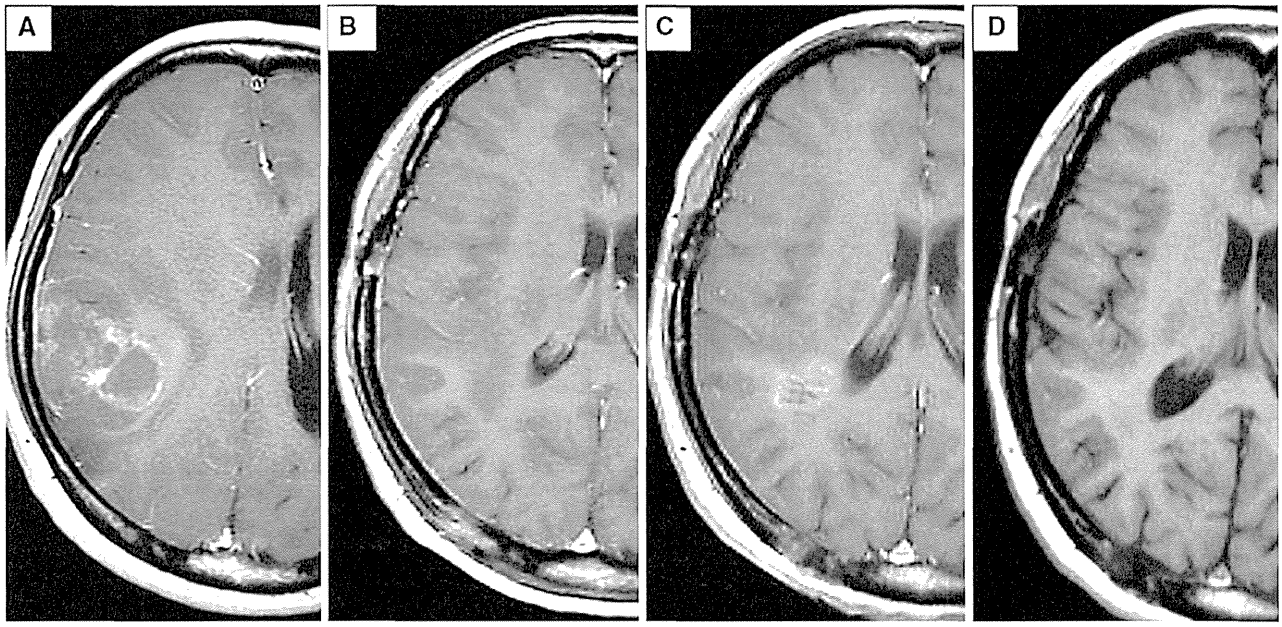

Fig. 1 Pseudoprogression in a 44-year-old woman with a right parietal glioblastoma with an oligodendroglioma component followed by concurrent 60-Gy radiotherapy and temozolomide therapy. Preoperative (**a**) and postoperative (**b**) contrast-enhanced MRIs are shown. The gadolinium-enhanced lesion was grossly resected, but early radiological progression was found 2 months after concurrent treatment (**c**) without clinical deterioration. Spontaneous improvement of the enhancing lesion was observed without further treatment other than adjuvant TMZ after another 2 months (**d**)

promoter and p53 overexpression are slightly weak biomarkers for distinguishing PP from early true progression in order to decide on courses of treatment. Although GBMO seems a good biomarker, it would be inadequate for making decisions because GBMO could include an early TP case and the diagnosis could fluctuate depending on the pathologist. Some patients might be diagnosed with GBM or anaplastic oligodendroglioma. There are few reports regarding PP and anaplastic gliomas, but Wit et al. [15] described 3 patients with PP out of 32 malignant glioma patients after radiotherapy; two had oligodendroglial features (one anaplastic oligodendroglioma and one anaplastic oligoastrocytoma). Oligodendroglial features may have some influence on developing PP. Regarding the IDH1 mutation, both the sensitivity and specificity for detecting PP were high at 66.7 and 100 %, respectively, within early radiological progression, and this biomarker provides a very objective means of assessment. However, there was no narrowly found statistical significance between the IDH1 mutation and pseudoprogression. If some additional inspections of larger populations revealed high specificity of the IDH1 mutation in pseudoprogression, we could then determine the need for second-look surgery when MRI detected early radiological progression.

