治療経過要約

対象:2009 年 7 月 31 日までの登録例 103 例

	A 群	B 群	合計
プロトコール治療中・治療終了報告用紙未回収など	13	31	44
プロトコール治療終了または中止	40	19	59
終了または中止の理由			
1.A 群のみ:プロトコール規定の治療完了	40	0	40
2.プロトコール治療無効と判断	0	18	18
全身状態などの悪化により治療継続不可能と判断 「再発した病変」の最大径が 3 cm以上 「定位照射病変数と再発した病変数」が合計 9 個以上 脳幹への進展・再発 定位照射病変が増悪、または照射病変消失後、再度同部位に再発			
3.有害事象	0	1	1
4.有害事象との関連が否定できない患者拒否	0	0	0
5.有害事象との関連が否定できる患者拒否	0	0	0
6.治療期間中の死亡	0	0	0
88.その他の理由	0	0	0

安全性の評価

重篤な有害反応/有害事象

治療中及び最終治療日から 30 日以内の死亡

なし

プロトコール治療終了 31 日以降の治療関連死疑い

なし

MMSE

対象:追跡調査に評価があった 6 ヵ月:68 例/12 ヵ月:31 例

得点	判定	登録後 6 ヵ月	登録後 12 ヵ月
30-24	正常	44	25
23-20	軽度知能低下	3	0
19-10	中程度知能低下	2	2
9-0	高度知能低下	3	0
転院・増悪等による未施行(死亡による未施行は除く)		14	3
欠損		2	1

PS

対象:追跡調査に評価があった 6 ヵ月:68 例/12 ヵ月:31 例

	登録後 6 ヵ月	登録後 12 ヵ月
神経症状含む		
0	31	17
1	18	7
2	5	3
3	3	1
4	3	1
転院・増悪等による未施行(死亡による未施行は除く)	7	2
欠損	1	0
神経症状含まず		
0	39	21
1	12	4
2	3	3
3	2	0
4	4	1
転院・増悪等による未施行(死亡による未施行は除く)	7	2
欠損	1	0