Can any possible assumption be made to account for the mechanisms involved in the relationship between the IDH1 mutation and pseudoprogression? Histopathologically, PP resembles radiation necrosis, i.e., a mixture of necrotic changes and gliosis with few viable tumor cells, in addition to endothelial thickening, hyalinization and thrombosis of vessels [5, 11]. Radiation-induced injury to endothelial cells is thought to be a main cause of acute and subacute radiation injury. Endothelial cell death induces destruction of the blood brain barrier (BBB) with vasogenic edema, ischemia and hypoxia [16]. Also, radiographically, PP shows enlarging contrast enhancement and edema on MRI, which suggests destruction of the BBB. The BBB is composed of capillary endothelial cells surrounded by basal lamina and astrocytic perivascular endfeet. Is there any relationship between the IDH1 mutation and MGMT promoter methylation status and blood-brain barrier damage? It is well known that MGMT promoter methylation status and IDH1 mutations in the GBM may sensitize tumors to irradiation and chemotherapy. Therefore, there could be two hypotheses for PP. The first is that the tumor vessels' endothelial cells came from GBM stem cells with methylated MGMT promoter or IDH1 mutation. Wang et al. [17] demonstrated the capability of GBM stem cells for differentiation along tumor and endothelial lineages. If this was correct, and the tumor vessels' endothelial cells also had methylated MGMT promoters or IDH1 mutations, DNA damage could easily increase, and clinically PP could develop. In our cases, immunostaining of IDH1 mutations could not be detected in any type of tumor vessels (Fig. 2c). Then, we state the second