有効性の評価

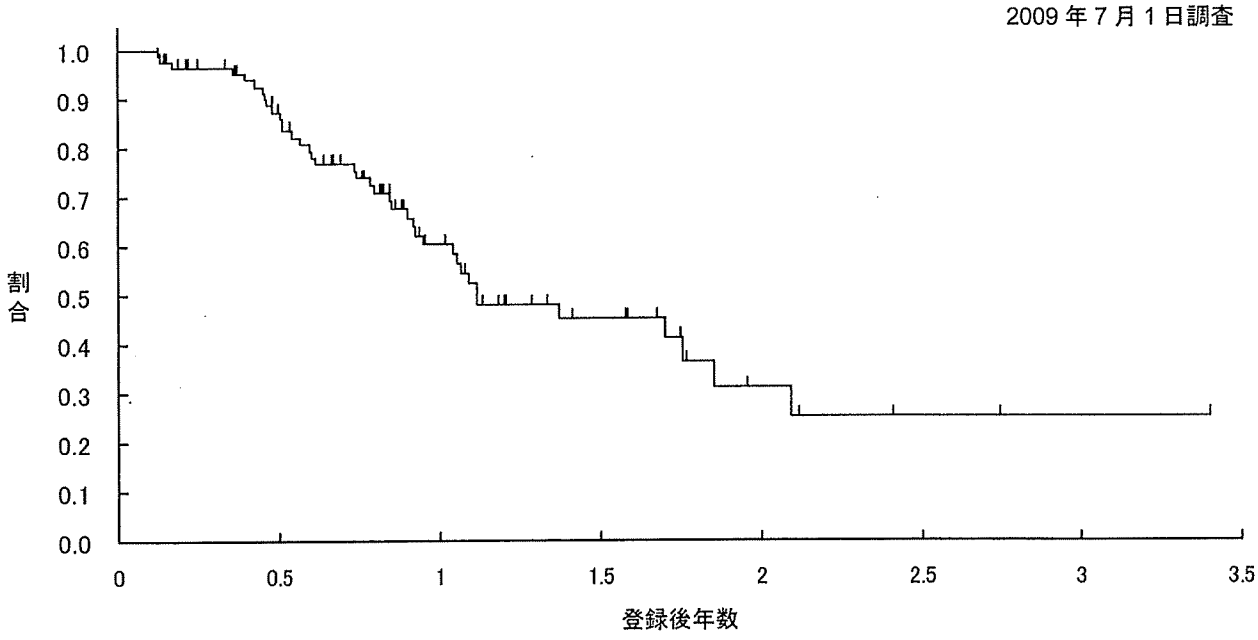
「1 年＝365.25 日」「1 カ月＝(365.25/12) 日」で計算

生存期間

解析対象：2009 年 5 月 31 日までの登録数 89 例
起算日：登録日
イベント：死亡
打ち切り：生存例、追跡不能例は最終生存確認日で打ち切り

Kaplan-Meier 法による推定生存曲線

2009 年 7 月 1 日調査



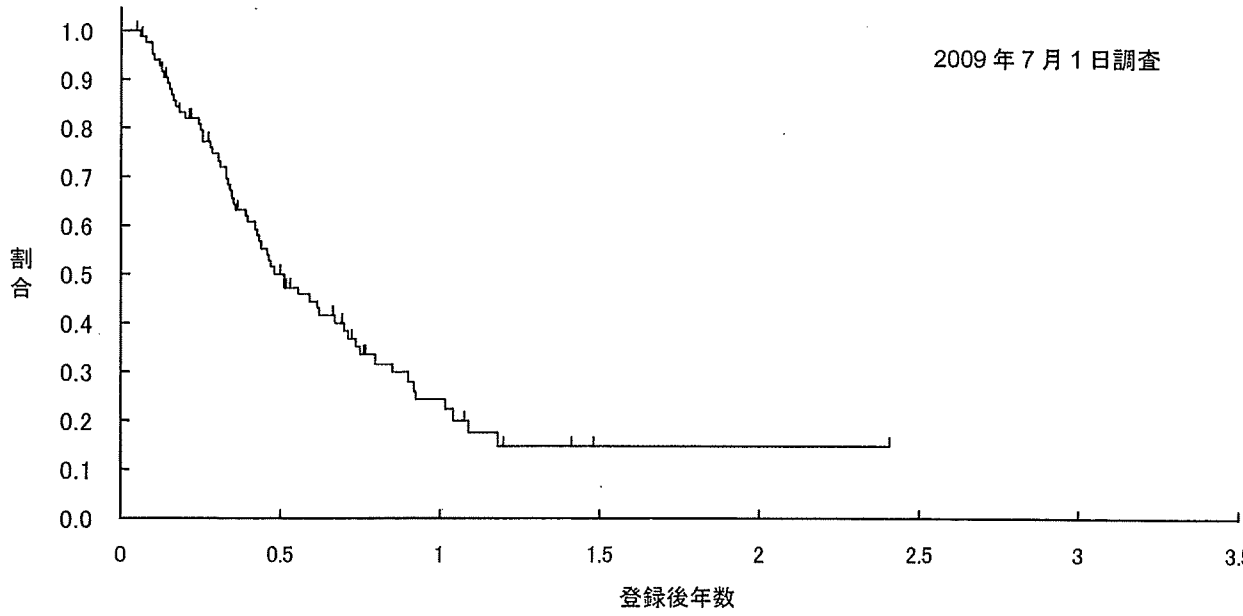
解析対象	イベント(死亡)	打ち切り例の最長追跡期間	最後の死亡が起こった時点での生存	生存期間中央値 (95%信頼区間)
89 例	39 例	3.40 年	4 例	1.12 年 (0.95 年－1.85 年)

1 年生存割合 (95%信頼区間)	2 年生存割合 (95%信頼区間)
60.5% (47.7%－71.0%)	31.4% (16.4%－47.5%)

無増悪生存期間

解析対象:2009 年 5 月 31 日までの登録数 89 例のうち、増悪情報のみ未回収の 1 例を除く 88 例
起算日:登録日
イベント:増悪もしくは死亡
打ち切り:無増悪生存例、追跡不能例は最終無増悪生存確認日で打ち切り

Kaplan-Meier 法による推定無増悪生存曲線



解析対象	イベント (増悪・死亡)	打ち切り例の 最長追跡期間	最後のイベントが起こった 時点での無増悪生存
88 例	60 例	2.41 年	5 例

無増悪生存期間中央値 (95%信頼区間)	1 年無増悪生存割合 (95%信頼区間)
0.51 年 (0.42 年−0.70 年)	24.3% (14.6%−35.3%)

＊ 無増悪生存期間中央値(95%信頼区間)の月換算:6.12 ヶ月年(5.04 ヶ月-8.40 ヶ月)

追跡調査のデータがアップデートされていない例

追跡調査用紙が締め切り日を過ぎて回収されたものも含まれる

なし

II. 分担研究報告

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書

放射線による認知機能障害を回避する転移性脳腫瘍の治療法に関する臨床研究

研究分担者：大西丘倫（愛媛大学医学部脳神経外科・教授）、小川　彰（岩手医科大学・学長／教授）、佐伯直勝（千葉大学医学部脳神経外科・教授）、澤村　豊（北海道大学医学部脳神経外科・講師）、渋井壮一郎（国立がんセンター中央病院脳神経外科・医長）、白土博樹（北海道大学病院放射線部・教授）、城倉英史（古川星陵病院・副院長／鈴木二郎記念ガンマハウス・施設長）、角　美奈子（国立がんセンター中央病院放射線治療部・医長）、藤堂具紀（東京大学医学部脳神経外科・特任教授）、冨永悌二（東北大学医学部脳神経外科・教授）、中川恵一（東京大学医学部放射線科・准教授）、西川　亮（埼玉医科大学包括的がんセンター脳脊髄腫瘍科・教授）、三國信啓（京都大学医学部脳神経外科・准教授）、若林俊彦（名古屋大学医学部脳神経外科・教授）。

研究要旨

研究代表者の指導のもとに、放射線による認知機能障害を回避する転移性脳腫瘍の治療法に関する研究という課題で、「転移性脳腫瘍に対する、腫瘍摘出術＋全脳照射と腫瘍摘出術＋Salvage Radiation Therapyとのランダム化比較試験」のプロトコールを作成し、登録施設として本臨床試験に参加した。また、脳腫瘍治療における脳機能評価、機能温存法についての基礎および臨床研究を行った。

A.よりH.までの報告は、研究代表者と同一であるため、省略する。

III. 研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sakurada K Saino M Mouri W Sato A Kitanaka C Kayama T	Nestin expression in central nervous system germ cell tumors.	Neurosurg Rev	31	173-177	2008
Wakabayashi T Kayama T Nishikawa R Takahashi R Yoshimine T Hashimoto N Aoki T Kurisu K Natsume A Ogura M Yoshida J	A multicenter phase I trial of interferon-beta and temozolomide combination therapy for high-grade gliomas (INTEGRAStudy).	Jpn J Clin Oncol	38	715-718	2008
S Maesawa M Fujii N Nakahara T Watanabe K Saito Y Kajita T Nagatani T Wakabayashi J Yoshida	Clinical indication for high-field 1.5T intraoperative magnetic resonance imaging and neuro-navigation for neurosurgical procedures -review of initial 100 cases-.	Neurol Med Chir(Tokyo)	49	340-350	2009
Shibui S	Treatment of metastatic brain tumors.	Int J Clin Oncol	14	273-274	2009
Narita Y Shibui S	Strategy of surgery and radiation therapy for brain metastases.	Int J Clin Oncol	14	275-280	2009
Nishioka K Abo D Aoyama H Furuta Y Onimaru R Onodera S Sawamura Y Ishikawa M Fukuda S Shirato H	Stereotactic Radiotherapy for Intracranial Nonacoustic Schwannomas Including Facial Nerve Schwannoma.	Int J Radiat Oncol Biol Phys	75	1415-1419	2009

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakajima T Kumabe T Kanamori M Saito R Tashiro M Watanabe M Tominaga T	Differential diagnosis between radiation necrosis and glioma progression using sequential proton magnetic resonance spectroscopy and methionine positron emission tomography.	Neurol Med Chir (Tokyo)	49	394-401	2009
Akamatsu Y Sugawara T Mikawa S Saito A Ono S Takayama K Jokura H Seki H	Ruptured pseudoaneurysm following Gamma Knife surgery for a vestibular schwannoma.	J Neurosurg.	110(3)	543-546	2009
Koga T Morita A Maruyama K Tanaka M Ino Y Shibahara J Louis DN Reifenberger G Itami J Hara R Saito N Todo T	Long-term control of disseminated pleomorphic xanthoastrocytoma with anaplastic features by means of stereotactic irradiation.	Neuro-Oncology	11(4)	446-451	2009
Haga A Nakagawa K Shiraishi K Itoh S Terahara A Yamashita H Ohtomo K Saegusa S Imae T Yoda K Pellegrini R	Quality assurance of volumetric modulated arc therapy using Elekta Synergy.	Acta Oncol	29	1-5	2009
Fujimoto N Sumi M Ito Y Imai A Kagami Y Sekine I et.al.	Relation between elective nodal failure and Irradiated volume in non-small-cell lung cancer (NSCLC) treated with radiotherapy using conventional fields and doses.	Radiotherapy and Oncology	91	433-437	2009

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
三國信啓 菊池隆幸 松本敦仁 横山洋平 高橋　潤 橋本信夫	Multimodality 画像を用いた脳腫瘍の手術戦略—脳腫瘍手術における解剖学的・生理学的脳機能評価法の臨床的意義.	CI 研究	30	115-121	2008
西川　亮	Low-grade glioma の治療における諸問題.	脳神経外科ジャーナル	18	418-422	2009

Ⅳ. 研究成果の刊行物・別刷

Neurosurg Rev (2008) 31:173–177
DOI 10.1007/s10143-007-0115-3

ORIGINAL ARTICLE

Nestin expression in central nervous system germ cell tumors

K. Sakurada · M. Saino · W. Mouri · A. Sato · C. Kitanaka · T. Kayama

Received: 21 April 2007 / Revised: 5 September 2007 / Accepted: 9 September 2007 / Published online: 19 December 2007

Abstract Central nervous system (CNS) germ cell tumors constitute a unique class of rare tumors that mainly affect children and adolescents. These tumors are believed to originate from displaced primordial germ cells. Recently, results of treatment of germ cell tumors have improved with use of radiotherapy and combination chemotherapy. However, some tumors have proven refractory to intensive treatment with surgery, radiation, and combination chemotherapy. Nestin is an intermediate filament protein expressed in undifferentiated cells during CNS development and in CNS tumors and is used as a marker of immature elements of tumors, including brain tumor stem cells. In this study, we examined for the first time nestin expression in 19 CNS germ cell tumors (nine pure germinomas, five germinomas with syncytiotrophoblastic giant cells, one yolk sac tumor, one choriocarcinoma, one embryonal carcinoma, and two mature teratomas). Nestin was expressed in 14 cases but was not expressed in three pure germinomas and two mature teratomas. Clinically, nestin-negative tumors did not exhibit dissemination, while all tumors that exhibited dissemination also strongly expressed nestin protein. These findings suggest that the detection of nestin expression could be useful in the management of CNS germ cell tumors, as an auxiliary predictor of dissemination and/or progression.

K. Sakurada (✉) · M. Saino · W. Mouri · A. Sato · T. Kayama
Department of Neurosurgery,
Yamagata University School of Medicine,
2-2-2 Iidanishi,
Yamagata, Yamagata 990-9585, Japan
e-mail: kasakura@med.id.yamagata-u.ac.jp

C. Kitanaka
Department of Molecular Cancer Science,
Yamagata University School of Medicine,
2-2-2 Iidanishi,
Yamagata, Yamagata 990-9585, Japan

Keywords CNS germ cell tumor · Nestin · Tumor stem cell · Dissemination · Immunohistochemistry

Introduction

Central nervous system (CNS) germ cell tumors constitute a unique class of rare tumors that affect mainly children and adolescents. These tumors originate from displaced primordial germ cells. Matsutani et al. [6, 7] reported that radiotherapy and combination chemotherapy improved the results of treatment of germ cell tumors, especially in patients in the group with good prognosis, with pure germinomas. However, some tumors have proven refractory to intensive treatment with surgery, radiation, and combination chemotherapy.

Nestin is an intermediate filament protein (IFP) expressed in undifferentiated cells during CNS development and in CNS tumors and used as a marker of immature elements of tumors, including brain tumor stem cells. There is increasing evidence that cancers might contain their own stem cells. Many cancers, like normal organs, seem to be maintained by hierarchical organization that includes slowly dividing stem cells, rapidly dividing transit amplifying cells, and differentiated cells. Similar to normal stem cells, cancer stem cells have drug export systems. This property has been used for selection of cancer stem cells from tumor specimens and cancer cell lines [4, 5]. We suspected that stem cell drug resistance contributes to cancer stem cell chemo-resistance. In addition, the presence of a small subpopulation of slowly dividing cancer stem cells might explain why primary CNS germ cell tumors recur after treatment with radiation or chemotherapy.

Given the possibility of resistance of stem cells to therapy, detection of nestin expression in CNS germ cell tumors might be useful as an indicator of dissemination and/or progression.

In this study, we therefore examined nestin expression in 19 CNS germ cell tumors (nine pure germinomas, five germinomas with syncytiotrophoblastic giant cells (STGCs), one yolk sac tumor, one choriocarcinoma, one embryonal carcinoma, and two mature teratomas) and their clinical course.

Materials and methods

Tissue specimens

CNS germ cell tumor specimens were obtained from 19 patients (3 women and 16 men) undergoing craniotomy or biopsy at Yamagata University Hospital. The histological diagnosis for 19 patients were: nine pure germinomas, five germinoma with STGCs, one yolk sac tumor, one choriocarcinoma, one embryonal carcinoma, and two mature teratomas.

Immunoperoxidase staining

All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 3-μm-thick sections. The tissue sections were immunostained with anti-Nestin (1: 1,500, Chemicon, California, USA) using the biotin–streptavidin immunoperoxidase method (Nichirei, Tokyo, Japan). Immunoreaction was visualized with diaminobenzidine, and tissue sections were briefly counterstained with hematoxylin. Nestin positivity was evaluated semiquantitatively, as follows: 0, no staining; 1+, less than 1–10% of total tumor cell population positive; 2+, 11–20% of total tumor cell population positive; 3+, more than 20% of total tumor cell population positive.

Results

Five patients (one with pure germinoma, three with germinomas with STGCs, one with embryonal carcinoma) exhibited dissemination. The time from diagnosis to dissemination ranged from 9 to 79 months (mean 24.2 months; Table 1).