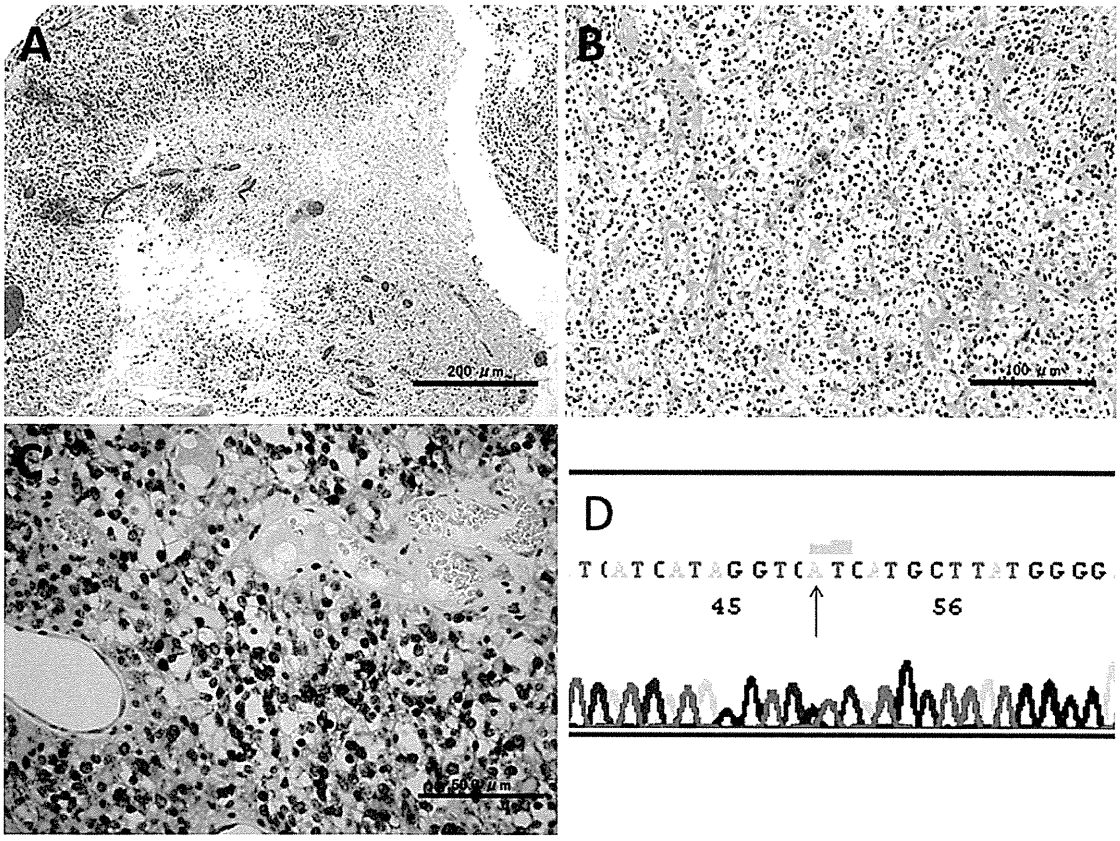

Fig. 2 Pathological findings and molecular biological detection of isocitrate dehydrogenase 1 (IDH1) mutation in a 44-year-old woman with pseudoprogression 2 months after concurrent 60-Gy radiotherapy and temozolomide therapy. Photomicrograph shows necrosis with pseudopalisading pattern (**a**, H&E, ×100), and tumor cells with clear cytoplasm and marked microvascular proliferation (**b**, H&E, ×200). Immunohistochemical examination with H09 (Dianova, Hamburg, Germany; 1:50), which specifically detects the mutant IDH1R132H, showed that almost all tumor cells were diffusely stained except for endothelial cells (**c**). Somatic mutations in IDH1 from CGT to CAT were detected by direct sequencing of tumor DNA (**d**)

hypothesis about PP, in which tumor cells with genetic or epigenetic anomalies, which might maintain the BBB, respond to initial therapy, resulting in the collapse of the BBB, and then PP would develop.

In summary, in our small series, there might be a correlation between PP and an IDH1 mutation. If this correlation were statistically confirmed in larger populations, we could avoid unnecessary and potentially harmful second-look surgery.

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Conflict of interest None declared.

References

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996
2. Hygino da Cruz LC, Jr., Rodriguez I, Domingues RC, Gasparetto EL, Sorensen AG (2011) Pseudoprogression and pseudoresponse: imaging challenges in the assessment of posttreatment glioma. AJNR Am J Neuroradiol 32:1978–1985
3. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
4. Sonoda Y, Kumabe T, Nakamura T, Saito R, Kanamori M, Yamashita Y, Suzuki H, Tominaga T (2009) Analysis of IDH1 and IDH2 mutations in Japanese glioma patients. Cancer Sci 100:1996–1998
5. Topkan E, Topuk S, Oymak E, Parlak C, Pehlivan B (2012) Pseudoprogression in patients with glioblastoma multiforme after concurrent radiotherapy and temozolomide. Am J Clin Oncol 35:284–289
6. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L,

Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003
7. Hoffman WF, Levin VA, Wilson CB (1979) Evaluation of malignant glioma patients during the postirradiation period. J Neurosurg 50:624–628
8. Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, Bertorelle R, Bartolini S, Calbucci F, Andreoli A, Frezza G, Leonardi M, Spagnolli F, Ermani M (2008) MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. J Clin Oncol 26:2192–2197
9. Brandes AA, Tosoni A, Spagnolli F, Frezza G, Leonardi M, Calbucci F, Franceschi E (2008) Disease progression or pseudoprogression after concomitant radiochemotherapy treatment: pitfalls in neurooncology. Neuro Oncol 10:361–367
10. Brandsma D, van den Bent MJ (2009) Pseudoprogression and pseudoresponse in the treatment of gliomas. Curr Opin Neurol 22:633–638
11. Chaskis C, Neyns B, Michotte A, De Ridder M, Everaert H (2009) Pseudoprogression after radiotherapy with concurrent temozolomide for high-grade glioma: clinical observations and working recommendations. Surg Neurol 72:423–428
12. Chamberlain MC (2008) Pseudoprogression in glioblastoma. J Clin Oncol 26:4359. author reply 4359–4360
13. Kang HC, Kim CY, Han JH, Choe GY, Kim JH, Kim IA (2011) Pseudoprogression in patients with malignant gliomas treated with concurrent temozolomide and radiotherapy: potential role of p53. J Neurooncol 102:157–162
14. Park CK, Kim J, Yim SY, Lee AR, Han JH, Kim CY, Park SH, Kim TM, Lee SH, Choi SH, Kim SK, Kim DG, Jung HW (2011) Usefulness of MS-MLPA for detection of MGMT promoter methylation in the evaluation of pseudoprogression in glioblastoma patients. Neuro Oncol 13:195–202
15. de Wit MC, de Bruin HG, Eijkenboom W, Sillevis Smitt PA, van den Bent MJ (2004) Immediate post-radiotherapy changes in malignant glioma can mimic tumor progression. Neurology 63: 535–537
16. Wong CS, Van der Kogel AJ (2004) Mechanisms of radiation injury to the central nervous system: implications for neuroprotection. Mol Interv 4:273–284
17. Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C, Tabar V (2010) Glioblastoma stem-like cells give rise to tumour endothelium. Nature 468:829–833