Nestin immunopositivity was found in 14 (73.6%) cases. Two mature teratomas were nestin negative. All cases of dissemination were strongly positive (2+/3+) for nestin immunostaining. Germinomas with STGCs expressed nestin protein more strongly than pure germinoma. This finding correlated with clinical outcomes; that is, germinomas with STGCs were more refractory to radiochemotherapy than pure germinomas. Other clinical parameters such as tumor size, location, and treatment modalities did not appear to correlate with tumor dissemination.

Representative case (case 6)

Clinical course A 14-year-old boy suffered from visual disturbance and pan-hypopituitarism. Magnetic resonance (MR) imaging revealed a suprasellar mass and optic nerve

Table 1 Summary of cases

Case	Age	Sex	Histological diagnosis	Size (mm)/location	Treatment	FT (M)/status	TTD (M)	Intensity of nestin staining
1	9	M	Pure germinoma	30/BG	SB+RT	158/alive	–	–
2	17	M	Pure germinoma	30/P	RS+RT	131/alive	–	+
3	31	M	Pure germinoma	20/P	SB+RT+CHT	101/alive	–	++
4	10	M	Pure germinoma	40/P	RS+RT+CHT	85/alive	–	+++
5	13	M	Pure germinoma	20/S+P	RS+RT+CHT	66/alive	–	–
6	14	M	Pure germinoma	30/S	RS+RT+CHT	51/alive	11	+++
7	8	F	Pure germinoma	30/S	RS+RT+CHT	39/alive	–	–
8	9	F	Pure germinoma	35/BG	RS+RT+CHT	35/alive	–	++
9	32	M	Pure germinoma	10/S+P	EB+RT+CHT	40/alive	–	++
10	10	M	Germinoma with STGC	30/BG	SB+RT+CHT	194/alive	–	+
11	17	M	Germinoma with STGC	30/P	RS+RT+CHT	59/dead	10	+++
12	20	M	Germinoma with STGC	50/BG-frontal	SB+RT+CHT	52/dead	12	++
13	24	M	Germinoma with STGC	40/S	EB+RT+CHT	98/alive	79	+++
14	17	M	Germinoma with STGC	20/S+P	EB+RT+CHT	55/alive	–	++
15	16	F	Yolk sac tumor	15/S+P	EB+RT+CHT	114/alive	–	+
16	12	M	Choriocarcinoma	40/P	RS+RT+CHT	123/alive	–	++
17	16	M	Embryonal carcinoma	30/P	RS+RT+CHT	13/dead	9	++
18	14	M	Mature teratoma	30/P	RS+RT+CHT	180/dead	–	–
19	35	M	Mature teratoma	40/S	RS	16/alive	–	–

FT Follow-up terminated month, *TTD* Time to dissemination (months), *S* suprasellar, *P* pineal, *BG* basal ganglia, *RS* radical surgery, *EB* endoscopic biopsy, *SB* stereotactic biopsy, *RT* radiation therapy, *CHT* chemotherapy

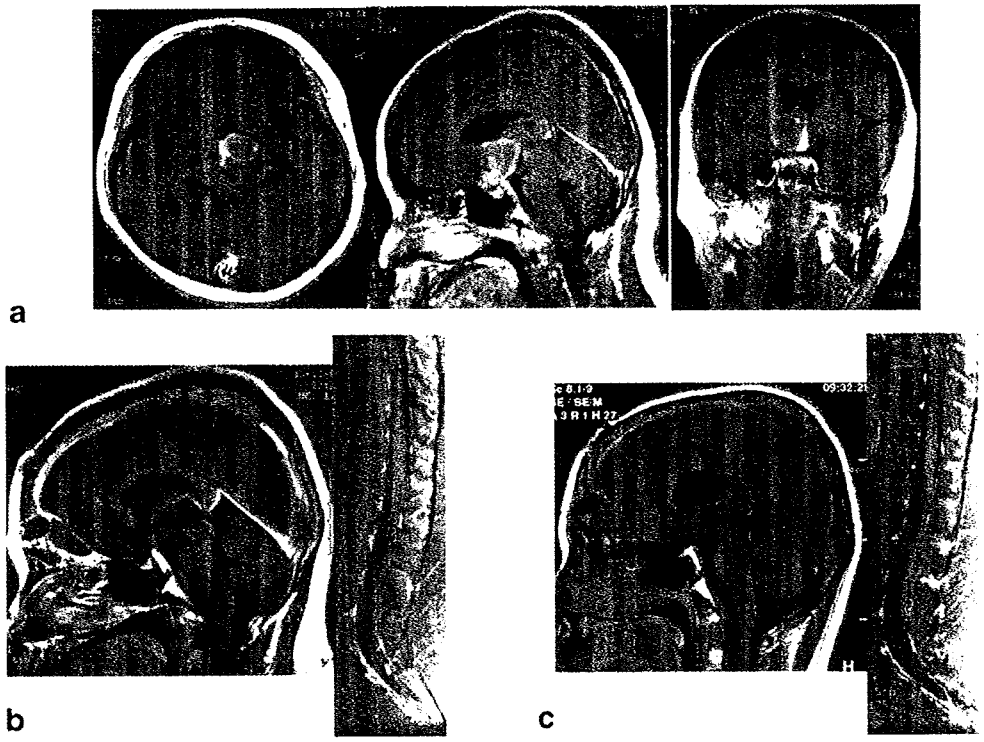


Fig. 1 MRI at first admission (**a**, *upper column*) shows a suprasellar mass and optic nerve swelling. MRI (**b**, *lower left*) shows two nodular disseminated lesions in floor of the fourth ventricle and the cauda equina. MRI (**c**, *lower right*) after additional chemotherapy and radiation demonstrates complete disappearance of the two disseminated lesions

swelling. The preoperative diagnosis was optic glioma. Craniotomy was performed, but because the intraoperative pathologic diagnosis was pure germinoma, we performed only biopsy. He was treated with extended local irradiation and three cycles of combination chemotherapy (ifosphamide–cisplatin–etoposide therapy), according to the protocol of the Japanese Pediatric Brain Tumor Study Group. The tumor completely disappeared after this treatment. However, 10 months after initial treatment, MR imaging revealed dissemination along the fourth ventricle and in the spinal cord. After additional chemotherapy and whole-brain and whole-spine irradiation, the disseminated lesions disappeared, and he has remained alive for 4 years with neither recurrence nor dissemination (Fig. 1).

Pathologic findings The tumor was composed of uniform cells resembling primitive germ cells with lymphoid cell infiltration. Nestin immunopositivity was strongly positive (Fig. 2).

Discussion

CNS germ cell tumors constitute a unique class of rare tumors that mainly affect children and adolescents. The incidence of intracranial germ cell tumors is higher in Asia than in Western countries. Matsutani and the Japanese Pediatric Brain Tumor Study Group reported excellent

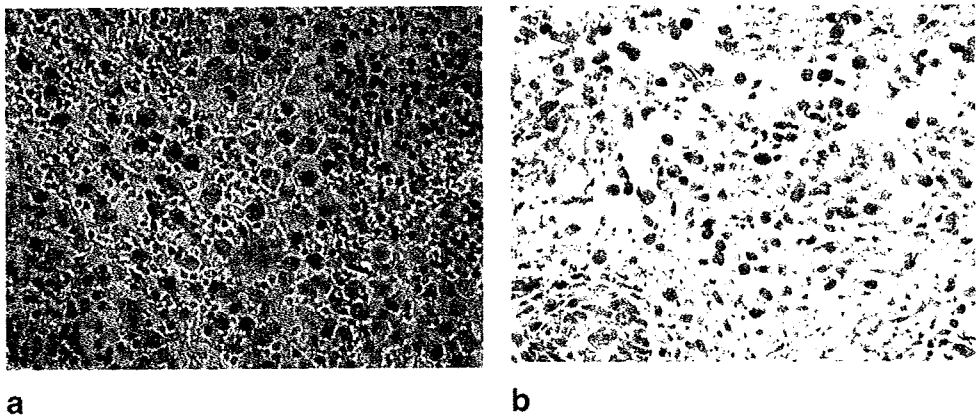


Fig. 2 Staining in case 6. **a** Hematoxylin and eosin staining (×400). Tumor cells are present with abundant clear cytoplasm, with infiltrating lymphocytic cells. **b** Immunostaining for nestin (×400). The intensity of nestin staining is +++

clinical results with use of radiation and combination chemotherapy [6, 7]. They treated germinoma with STGC as an intermediate prognosis group, providing more intensive treatment than would be given to patients of pure germinoma. Although intracranial germ cell tumors are believed to originate from displaced primordial germ cells, whether the "germ cells" correspond to the primordial germ cells known in embryology or to a certain stage of differentiation of primordial germ cells is not clear. Sano [8] propounded the hypothesis that tumors composed of cells resembling the cells that appear in the earlier stages of embryogenesis are more malignant than those composed of cells resembling the cells that appear in the later stages of embryogenesis [9].

Nestin is an IFP expressed in undifferentiated cells during CNS development and in CNS tumors and is used as a marker of immature elements of tumors, including brain tumor stem cells. Many studies have revealed that various brain tumors such as glioblastomas, anaplastic astrocytomas, ependymomas, and medulloblastomas contain cancer stem cells [1, 11, 12]. Nestin was originally thought to be a marker of immature neural lineages. However, recently, many studies have revealed expression of nestin in various lineages, such as melanomas, gastrointestinal stromal tumors, leiomyomas, and others [3, 10]. In a review, Wiese et al.[13] noted that nestin expression is a property of multilineage progenitor cells. Cancer stem cells have the same abilities as normal stem cells and are tumorigenic. Similar to normal stem cells, cancer stem cells have drug export systems. We suspected that stem cell drug resistance contributes to cancer stem cell chemoresistance. In addition, the presence of a small subpopulation of slowly dividing cancer stem cells might explain why malignant primary CNS germ cell tumors recur (dissemination and metastasis) after treatment with radiation or chemotherapy. Recently, Ehrmann et al. [2] reported that immunohistochemical detection of nestin expression could be useful as a diagnostic and prognostic marker of gliomas.

In this study, we examined nestin expression in 19 CNS germ cell tumors and compared nestin expression and tumor dissemination and/or recurrence. This is the first study to examine nestin expression in intracranial germ cell tumors. Nestin was expressed in 14 cases but not expressed in three pure germ cell tumors and two mature teratomas. Nestin-negative tumors did not exhibit dissemination, whereas all tumors with dissemination (one pure germ cell tumor and three germinomas with STGC, one embryonal carcinoma) expressed nestin strongly. The Japanese Pediatric Brain Tumor Study Group separated intracranial germ cell tumors into three groups (good prognosis group: pure germinomas, intermediate group: germinomas with STGCs, poor prognosis group: embryonal carcinoma, choriocarcinoma, yolk sac tumor)[7]. We treated tumors on an individual basis according to group with radiation and multidrug chemotherapy and obtained mostly excellent results. However, a few patients had poor results despite being in the good prognosis group. Although more precise and long follow-up is needed, the present findings indicate that detection of nestin expression could be useful in the treatment of CNS germ cell tumors, as an auxiliary indicator of dissemination and/or progression.

References

1. Almqvist PM, Mah R, Lendahl U, Jacobsson B, Hendson G (2002) Immunohistochemical detection of nestin in pediatric brain tumors. J Histochem Cytochem 50:147–58
2. Ehrmann J, Kolar Z, Mokry J (2005) Nestin as a diagnostic and prognostic marker: immunohistochemical analysis of its expression in different tumours. J Clin Pathol 58:222–223
3. Grichnik JM, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL, Vervaert CE, Seigler HF (2006) Melanoma, a tumor based on a mutant stem cell? J Invest Dermatol 126:142–53
4. Kondo T, Setoguchi T, Taga T (2004) Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 101:781–786
5. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 5:67
6. Matsutani M (2001) Combined chemotherapy and radiation therapy for CNS germ cell tumors—the Japanese experience. J Neurooncol 54:311–316
7. Matsutani M, Ushio Y, Abe H, Yamashita J, Shibui S, Fujimaki T, Takakura K, Nomura K, Tanaka R, Fukui M, Yoshimoto T, Hayakawa T, Nagashima T, Kurisu K, Kayama T (1998) Combined chemotherapy and radiation therapy for central nervous system germ cell tumors: preliminary results of a Phase II study of the Japanese Pediatric Brain Tumor Study Group. Neurosurg Focus 5:e7
8. Sano K (1999) Pathogenesis of intracranial germ cell tumors reconsidered. J Neurosurg 90:258–264
9. Sano K, Matsutani M, Seto T (1989) So-called intracranial germ cell tumours: personal experiences and a theory of their pathogenesis. Neurol Res 11:118–126
10. Sarlomo-Rikala M, Tsujimura T, Lendahl U, Miettinen M (2002) Patterns of nestin and other intermediate filament expression distinguish between gastrointestinal stromal tumors, leiomyomas and schwannomas. APMIS 110:499–507
11. Schiffer D, Manazza A, Tamagno I (2006) Nestin expression in neuroepithelial tumors. Neurosci Lett 400:80–85
12. Sugawara K, Kurihara H, Negishi M, Saito N, Nakazato Y, Sasaki T, Takeuchi T (2002) Nestin as a marker for proliferative endothelium in gliomas. Lab Invest 82:345–351
13. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM (2004) Nestin expression—a property of multi-lineage progenitor cells? Cell Mol Life Sci 61:2510–2522

Comments

Matthias Kirsch, Gabriele Schackert, Dresden, Germany

In the present paper, Sakurada et al. demonstrated the preferential appearance of Nestin-positive tumor cells in disseminated versus local

germ cell tumors. This finding might be relevant for therapeutic decisions upon early, although radiologically unproven dissemination.

In addition, the authors relate to the possibility of a potential germinoma stem-like cell population. For this purpose, CD133 or prominin-1 is most prominently used as a tumor stem cell marker although neither function nor pathogenetic role is known. Recently, CD133+ glioma-initiating cells are tumorigenic in vivo even after serial transplantation (reviewed in [1, 3]. These brain tumor stem cells represent 1% to 30% of the total cell number and show characteristics of neural stem cells. Their unique potential for chemotherapy and radiation therapy resistance has enormous implications on our current understanding of tumor biology and treatment rationals. Regarding germ cell tumors, up-to-date, only one additional embryonic stem cell factor has been associated with another type of germ cell tumors: sox2 is expressed in embryonal carcinoma but not pure seminoma [2]. Therefore, an exciting road of future investigations lies ahead: characterization of CD133 and other stemness markers in germinomas, isolation and propagation of tumor-initiating cells, as well as a demonstration of their tumorigenicity in vivo.

References

1. Dirks PB (2006) Stem cells and brain tumours. Nature 444 (7):687

2. Santagata S, Ligon KL, Hornick JL (2007) Embryonic stem cell transcription factor signatures in the diagnosis of primary and metastatic germ cell tumors. Am J Surg Pathol 31(6):836

3. Vescovi AL, Galli R, Reynolds BA (2006) Brain tumour stem cells. Nature Reviews Cancer 6(June):425

Jpn J Clin Oncol 2008;38(10)715–718
doi:10.1093/jjco/hyn095

Clinical Trial Note

A Multicenter Phase I Trial of Interferon-β and Temozolomide Combination Therapy for High-grade Gliomas (INTEGRA Study)

Toshihiko Wakabayashi[1], Takamasa Kayama[2], Ryo Nishikawa[3], Hiroshi Takahashi[4], Toshiki Yoshimine[5], Nobuo Hashimoto[6], Tomokazu Aoki[7], Kaoru Kurisu[8], Atsushi Natsume[1], Masatoshi Ogura[1] and Jun Yoshida[1]

[1]Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, [2]Department of Neurosurgery, Yamagata University School of Medicine, Yamagata, [3]Department of Neurosurgery, Saitama Medical University, Saitama, [4]Department of Neurosurgery, Nippon Medical School, Tokyo, [5]Department of Neurosurgery, Osaka University School of Medicine, Osaka, [6]Department of Neurosurgery, Kyoto University School of Medicine, Kyoto, [7]Department of Neurosurgery, Kitano Hospital, Osaka and [8]Department of Neurosurgery, Hiroshima University School of Medicine, Hiroshima, Japan

Received June 24, 2008; accepted August 14, 2008

A multicenter phase I clinical trial, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA Study), is being conducted for patients with high-grade glioma in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of interferon-β and temozolomide. The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with the measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by magnetic resonance imaging. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors. Ten newly diagnosed and 10 recurrent patients will be enrolled in this study.

Key words: chemo-phase I-II-III — clinical trials — CNS

INTRODUCTION

Gliomas account for ~40% of all brain tumors and are thus the most common primary tumors of the central nervous system. Primary brain tumors are classified according to their cell type and histological grade into categories defined by the World Health Organization (WHO) (1). High-grade (WHO grades III and IV) gliomas, which include anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA) and glioblastoma multiforme (GBM), are often resistant to treatment; GBM, the most common glioma in adults, kills patients within a median time span of a year after diagnosis despite treatment with aggressive surgical resection, nitrosourea-based chemotherapy and radiotherapy (2–4). A number of studies by large cooperative groups have shown the benefits of radiation therapy in doses up to 60 Gy after surgery for improving overall survival and time to progression (5). In Japan, nitrosourea agents such as 1-(4-amino-2-methyl-5-pyridiminyl)methyl-3-(2-chloroethyl)-3-nitrosourea and methyl-6-[3-(2-chloroethyl)-3-nitrosoureido]-6-deoxy-alpha-D-glucopyranoside have been used to treat malignant gliomas for a long time; however, this treatment offered few clinical benefits. Temozolomide (TMZ), an oral alkylating agent, has been demonstrated to possess antitumor activity against malignant gliomas, with minimal additional toxicity; furthermore, in a previous study of concomitant radiation therapy and chemotherapy with TMZ followed by adjuvant TMZ, survival duration substantially improved (6). In 2006, TMZ

For reprints and all correspondence: Toshihiko Wakabayashi, Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Japan.
E-mail: wakabat@med.nagoya-u.ac.jp

was certified as the treatment agent for malignant gliomas by the National Ministry of Health and Welfare of Japan, and a combination of radiotherapy and chemotherapy with TMZ is now used as the first-line therapy. However, its clinical outcomes depend on the *O*-(6)-methylguanine-DNA methyltransferase (MGMT) status, and MGMT modification is one of the key factors to obtain greater clinical benefits in the future.

Interferon-β (IFN-β) exhibits pleiotropic biological effects and has been widely used either alone or in combination with other antitumor agents in the treatment of malignant gliomas and melanomas (7). In the treatment of malignant gliomas, IFN-β can act as a drug sensitizer, enhancing toxicity against various neoplasms when administered in combination with nitrosourea. IFN-β and nitrosourea combination therapy has been particularly used for the treatment of gliomas in Japan (8). Previously, we demonstrated that IFN-β markedly enhanced chemosensitivity to TMZ in an *in vitro* study of human glioma cells (9); this finding suggested that one of the major mechanisms by which IFN-β enhances chemosensitivity is the downregulation of MGMT transcription via *p53* induction. This effect was also observed in an experimental animal model (10). These two studies suggested that chemotherapy with IFN-β and TMZ plus radiation might further improve the clinical outcome in malignant gliomas when compared with TMZ plus radiation therapy. Here, in order to evaluate the safety, feasibility and preliminary clinical effectiveness of the combination of IFN-β and TMZ, we are conducting a clinical study, namely, Integrated Japanese Multicenter Clinical Trial: A Phase I Study of Interferon-β and Temozolomide for Glioma in Combination with Radiotherapy (INTEGRA study). This study involves eight medical institutions, covering the entire regional population of Japan.

PROTOCOL DIGEST OF THE STUDY

Purpose

The main aim of this study is to evaluate the safety, feasibility and preliminary clinical effectiveness of IFN-β and TMZ for the treatment of malignant gliomas.

Study Setting and Protocol Review

This is a multicenter clinical trial involving eight neurosurgical institutions: Yamagata, Saitama Medical, Nippon Medical, Nagoya, Osaka, Kyoto, and Hiroshima Universities and Kitano Hospital. The protocol has been reviewed and approved by institutional review boards of each of these institutions.

Registration and Monitoring

Participating investigators are instructed to send an eligibility criteria report to the Data Center at Nagoya University, which is a third party different from the study director. Ten newly diagnosed and 10 recurrent patients are registered for a period of 6 months from December 2007. Data, including those of magnetic resonance imaging (MRI), blood tests, and pathology, will be collected at the data center. The quality of data will be checked and verified at the data center. If required, the data center would provide feedback to the institutions. The data center will send high-quality data to the study director. Committees of safety and efficacy (Dr Kazuo Tabuchi, Koyanagi Memorial Hospital, Saga), radiotherapy (Dr Shinji Naganawa, Department of Radiology, Nagoya University School of Medicine), pathological review (Dr Youichi Nagasato, Department of Pathology, Gunma University School of Medicine) and statistics (Dr Kunihiko Hayashi, Gunma University School of Health Science) will send their reports to the head office.

Endpoints

The primary endpoint is incidence of adverse events. The secondary endpoints are progression-free survival time and overall survival time. In addition, objective tumor response will be evaluated in a subpopulation of patients with measurable disease. The reduction rate of tumor will be calculated according to Response Evaluation Criteria In Solid Tumors for measurable tumors as determined by MRI. Non-measurable tumors are classified into four grades: complete remission, partial response, progression and not evaluable. Subsequently, the overall response will be evaluated based on the results of measurable and non-measurable tumors.

Eligibility Criteria

The eligibility criteria are as follows:

(i) Histologically confirmed diagnosis of newly diagnosed or recurrent high-grade glioma (AA, AO, AOA or GBM). More than 50% volume of tumor is located in the supratentorial region.
(ii) No tumor recognized in the optic nerve, olfactory nerve and pituitary gland on pretreatment MRI.
(iii) No dissemination detected by MRI. Age between 18 and 75 years at the time of registration.
(iv) Performance status is 0–2, 3 only due to neurological deficits.
(v) Sufficient organ function before chemotherapy according to the following laboratory data: WBC $\geq$ 3000/mm^3 or neutrophils $\geq$1500/mm^3, platelets $\geq$100 000/mm^3, hemoglobin $\geq$8.0 g/dl, bilirubin $\leq$1.5 mg/dl, serum glutamic oxaloacetic transaminase $\leq$ 100 IU, serum glutamic pyruvic transaminase $\leq$ 100 IU, creatinine $\leq$1.5 mg/dl, creatinine clearance $\geq$50 ml/min and electrocardiogram showing no serious arrhythmia and no serious ischemic heart disease.
(vi) No prior chemoradiotherapy for newly diagnosed patients.

(vii) The interval from the end of prior anti-tumor therapy (e.g. chemotherapy, radiotherapy, immunotherapy) must be at least 4 weeks for recurrent patients, regardless of the regimen.
(viii) Written informed consent.

Exclusion Criteria

The exclusion criteria are as follows:

(i) synchronous double cancer or metachronous double cancer in last 5 years; carcinoma *in situ* accepted;
(ii) meningitis or pneumonia;
(iii) pregnant, possibly pregnant, or nursing women;
(iv) mental disorder;
(v) uncontrolled diabetes mellitus (DM) or under treatment with insulin for DM;
(vi) myocardial infarction in last 3 months;
(vii) history of pulmonary fibrosis or interstitial pneumonia.

Treatment Methods

For newly diagnosed patients:

Radiotherapy	60 Gy/30 fr, 2 Gy ×5 days/week;
IFN-β	3 MIU/body, administered intravenously on alternate days during radiotherapy;
TMZ	75 mg/(m^2 day), daily from the first day to the last day of radiotherapy.

After completing this induction period, all patients will have 4 weeks of washout period, and they will be then shifted to adjuvant period.

IFN-β	3 MIU/body, administered on the first day morning every 4 weeks;
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days in the absence of tumor progression, serious adverse events such as grade 4 hematological toxicity, refusal of therapy and deviation from the protocol.

For recurrent patients:

IFN-β	3 MIU/body, administered the first day morning every 4 weeks (day 1);
TMZ	150 mg/(m^2 day) (days 1–5: first cycle); 200 mg/(m^2 day) (days 1–5: second to sixth cycle).

In the absence of hematologic toxicity, the dose is increased to 200 mg/(m^2 day), beginning with the second cycle to the sixth cycle.

This cycle is repeated six times every 28 days.

This regimen has been considered to be the most promising based on previous clinical studies (8,11–14). Thus, dose-limiting toxicity was not evaluated in this study.

Follow-up and Statistical Methods

Disease progression and occurrence of new disease will be examined by MRI performed at baseline and at least after every 4–5 weeks during treatment. Blood tests and symptom checks will be carried out before treatment and at least after every 2 weeks during treatment. Follow-up will continue for 3 months from the end of treatment. In cases wherein therapy is discontinued due to toxicity, clinicians would follow-up patients until they recover from toxicity. In addition, overall survival, progression-free survival and treatment success curves are constructed as time-to-event plots by the Kaplan–Meier method.

Acknowledgement

We would like to thank Dr Junichi Sakamoto for his helpful comments and suggestions.

Funding

This work was supported in part by Japan Brain Foundation.

Conflict of interest statement

None declared.

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007;114:97–109.
2. Nagane M, Levitzki A, Gazit A, Cavenee WK, Huang HJ. Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases. *Proc Nat Acad Sci USA* 1998;95:5724–9.
3. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res* 2004;64:6892–9.
4. Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J Neuropathol Exp Neurol* 2005;64:479–89.
5. Walker MD, Green SB, Byar DP, Alexander E Jr, Batzdorf U, Brooks WH, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med* 1980;303:1323–9.
6. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
7. Chawla-Sarkar M, Lindner DJ, Liu YF, Williams BR, Sen GC, Silverman RH, et al. Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. *Apoptosis* 2003;8:237–49.
8. Wakabayashi T, Hatano N, Kajita Y, Yoshida T, Mizuno M, Taniguchi K, et al. Initial and maintenance combination treatment with interferon-beta, MCNU (Ranimustine), and radiotherapy for patients with previously untreated malignant glioma. *J Neurooncol* 2000;49:57–62.

9. Natsume A, Ishii D, Wakabayashi T, Tsuno T, Hatano H, Mizuno M, et al. IFN-beta down-regulates the expression of DNA repair gene MGMT and sensitizes resistant glioma cells to temozolomide. *Cancer Res* 2005;65:7573–9.
10. Natsume A, Wakabayashi T, Ishii D, Maruta H, Fujii M, Shimato S, et al. A combination of IFN-beta and temozolomide in human glioma xenograft models: implication of p53-mediated MGMT downregulation. *Cancer Chemother Pharmacol* 2007.
11. Aoki T, Takahashi JA, Ueba T, Oya N, Hiraoka M, Matsui K, et al. Phase II study of nimustine, carboplatin, vincristine, and interferon-beta with radiotherapy for glioblastoma multiforme: experience of the Kyoto Neuro-Oncology Group. *J Neurosurg* 2006;105:385–91.
12. Hatano N, Wakabayashi T, Kajita Y, Mizuno M, Ohno T, Nakayashiki N, et al. Efficacy of post operative adjuvant therapy with human interferon beta, MCNU and radiation (IMR) for malignant glioma: comparison among three protocols. *Acta Neurochir* 2000;142:633–8; discussion 9.
13. Watanabe T, Katayama Y, Yoshino A, Fukaya C, Yamamoto T. Human interferon beta, nimustine hydrochloride, and radiation therapy in the treatment of newly diagnosed malignant astrocytomas. *J Neurooncol* 2005;72:57–62.
14. Yoshida J, Kajita Y, Wakabayashi T, Sugita K. Long-term follow-up results of 175 patients with malignant glioma: importance of radical tumour resection and postoperative adjuvant therapy with interferon, ACNU and radiation. *Acta Neurochir* 1994;127:55–9.

Neurol Med Chir (Tokyo) 49, 340~350, 2009

Clinical Indications for High-Field 1.5 T Intraoperative Magnetic Resonance Imaging and Neuro-navigation for Neurosurgical Procedures

—Review of Initial 100 Cases—

Satoshi MAESAWA, Masazumi FUJII*, Norimoto NAKAHARA, Tadashi WATANABE, Kiyoshi SAITO*, Yasukazu KAJITA*, Tetsuya NAGATANI*, Toshihiko WAKABAYASHI*, and Jun YOSHIDA*

Department of Neurosurgery, Nagoya Central Hospital, Nagoya, Aichi;
**Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi*

Abstract

Initial experiences are reviewed in an integrated operation theater equipped with an intraoperative high-field (1.5 T) magnetic resonance (MR) imager and neuro-navigation (BrainSUITE®), to evaluate the indications and limitations. One hundred consecutive cases were treated, consisting of 38 gliomas, 49 other tumors, 11 cerebrovascular diseases, and 2 functional diseases. The feasibility and usefulness of the integrated theater were evaluated for individual diseases, focusing on whether intraoperative images (including diffusion tensor imaging) affected the surgical strategy. The extent of resection and outcomes in each histological category of brain tumors were examined. Intraoperative high-field MR imaging frequently affected or modified the surgical strategy in the glioma group (27/38 cases, 71.1%), but less in the other tumor group (13/49 cases, 26.5%). The surgical strategy was not modified in cerebrovascular or functional diseases, but the success of procedures and the absence of complications could be confirmed. In glioma surgery, subtotal or greater resection was achieved in 22 of the 31 patients (71%) excluding biopsies, and intraoperative images revealed tumor remnants resulting in the extension of resection in 21 of the 22 patients (95.4%), the highest rate of extension among all types of pathologies. The integrated neuro-navigation improved workflow. The best indication for intraoperative high-field MR imaging and integrated neuro-navigation is brain tumors, especially gliomas, and is supplementary in assuring quality in surgery for cerebrovascular or functional diseases. Immediate quality assurance is provided in several types of neurosurgical procedures.

Key words: intraoperative magnetic resonance imaging, navigation, glioma

Introduction

Intraoperative magnetic resonance (MR) imaging has gradually become widespread since Brigham and Women's Hospital in Boston introduced a 0.5 Tesla (T) open MR imager to the operating theater in 1994.[2,5,24,40,41] Our institution installed a fully integrated neurosurgical system including neuro-navigation and an intraoperative 1.5 T high-field MR imager (BrainSUITE®; BrainLAB AG, Heimsteten, Germany), the first such unit in Asia, in 2006. Compared to intraoperative low- or medium-field MR imaging, this system provides high quality images with short scan times, and various sequences are available intraoperatively including MR spectroscopy, diffusion tensor imaging, diffusion-weighted imaging, and MR angiography.[27,28,31,32] Using an image-fusion technique, the integrated navigation system can combine MR imaging data with functional and/or metabolic information obtained by other modalities such as positron emission tomography (PET) and magnetoencephalography.[9,27] Overall, the fully integrated architecture facilitates straightforward workflow including patient transport, image

Received May 23, 2008; Accepted February 13, 2009
Author's present address: S. Maesawa, M.D., Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan.